Population Genetics of Bovine and Human Schistosomes in Côte d'Ivoire

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Dekan

Table of Contents

List of Figures

List of Tables

List of Abbreviations

A	Alleles
AM	Arithmetic Mean
A_r	Allelic Richness
BCE	Before Common Era
BLAST	Basic Local Alignment Search Tool
BP	Base pairs
CI	Confidence Interval
COX1	Cytochrome Oxidase Subunit I
CSRS	Centre Suisse de Recherches Scientifiques
DALYs	Disability Adjusted Life Years
DAPC	Discriminant Analysis of Principal Components
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediamine Tetraacetic Acid
EPG	Eggs per Gram of Feces
ERR	Egg Reduction Rate
ETOH	Ethanol Alcohol
ETS	External Transcribed Spacer
FECT	Fecal Egg Count Reduction Test
F_{IS}	Inbreeding Coefficient
F_{ST}	Fixation Index
GBP	Global Burden of Disease
GDP	Gross Domestic Product
GEE	Generalized Estimating Equation
GE	Geometric Mean
H_e	Expected Heterozygosity
H_o	Observed Heterozygosity
HSES	Health in Social-Ecological Systems
ICOSA	Integrated Control of Schistosomiasis in sub-Saharan Africa
ITS	Internal Transcribed Spacers
LANADA	Laboratoire National d'Appui au Développement Agricole
MCMC	Markov Chain Monte Carlo
MDA	Mass Drug Administration
MIRAH	Ministère des Ressources Animales et Halieutiques
MtDNA	Mitochondrial DNA
NAD	NADH-dehydrogenase Subunit 1

NTD	Neglected Tropical Disease
NTS	Non-transcribed Spacer
OR	Odds Ratio
PBS	Phosphate Buffered Saline
PCA	Principal Components Analysis
PCR	Polymerase Chain Reaction
P_{HWE}	Hardy-Weinberg Equilibrium
PNES	Proportion of Non-egg Shedding Cattle
POC-CAA	Point-of-care Circulating Cathodic Antigen
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
RR	Reinfection Rate
R_{ST}	Fixation Index
SDGs	Sustainable Development Goals
SNPs	Single Nucleotide Polymorphism
SNSF	Swiss National Science Foundation
STR	Short Tandem Repeats
Swiss TPH	Swiss Tropical and Public Health Institute
TAE	Tris-acetate-EDTA
TBE	Tris-borate-EDTA
UN	United Nations
WASH	Water, Sanitation and Hygiene
WHO	World Health Organization
YLD	Years Lived with Disability

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I would like to dedicate this thesis to my paternal Grandparents and Uncles: Steve, Anna, Jaroslaw (Jerry) and Jan (John) Ewasiechko. I am forever grateful for the substantial sacrifices you made that allowed me a life of such possibilities. Your courage, persistence and resilience are a daily inspiration.

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Summary

Neglected tropical diseases (NTDs) are a diverse group of infectious disease that severely affect the health of over one billion people in 149 countries across the globe. Schistosomiasis and fascioliasis are among the 20 NTDs identified by the World Health Organization (WHO) and are identified for control in the WHO's Roadmap, *Ending the neglect to attain the sustainable development goals: A road map for neglected tropical diseases 2021-2030.*

These two NTDS caused by trematode parasites, are also classified as zoonotic neglected tropical diseases and are, therefore, not only of great concern to human health professionals but also to veterinary health professionals, as they cause considerable human morbidity and economic loss to the livestock industry. Furthermore, as these are diseases of poverty, their endemicity in low-resource settings endanger the attainment of United Nation's Sustainable Development Goal number one: end poverty.

Many resources are devoted to the control and prevention of human fascioliasis and schistosomiasis and, while these programs have had success, continuous adaption of the parasites to evade control measures is a constant risk. Hybridization of species is an opportunity for organisms to acquire advantageous traits. In the case of parasites, examples include expanded host range and increased pathogenicity. To date numerous combinations of schistosome species from livestock and humans, as well as *Fasciola* hybrids have been documented. In the context of NTD control, hybridization poses a threat to expensive gains made by control and prevention programs, especially if it leads to persistent animal reservoirs or drug resistance.

Population genetics offers insight into genetic diversity, population structure and gene flow, which influence how alleles spread throughout a population. Understanding the extent of, and barriers to gene flow can help forecast the direction and rate of spread of advantageous alleles such as drug-resistance and inform efforts to reduce this risk.

This project used a One Health approach to examine transmission dynamics and hybridization of human and animal trematodes in sub-Saharan Africa. One Health is a holistic approach to health that addresses the links between human and animal medicine, public health and environmental sustainability for the betterment of the health and well-being of humans, animals and the environment. It emphasizes transdisciplinarity, collaboration and systems thinking and is particularly useful when tackling control of zoonotic diseases.

The aims of this thesis were to identify *Schistosoma* and *Fasciola* species found in Ivorian humans, cattle, sheep and goats using mitochondrial and nuclear loci, quantify genetic diversity and infer transmission dynamics of schistosomes within and between definitive hosts and geographical locations in Côte d'Ivoire. In addition to these aims, the sensitivity of the sedimentation method for diagnosis of cattle schistosomiasis was assessed and a clinical trial testing the effectiveness of triclabendazole in *Fasciola* infected Ivoirian cattle was conducted.

In order to meet these goals *Schistosoma* and *Fasciola* flukes from slaughtered cattle, sheep and goats at five abattoirs across Côte d'Ivoire were collected. Additionally, *Schistosoma* miracidia were hatched from the urine of humans and feces of cattle, sheep and goats in villages and farms sharing the same water source in Northern Côte d'Ivoire. DNA was extracted from all biological samples for genetic analysis of mitochondrial and nuclear loci as well as microsatellites.

High prevalences of schistosome hybrids were observed in humans in Côte d'Ivoire. Various degrees of hybridization were observed, indicating that this region is a stable hybrid zone. It would appear that most hybrids are a cross between human (*Schistosoma haematobium)* and livestock species (*S. bovis)*, however, in the North we observed traces of *S. curassoni* (a schistosome species of livestock) in hybrids, indicating that *S. curassoni* may also play a role in human schistosomiasis. Cattle did not harbour any hybrids, and all cattle across the five sites were infected with *S. bovis*.

Schistosomes in Côte d'Ivoire were genetically diverse across all locations. Geographical structuring was observed in human schistosome populations, while cattle schistosome populations appear to be panmictic, with very little structuring.

The molecular investigation of *Fasciola* flukes from Chad and Côte d'Ivoire revealed the first *Fasciola* hybrid (*F. gigantica* x *F. hepatica*) in Africa, from Chad, and a clinical trial demonstrated that triclabendazole is an effective treatment for *Fasciola* infected Ivorian cattle.

Observations during the fieldwork indicated that sedimentation, the common diagnostic method for fascioliasis and schistosomiasis in livestock, was misclassifying schistosomiasis cases (false negatives). Consequently, the sensitivity of sedimentation for schistosomiasis diagnosis in cattle was tested and shown to be somewhat low due to eggs hatching during the sedimentation process, although sensitivity can be increased by adjusting the protocol in ways that inhibit egg hatching.

Schistosomiasis and fascioliasis have a high cost to society in terms of human morbidity and economic losses to farmers, especially in low-resource setting. They are complex problems that are influenced by biological and environmental factors, but also social factors such as politics, culture, economics and determinants of health. Further research is needed in West Africa, and specifically in Côte d'Ivoire, to ascertain the extent to which schistosomiasis and fascioliasis in livestock, and other definitive hosts, pose a risk to human health and NTD control and prevention goals. Human and animal health are intricately linked, especially in low-resources settings with sustenance farming. An integrated One Health approach is essential to tackle the complexity of NTDs and the SDGs if progress towards these goals is to be made.

1. Introduction

Neglected tropical diseases (NTDs) are "a diverse group of communicable diseases that prevail in tropical and subtropical conditions in 149 countries, affect more than one billion people and cost developing economies billions of dollars every year" (World Health Organization, 2020). They cause a great burden in terms of social, health and economic consequences among the world's most vulnerable and marginalized people (WHO, 2020). Populations most at risk are those who lack sanitation, living in poverty and in close contact with disease vectors and domestic animals and livestock (World Health Organization, 2020).

The WHO has set goals for the elimination and control of NTDs over the last 20 years, however, attainment of these goals has proven exceedingly difficult and complex due to the complicated biology of the pathogens (intermediate hosts, vectors and animal reservoirs) as well as the profound influence of social determinants of health, such as health practices and coping, health services, physical environments and education. Ultimately, many NTDs cannot be eliminated with single-pronged interventions. Integrated approaches that effectively interrupt transmission at the interface of the environment and human host, through treatment, environmental sanitation and hygiene, vector control and behaviour change are required (Campbell et al., 2020; Colley et al., 2020; King et al., 2020).

Schistosomiasis and fascioliasis are caused by parasitic blood and liver flukes, respectively, of the *Schistosoma* and *Fasciola* genera. They are among the 20 NTDs listed by the WHO. In addition to causing NTDs, these two parasites are zoonotic and are, therefore, not only of great concern to human health professionals but also to veterinary health professionals. It is estimated that schistosomiasis costs 1.43 million disability adjusted life years (DALYS) globally, while food born trematodiasis, which includes fascioliasis, were estimated to cost 1.87 million DALYS globally in 2018 (GBD 2017 DALYs and HALE Collaborators, 2018). In addition to the human health burden, they cause considerable economic loss to the livestock industry (MSD, 2016; Robinson and Dalton, 2009) and animal reservoirs pose a risk to expensive gains made in prevention and control and future goals of elimination.

Prevention and control of zoonotic diseases is multifaceted and requires systems thinking that traditional methods lack. One Health is a holistic approach used to tackle complex issues that affect humans and animals. It considers the health of both animals and humans as well as financial and environmental implications and emphasizes transdisciplinarity, involving not only human and veterinary medical professionals, but also social scientists, stakeholders and communities. This PhD thesis uses a One Health approach to address transmission dynamics of *Schistosoma* and *Fasciola* in Côte d'Ivoire.

Schistosomiasis

Schistosomiasis has plagued humankind for millennia; Egyptian mummies as far back as 1250 BCE, have been found to harbour *Schistosoma haematobium* eggs. Yet it was only in 1852, also in Egypt, that Theodor Bilharz, a German medical doctor, first described the adult stage of *S. haematobium*, along with his colleague, Ernst von Siebold, giving rise to the name Bilharzia (Despommier et al., 2019). Since this time many more *Schistosoma* species have been discovered and many advances in the fight against schistosomiasis have been made. Nevertheless, this affliction continues to cause great suffering to many people in Africa, Asia and South America and the path to eradication still eludes humanity.

Life cycle and Biology

Schistosoma, also known as blood flukes, are unlike most other trematodes in that they are dioecious, with the female adult worm living inside the gynaecophoric canal of the male (Colley et al., 2014). Adult couples live in the blood vessels of their definitive mammalian host, but the site depends on the species of schistosome. Five to seven weeks after penetration of the skin by *Schistosoma* cercariae, adult pairs mate and begin to excrete hundreds of eggs a day (Goater et al., 2014), which are either excreted into the urine or feces, or get lodged in the tissue around the body as they flow back into the circulation with the blood (Colley et al., 2014; Walker, 2011). The number of eggs produced per day, the shape of the eggs and the exit route from the body (feces or urine) vary by species (Colley et al., 2014). Only about one third of all eggs eventually make it out of the body and into the environment (Schmidt and Roberts, 2013).

Once eggs are exposed to fresh water and ambient temperatures, ciliated miracidia (150-160 µm long and 60-70 µm wide, depending on the species) hatch from the eggs in as little as 20 minutes. Miracidia have a short lifespan, and consequently, little time to find a suitable intermediate snail host. They do not feed and will eventually exhaust their energy reserves, surviving for only 5-6 hours under optimal conditions (Jones et al., 2008; Schmidt and Roberts, 2013; Sturrock, 1993). Eggs that do not reach water can survive in the environment for approximately one week, as long as they are not exposed to excessive heat or desiccation (Sturrock, 1993).

Snail host species differ by *Schistosoma* species (Tab. 1 and 2). Once the miracidia locate a suitable snail host, they infect it, and undergo asexual replication. After 4-6 weeks tens of thousands of cercariae (1mm long) emerge from the snail host, into the water (Colley et al., 2014; Sturrock, 1993). The release of cercariae usually occurs diurnally, with the stimulation of light and like miracidia, they do not feed and therefore, survive only for 48-72 hours. The cercariae infect the definitive mammalian host by penetrating the skin, after which they lose their tail, are transformed to schistosomulae and travel with the blood through the heart and lungs to the liver where they mature and find a mate

(Goater et al., 2014). Once paired off, the schistosome couple move to the vessels of the urinary or intestinal tracts, depending on the species, where they will lay their eggs (Goater et al., 2014).

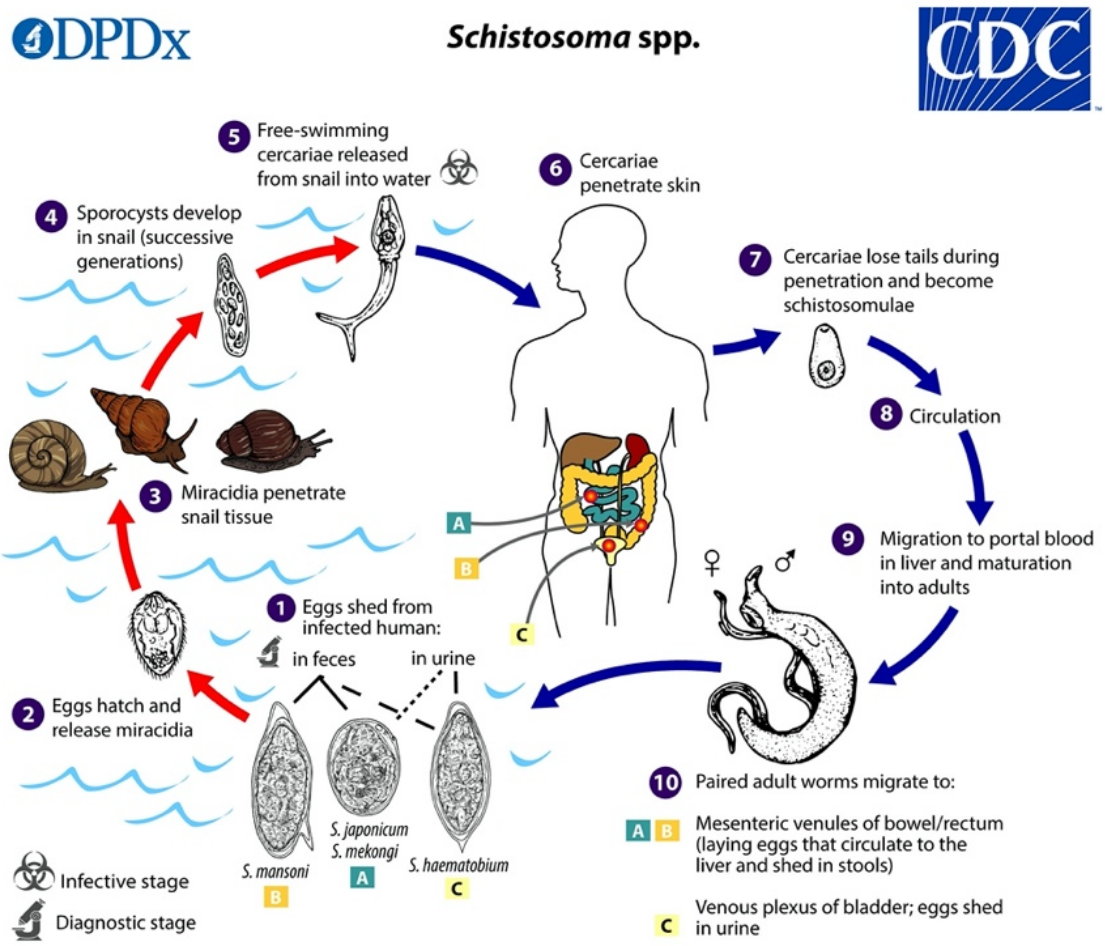

Figure 1.1. Life cycle of schistosome species (CDC, 2019)

Schistosoma Species

Twenty-two *Schistosoma* species are known to naturally infect mammals, of which six are thought to be of medical concern and eight of veterinary concern (Tab. 1.1 and 1.2) (MSD, 2016; Standley et al., 2012). While most *Schistosoma* species reside in the mesenteric veins around the intestinal tract, some inhabit other vessels. Most notably, *S. haematobium* are found in the vessels around the bladder or female genital tract and *S. nasale* are found in the nasal passages of livestock (Agrawal and Rao, 2018; Colley et al., 2014).

Table. 1.1 *Schistosoma* species of medical concern. Adapted from: WHO, Factsheet, 2017; The Australian Society for Parasitology, 2017; Murniello, 2006; Weerakoon et al. 2015 and WHO Schistosomiasis Factsheet, 2017.

Species	Definitive Hosts	Location	Egg Excretion	Snail Host	Geographical Distribution
S. haematobium	humans, primates	veins of urogenital system	urine	*Bulinus*	Africa
S. mansoni	humans, rodents	intestinal mesenteric veins	feces	*Biomphalaria*	Africa, America
S. japonicum	humans, ruminants, carnivores	intestinal mesenteric veins	feces	*Oncomelania*	SE Asia, endemic in some countries in far SE
S. intercalatum	humans, rodents, cattle	intestinal mesenteric veins	feces	*Bulinus, Physopsis*	Africa
S. mekongi	dogs, cats, humans	intestinal mesenteric veins	feces	*Neotricula*	SE Asia
S. guineensis	Humans	intestinal mesenteric veins	feces	*Bulinus*	Rain Forest areas of central Africa

Table. 1.2 *Schistosoma* species of veterinary concern. Adapted from: The Australian Society for Parasitology, 2017. Kaufmann, 1996. Latchumikanthan et al. (2014); MSD VetManual, 2017. Schmidt and Roberts (2013); Sinha and Srivastava, 1960); WHO, Factsheet, 2017.

Species	Definitive Hosts	Location	Egg Excretion	Snail Host	Geographical Distribution
S. mattheei	cattle, small ruminants	intestinal mesenteric veins	feces	*Bulinus*	SE Africa
S. bovis	ruminants small ruminants	intestinal mesenteric veins	feces	*Bulinus*	Mediterranean region, middle east, northern, western and eastern Africa
S. curassoni	cattle, small ruminants	intestinal mesenteric veins	feces	*Bulinus*	Senegal, Mauritania, Mali, Niger, Nigeria
S. spindale	cattle, sheep, horses & donkeys	intestinal mesenteric veins	feces	*Indoplanorbis*	India, Sri Lanka, Indonesia, Malaysia, Thailand and Vietnam
S. indicum	cattle, sheep, horses & donkeys	intestinal mesenteric veins	feces	*Indoplanorbis*	Indian sub-continent
S. nasale	cattle, buffalo, sheep, goat, horses	veins of nasal mucosa	nasal secretions	*Indoplanorbis*	India, Sri Lanka, Bangladesh and Myanmar
S. incognitum	pigs, dogs	intestinal mesenteric veins	feces	*Lymnea/Radix*	India, Thailand, Indonesia
S. japonicum	humans, ruminants, carnivores	intestinal mesenteric veins	feces	*Oncomelania*	endemic in several countries in the far East

Traditionally, *Schistosoma* species have been distinguished by egg morphology (Fig. 2 and 3) and the route of excretion of eggs in the case of coprology or uroscopy, indicating the location of the adult flukes (MSD, 2016; The Australian Society for Parasitology, 2017). With the development and advancement of molecular methods, it is now possible to distinguish *Schistosoma* species at the molecular level.

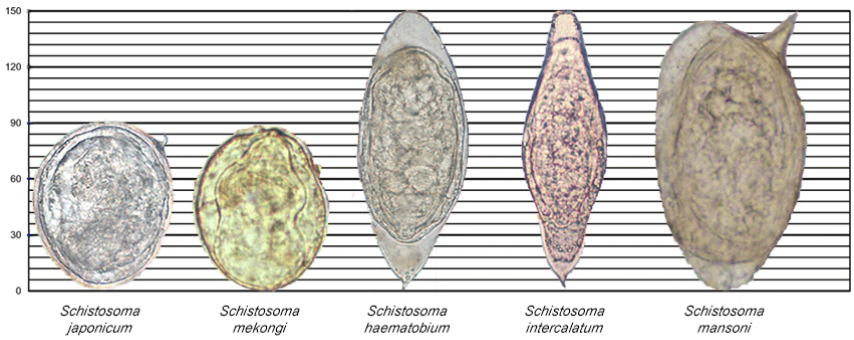

Figure 1.2. Eggs of different *Schistosoma* species ("CDC - DPDx - Diagnostic Procedures - Stool Specimens," 2019)

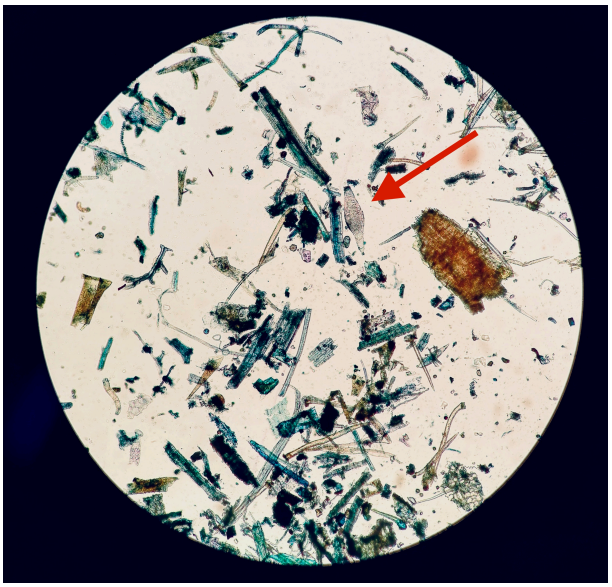

Figure 1.3. *S. bovis* egg (picture courtesy of Jules Kouadio, 2018).

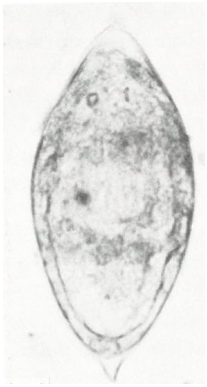

Figure 1.4. *S. curassoni* egg (Rollinson and Simpson 1987).

The only schistosome species officially recognized as a zoonosis is *S. japonicum,* as it infects livestock and humans (Standley et al., 2012). Most species of schistosomes are thought to be host specific, although there have been numerous reports of *S. mansoni* infecting rodents, and two recent report of *S. haematobium* and hybrids of *S. haematobium* x *S. bovis* infecting rodents in Corsica and Senegal (Catalano et al., 2018; Odeniran and Ademola, 2016; Oleaga et al., 2019) as well as *S. haematobium* infecting non-human primates (Odeniran and Ademola, 2016; Standley et al., 2012). Researchers have suspected natural hybridization within and between human and animal schistosomes since the 1940s and more recently, evidence of this phenomenon has been documented in the literature, mostly in West Africa (Tab. 1.3) (Léger et al., 2016). A recent outbreak of schistosomiasis in humans in Corsica, France revealed patients were infected with *S. haematobium*, *S. haematobium* x *S. bovis* hybrids and *S. bovis* of Senegalese origin, indicating that *Schistosoma* are, to some extent, zoonotic (Boissier et al., 2016). Furthermore, cases of human infection with hybridized and introgressed *S. bovis* x *S. curassoni* in Niger have been reported (Léger et al., 2016).

Hybridization makes identifying species by route of egg excretion difficult, as hybrids could be excreted by either route.

Table 1.3 Hybridized *Schistosoma* species determined by molecular methods reported in the literature. Adapted from Léger and Webster, 2017; Boon, 2019; Tian Bi, 2019; Angora, 2019; Savassi 2020.

Hybridized Species	Host Species	Location(s)
S. bovis (livestock) x *S. haematobium*	Humans, cattle (Benin only), *Bulinus truncatus*, *Bu. globosus*	Senegal, Niger, Corsica, Côte d'Ivoire, Benin
S. haematobium x S. guineenis	Humans, *B. forskalii*	Cameroon, Mali, Zambia
S. mansoni x S. rodhaini (wildlife)	*Biomphalaria sudanica*, *Bi. pfeifferi*	Kenya, Cameroon, Tanzania
S. bovis (livestock) *x S. curassoni* (livestock)	Cattle, sheep	Senegal, Niger, Mali
S. haematobium x S. mattheei (livestock)	Humans	South Africa, Mali, Zambia
S. mansoni x S. haematobium	Humans	Mali, Senegal
S. curassoni (livestock) *x S. haematobium*	Humans	Senegal, Niger

Epidemiology

Schistosomiasis in Humans

Schistosomiasis affects more than 250 million human individuals in tropical and subtropical regions of Sub-Saharan Africa, Asia and South America (Fig. 1.5). *Schistosoma* transmission has been reported in 78 countries worldwide, with 90% of people requiring treatment residing in Africa (Colley et al., 2014; De Bont and Vercruysse, 1997; WHO, 2017). Contact with fresh water for domestic activities (e.g. washing clothes), fishing and swimming are risk for factors for schistosomiasis (WHO, 2017a).

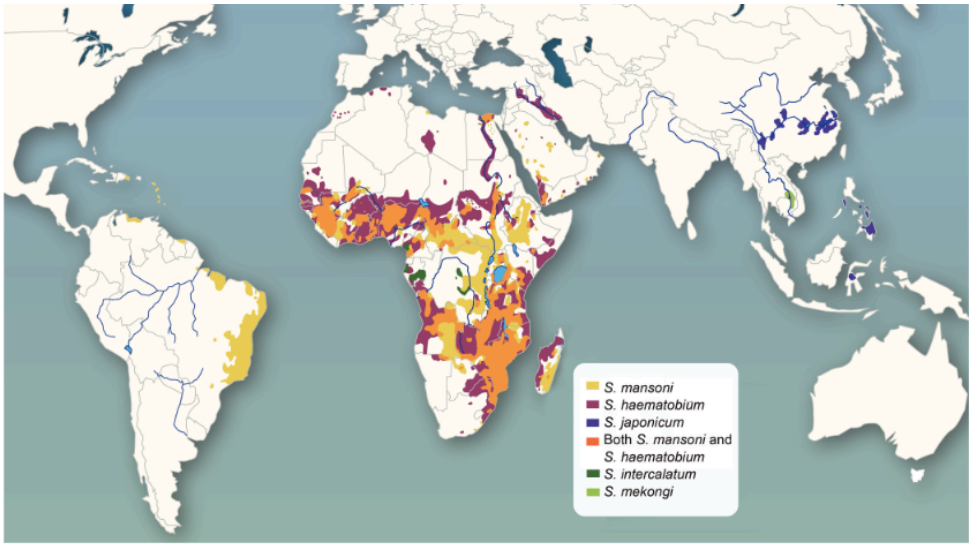

Figure 1.5. Global distribution of schistosomiasis globally (Gryseels et al., 2006; Weerakoon et al., 2015).

Schistosomiasis is highly focal with various species sometimes occurring together (Colley et al., 2014; Vos et al., 2016), depending on the presence of the intermediate host snail. In West Africa, and Côte d'Ivoire, *S. haematobium* and *S. mansoni* are species of human health concern. The presence of intermediate host snails of *S. mansoni* (*Biomphalaria pfeifferi*), *S. haematobium (Bulinus globosus* and *Bu. truncatus*) and *S. bovis (Bu. forskalii, Bu. globosus* and *Bu. truncatus*) have been documented across Côte d'Ivoire, although not all species occur ubiquitously across the country (Diakité et al., 2018, 2017; Krauth et al., 2017; Tian-Bi et al., 2019).

A survey among 5,104 children in 92 schools in Côte d'Ivoire revealed a mean observed prevalence of 5.7% for *S. haematobium*, 3.5% for *S. mansoni* and 0.3% for co-infection of the two species (Chammartin et al., 2014; Yapi et al., 2014) (Fig 1.6). However, *S. mansoni* prevalences between 12.8% and 39.9% amongst school-aged children have been reported in the western region, where prevalences of *S. haematobium* are known to be low (Assaré et al., 2015, 2016a, 2016b; Angora et al., 2019). The prevalences of *S. haematobium* ranges between 9.0 and 35.6% in south-central region of Côte d'Ivoire (Angora et al., 2019; Hürlimann et al., 2014).

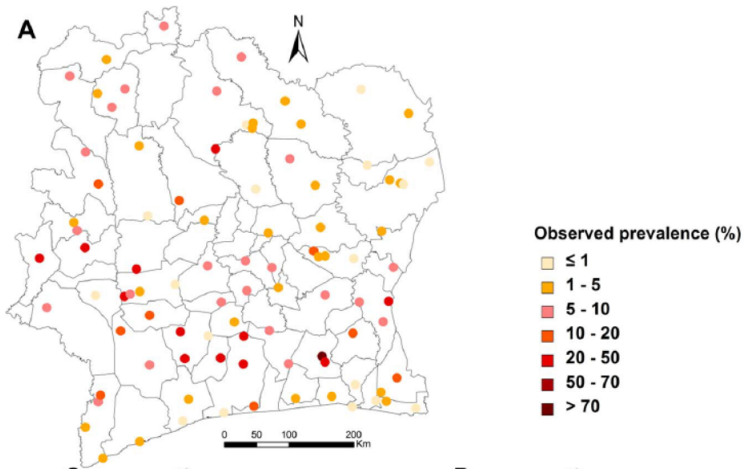

Figure 1.6. Prevalence of schistosomiasis in Côte d'Ivoire (Chammartin et al., 2014).

Schistosomiasis in Animals

S*chistosoma bovis* is located throughout Africa, but mostly north of 10 degrees south, south of this latitude it is replaced by *S. matthei*, while *S. curassoni* is located in West Africa (Standley et al., 2012). Prevalence data of schistosomiasis in animals are sparse compared to that of humans but have been found to range between 31-81% in Africa (De Bont and Vercruysse, 1997). More recent data show prevalences of 19.5% in cattle in Cameroon (Djui, 2020), while Léger et al. (2020) found that 92% of cattle, 14% of sheep and 15% of goats were infected with *S. bovis* in Senegal. In the same investigation *S. curassoni* prevalences were found to be 8% in cattle, 73% in sheep and 84% in goats. There is limited published data regarding prevalence of animal schistosomiasis in Côte d'Ivoire. Achi et al. (2003) found that 35% of cattle were found to be infected with *S. bovis* in the northern Savannah region of Côte d'Ivoire (Achi et al., 2003a) while prevalences in other regions of Côte d'Ivoire were, until recently, not known and thought to be low (Achi et al. 2003a).

Diagnosis

Diagnosis of schistosomiasis is commonly done by coprology: the Kato Katz method in humans and sedimentation in animals, or by uroscopy: urine filtration and concentration. However, these methods are known to lack sensitivity, especially in areas where prevalences are low (Weerakoon et al., 2015). Factors such as the patent period, egg quantity, dispersion of eggs in stool and infection intensity present issues for sensitivity and reliability (Weerakoon et al., 2015). The determination of infecting *Schistosoma* species is done by egg morphology during coprology or uroscopy.

The Kato Katz method was first developed in 1972 and is currently the standard recommended technique for diagnosis of human intestinal schistosomiasis by WHO (Weerakoon et al., 2015). Urinalysis, commonly, done with reagent strips, is another method used for diagnosis of urinary schistosomiasis, as hematuria and proteinuria are associated with the infection (Utzinger et al., 2015; Weerakoon et al., 2015). Recently the point-of-care circulating cathodic antigen (POC-CCA) test has become available and presents a sensitive tool for detecting *S. mansoni* when infection intensities are low, especially after anthelminthic treatment and as control programs move from morbidity control to elimination (Utzinger et al., 2015). Other diagnostic methods in use for humans include FECT, FLOTAC, DNA detection and serology (including antibodies and antigens) (Utzinger et al., 2015).

Diagnosis of schistosomiasis in livestock is routinely done by sedimentation but has varying sensitivity due to low eggs counts per gram of feces (Aradaib et al., 1995).

Hatching miracidia by filtering feces and urine has been used in humans to acquire miracidia for molecular analysis as well as diagnosis (Webster, 2009; Weerakoon et al., 2015). While miracidia hatching has been used to diagnose livestock schistosomiasis, there is only one report of its use for molecular use (Léger et al., 2020).

Health Sequelae

The majority of symptoms and morbidity are related to the inflammation caused by eggs that become lodged in the intestinal walls, liver, bladder or urogenital tract. Over the course of the 3-10 year life span (in some cases as long as 40 years), many eggs may migrate and cause serious sequelae such as anemia, malnutrition, impaired childhood development, female infertility, renal dysfunction, bladder cancer, hepatomegaly, splenomegaly, pulmonary hypertension or neuro-schistosomiasis (Colley et al., 2014; King and Dangerfield-Cha, 2008; van der Werf et al., 2003). Furthermore, there are increasing case reports of colorectal cancer caused by schistosomiasis (Herman et al., 2017). The sequelae in animals are similar due to the similar pathology and include diarrhea, emaciation, anemia, eosinophilia and sometimes even death (MSD, 2016; Zangana and Aziz, 2012).

Treatment, Prevention and Control

Praziquantel is the treatment of choice for schistosomiasis and is active against adult worms, but not against the young developing stages. The mechanism of action is unknown, and while evidence of resistance is scarce, it has been induced in a laboratory setting and a few cases have been reported in humans (Colley et al., 2014).

In 2003, the WHO initiated a paradigm shift in the control and elimination of NTDs, moving from focusing on disease to focusing on the health needs of marginalized populations, thus creating a more holistic approach. The WHO aimed to use resources more efficiently and address health determinants that put marginalized populations at risk of disease, such as poverty. This shift impacts the achievement of the Sustainable Development Goals (SDGs) and supports an integrated approach to NTD control and elimination, whereby treatment interventions are integrated with control measures, including preventive chemotherapy for schistosomiasis (World Health Organization, 2010).

The WHO (World Health Organization, 2010) recommends five strategies for the control and prevention of NTDs:

a) preventive chemotherapy
b) intensified case-management
c) vector control
d) provision of safe water, sanitation and hygiene
e) veterinary public health

Currently, regular mass drug administration (MDA) is the mainstay of control efforts for schistosomiasis in humans, in combination with improved access to safe water, improved sanitation and hygiene (WASH), snail control, health education and behavior change (Rollinson et al., 2013; WHO, 2017a). The aim is reduction of morbidity and transmission and eventual elimination through integrated control interventions (Weerakoon et al., 2015; WHO, 2017a).

MDA programs are regular, coordinated administration of anthelminthics, often coordinated for more than one disease, depending on the geographical overlap of the diseases. Often, if occurrence overlaps,

the four main helminthic infections (soil transmitted helminthiases, lymphatic filariasis, onchocerciasis and schistosomiasis) are included together in an integrated MDA. To date MDA programs have made a significant dent in reducing prevalence, intensity and morbidity of schistosomiasis (World Health Organization, 2010).

The WHO aims to have 96% of countries that are endemic for schistosomiasis, validated for "elimination of schistosomiasis as a public health problem" (Fig. 1.7). This term means that the targets set by WHO are met, but further action is required in order to maintain the targets or make further progress (WHO, 2020).

A national schistosomiasis control program was first drafted in Côte d'Ivoire in the mid-1990s, however due to numerous factors, including the socio-political crisis of the time and a lack of financial resources, it was not able to gain a foothold (Tchuenté and N'Goran, 2009). Numerous small-scale activities were carried out in the 2000s, however, there was renewed interest in 2010 when Côte d'Ivoire was identified as a country which urgently required preventive chemotherapy by the "Integrated control of schistosomiasis in sub-Saharan Africa" (ICOSA) project, and recommended to follow WHO guidelines (Chammartin et al., 2014).

Currently, only two health districts have been found not endemic for schistosomiasis in Côte d'Ivoire. All other 81 health districts are being targeted for MDA in school age children and sometimes adults once a year or every two or three years, depending on the prevalence (Family Health International, 360 2017). In 2016, 81% of people targeted for treatment received preventive chemotherapy (Family Health International 360, 2017).

While the efforts for schistosomiasis control in humans are significant, at this time, there is a lack of action concerning livestock schistosomiasis. There is no official program to control schistosomiasis in livestock in West Africa, including in Côte d'Ivoire (Gower et al., 2017).

Research into a vaccine is ongoing, and while there are a number of vaccine candidates at different stages of clinical trials, an effective vaccine has yet to be identified (McManus et al., 2020).

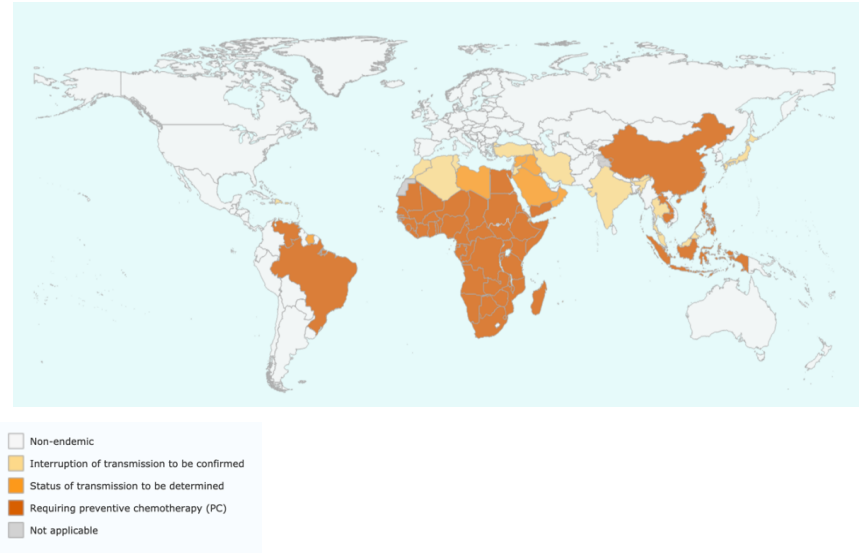

Figure 1.7. Countries endemic for schistosomiasis, 2018 (WHO, 2018).

Population structure, gene flow and transmission

Genetic diversity, population structure and gene flow can help determine how alleles that are advantageous to parasites, such as drug resistance, may spread throughout a population (Webster et al., 2013). Understanding the extent of gene flow and barriers to gene flow is important to predicting the spread of drug-resistant genes, as well as assessing the genetic consequences of control measures (Gower et al., 2013; Shiff et al., 2000). MDAs put pressure on the population, however there are not a great number of molecular studies of *S. haematobium,* making it difficult to compare over space and time (Gower et al., 2011).

The genotyping methods used to study schistosomes have mirrored the trends of markers used for other species over time. The first method developed was isoenzyme analysis in the 1970s, followed by restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and mitochondrial markers. Of these only mitochondrial markers continue to be used today. The most commonly used methods today are short tandem repeats (STRs) which include microsatellites and single nucleotide polymorphism (SNPs) (Blanton, 2019).

Genetic diversity and population structure of *Schistosoma* species in Africa has been sporadically investigated. *Schistosoma mansoni* has been relatively frequently studied, *S. haematobium* less so and *S. bovis* rarely. Currently there are no investigations reported on population diversity and structure of *S. curassoni*.

The origin of the genus *Schistosoma* is thought to be in Asia, as the most basal clade, *S. japonicum,* is Asian (Webster et al., 2013). Investigators speculate that *S. mansoni* split from *S. rodhaini* 107,000-147,000 years ago in East Africa, it then experienced a population size decrease 20,000-90,000 years ago before spreading across the continent. There is strong evidence to suggest it migrated to the Americas during the slave trade in the 16^{th} – 19^{th} centuries (Crellen et al., 2016).

Recent investigations in Africa have shown that while both *S. haematobium* and *S. mansoni* are highly genetically variable, *S. mansoni,* but not *S. haematobium,* were more diverse in East Africa than in West Africa. This suggests that *S. mansoni* originated in East Africa. Furthermore, there was a lack of *S. haematobium* heterozygotes in East Africa, which was not the case in West African samples nor for *S. mansoni* populations, indicating inbreeding within East African *S. haematobium* populations (Gower et al., 2013; Webster et al., 2013).

Genetic diversity and population structure of *Schistosoma* species in Côte d'Ivoire are not well understood and to date there is one published Ivorian study concerning this topic (Angora et al., 2022).

Transmission of this parasite within and between human and animal species depends greatly on social, behavioural and ecological conditions, including but not limited to: activities of daily living done in open water (fishing, washing, etc.), normalization of open defecation, contact with water sources used by animals, wild-life reservoirs, size and density of nearby human populations, local environmental conditions that affect snail populations and man-made environments such as large and small

multipurpose dams and irrigation systems that allow for snail populations to flourish (Diakité et al., 2017; Rollinson et al., 2013; Steinmann et al., 2006). Therefore, while publications from other areas of the continent may provide some insight into the situation in Côte d'Ivoire, local data from humans and animals are needed to design, implement and monitor control programs as transmission trends vary greatly depending on the local situation

Fascioliasis

Fascioliasis is a zoonotic liver fluke infection most commonly found in animals, but also in humans. It is caused by two species of the genus *Fasciola*; *F. hepatica* and *F. gigantica* (Mas-Coma et al., 2007). Both *Fasciola* species cause considerable economic loss to the meat and dairy industry worldwide and have traditionally been thought of mainly as a veterinary concern (Mas-Coma et al., 2009). Recent reports indicate that human fascioliasis may be more prevalent and pathogenic than previously thought (Mas-Coma et al., 2009; WHO, 2015a; Xia et al., 2015).

Food-borne trematode infections are a group of zoonotic parasitic infections, including clonorchiasis, opisthorchiasis, paragonimiasis and fascioliasis. They are caused by more than 100 species of trematodes (intestinal, lung and liver flatworms or "flukes"). Food-borne trematodiasis have been identified as one of the 20 neglected tropical diseases by the World Health Organization (WHO, 2011, 2014a, 2015a, 2016).

Life cycle and Biology

Fasciola species have a complex life cycle that includes a final host (cattle, small ruminants, humans and rodents), an intermediate snail host, and a carrier (aquatic plants or contaminated water) (Fig. 1.8) (Webb and Cabada, 2018). In areas where water treatment and sanitation are present, humans are a dead-end host (Mas-Coma et al., 2009). *Fasciola* species are hermaphroditic and although self-fertilization may occur, normally cross-fertilization is the means of sexual reproduction in the definitive host (Andrews, 1999).

The mammalian hosts who are infected with *Fasciola* flukes pass immature eggs into the environment via their feces. After a few weeks in fresh water, miracidia hatch from the eggs and infect a suitable intermediate snail host, where they remain for 5-7 weeks (Andrews, 1999). Hosts are freshwater snails of the *Lymnaeidae* family, specifically small sized species of the genus *Galba/Fossaria* for *F. hepatica* and species of the genus *Radix* in the case of *F. gigantica*. During this time, they undergo several developmental stages, finally being shed from the snail into the surrounding water as free-swimming cercariae (Andrews, 1999). Anywhere from a few minutes to two hours later, the cercariae encyst on an aquatic plant, blade of grass or other surface (Andrews, 1999). Metacercariae may survive for more than one year in pasture. However, it is thought that a high level of infectivity does

not persist this long. Long-term survival relies heavily on environmental conditions; sufficient moisture and moderate temperatures (Andrews, 1999).

Fasciola metacercariae enter the body during consumption of contaminated vegetation or drinking water. Within one hour, the *Fasciola* metacercariae begin the process of excystation and emerge into the small intestine. The newly excysted juvenile flukes rapidly penetrate the intestinal mucosa, moving into the abdominal cavity where they make their way to the liver, possibly penetrating other organs on the way, and can therefore, be found in other organs (e.g. lungs, pancreas, lymph nodes, etc.) (Andrews, 1999; CDC, 2013). After penetrating the liver capsule, the flukes burrow through the liver feeding on the tissue for 5-6 weeks, causing extensive damage. The significantly larger flukes then proceed into the bile ducts (Andrews, 1999). Approximately 7-8 weeks after infection, the flukes mature into adults in the bile ducts, where they permanently establish themselves and begin excreting eggs (Andrews, 1999; CDC, 2013; Mas-Coma et al., 2007). Liver flukes are capable of living for many years (11-year-old flukes were recorded in sheep in Durbin) in the bile duct where they can continue releasing up to 20,000 eggs per day (Andrews, 1999).

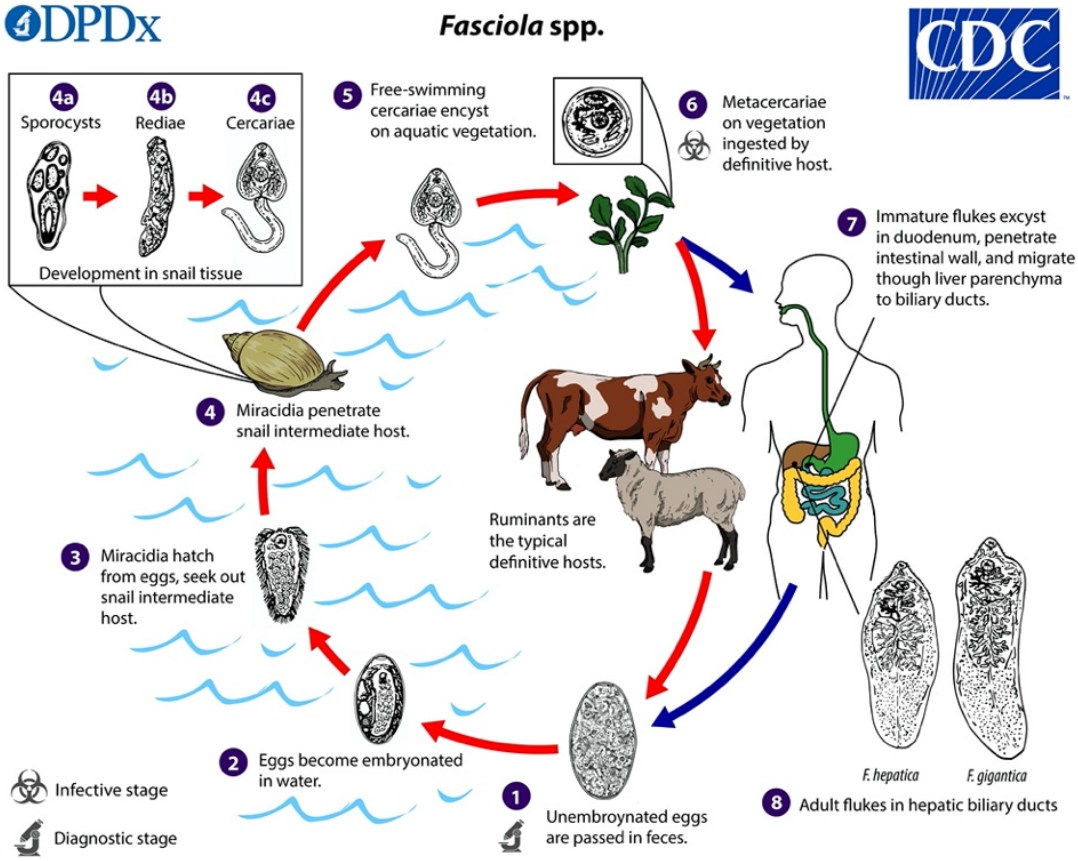

Figure 1.8. Life cycle of *Fasciola* (CDC, 2020).

Fasciola Species

Traditionally, it has been thought that *F. hepatica* exists in the temperate regions of Europe, the Americas and Oceania, while *F. gigantica* is present across Asia and Africa. However, these long held

beliefs have been challenged recently as reports of *F. hepatica* in Africa and Asia emerge (Ali et al., 2008; Amer et al., 2011; Dar et al., 2012; Inoue et al., 2006; Mucheka et al., 2015; Walker, 2008). To complicate matters, morphology, the only technique available to distinguish *F. gigantica* and *F. hepatica* before molecular characterization became available, has often been used despite evidence that measurements of the two species can overlap (Inoue et al., 2006).

There are numerous reports of fascioliasis from Africa, however many either assume the species is *F. gigantica* based on morphology or simply report fascioliasis. While in Asia molecular reports of both species are common, co-occurrence of the two species in Africa has been proven by molecular means only in Egypt (Amer et al., 2011; Dar et al., 2012), Iran (Amor et al., 2011b), Niger (Ali et al., 2008), Nigeria (Castilla Gómez de Agüero et al., 2020), Tanzania (Walker, 2008), South Africa and Zimbabwe (Mucheka et al., 2015). Reports of *F. hepatica* from Africa (based on morphology only) are available from Algeria, Côte d'Ivoire, Morocco, Tunisia, Ethiopia and Tanzania (Esteban et al., 1998; Petros et al., 2013; Walker et al., 2012). One potential human case was identified by coprology in Côte d'Ivoire, but the extent *of F. hepatica* in Côte d'Ivoire remains unclear at this time (Utzinger et al., 2010).

Given the presence of *F. hepatica* in the wider region and the difficulties distinguishing it from *F. gigantica* by clinical, coprological or immunological methods, it is possible that is has been overlooked (Mas-Coma et al., 2005).

Fasciola hybrids are the result of interbreeding between *F. gigantica* and *F. hepatica* in regions where they co-exist. Hybrids have been confirmed by molecular methods in Asia and the Middle East (Agatsuma et al., 2000; Huang et al., 2004; T. Itagaki et al., 2005; T Itagaki et al., 2005; Lyngdoh et al., 2016; Mufti et al., 2014; Nguyen et al., 2012; Peng et al., 2009; Shalaby et al., 2013; Wannasan et al., 2014). Suspicion of the presence of hybrids in Africa exists, Shalaby et al. (2013) found hybrids in animals in Saudi Arabia, although all the animals had been imported from Sudan and it is impossible to say from which country the hybrids came. There is also a report of hybrids by morphology in Egypt (Periago et al., 2008). *F. hepatica* has shown great adaptability to new hosts, environmental conditions and medications raising concerns that a hybrid form may have greater propensity for geographical and host expansion (Cwiklinski et al., 2015; Seehausen, 2004; Walker, 2008).

Epidemiology

Fascioliasis is reported in over 81 countries across the globe and is the most widely distributed trematode (Webb and Cabada, 2018). Epidemiological trends of fascioliasis can vary widely depending on the geographical and environmental characteristics of the transmission area and are sensitive to sudden changes in climate changes (WHO, 2015b). Fascioliasis is an important zoonosis that is emerging or re-emerging in many countries due to man-made modifications and environmental changes (Mas-Coma et al., 2007).

Human Fascioliasis

While traditionally thought to be a disease of veterinary concern, human cases of fascioliasis are not infrequent and many likely go undetected due to the mild and unspecific nature of the symptoms and a lack of medical awareness in combination with the challenges of diagnosing (Mas-Coma et al., 2009; Webb and Cabada, 2018). Furthermore, it is thought that fascioliasis is under-reported due to the fact that it is not a notifiable disease in most countries (Mas-Coma, 2005). It is estimated that over 2.6 million people globally suffered from fascioliasis in 2005, with almost 300,000 of those being "heavy" infections. In terms of morbidity, this translates to 35,206 years lived with disability (YLD) and the same number of DALYs, as there were zero estimated deaths (Fürst et al., 2012).
Human fascioliasis has been reported primarily in Southeast and Central Asia, Western Europe, the Middle East and South America, mostly where *F. hepatica* is found and where the highest prevalences are found (Robinson and Dalton, 2009; Tolan, 2011; WHO, 2007; Mas-Coma et al., 2005; Webb and Cabada, 2018). While there are case reports of human fascioliasis worldwide, there is a paucity of formal epidemiological studies (Esteban et al., 1998).
There is very little data concerning fascioliasis in African people, Fentie et al. (2013) found 3.3% of school children had *Fasciola* eggs in their stool in Ethiopia (no distinction between *F. gigantica* and *F. hepatica*). This was surprising, as previously only sporadic cases of human fascioliasis had been reported. In Egypt the overall prevalence from four rural villages was found to be 12.8% (no species distinction) (Esteban et al., 2003). At this time there is only one report of fascioliasis in humans from Côte d'Ivoire, *F. hepatica* by coprology (Utzinger et al., 2010).

Animal Fascioliasis in Africa

Fasciola gigantica has been documented extensively in African livestock, but there is a paucity of data from Côte d'Ivoire (Adewunmi et al., 1993; Alehegne et al., 2013; Ali et al., 2008; Ekong et al., 2012; Howell et al., 2012; Jean-Richard et al., 2014; Mungube et al., 2006). A recent review found prevalences of fascioliasis in African livestock from 1.2 - 91.0% in cattle, 0.19 - 73.7% in sheep 0.28 - 68.4% in goats and 9.73 - 33-7% in Buffalo (Mehmood et al., 2017). The prevalences vary greatly and are from across Africa: Chad, Egypt, Ethiopia, Kenya, Nigeria, Sudan, Tanzania, Tunisia, Uganda, Zambia and Zimbabwe and the authors speculate these differences are likely due to differing climatic conditions, use or non-use of anthelminthics, farmer awareness and use of proper control measures.
An investigation in northern Côte d'Ivoire, not included in the review, revealed 12% of sheep, 1.4% of goats and 4% of cattle harboured *F. gigantica,* as determined by morphology (Achi et al., 2003a, 2003b).

Diagnosis

Diagnosing fascioliasis is challenging. Coprology and serology are the mainstays of diagnosis, however both have their limitations (Webb and Cabada, 2018). There are numerous methods of coprology such as Kato Katz (mentioned above for schistosomiasis), Flotac, Lumbreras rapid sedimentation (Webb and Cabada, 2018). Diagnostics tests involving coprology cannot be used in the acute phase of the infection as eggs will not yet be present in the stool. Furthermore, they also can produce false negative results depending on infection intensity, require experienced laboratory personnel and cannot decipher *Fasciola* species (eggs do not differ morphologically) (Webb and Cabada, 2018).

Serological tests are more sensitive and can be used before eggs are present in the stool. ELISA tests have been developed and are quite sensitive and specific (above 95%) (Webb and Cabada, 2018). Molecular tests, specifically PCR of feces are also being developed and are promising with high sensitivity and specificity (88% and 100%, respectively) (Webb and Cabada, 2018). However, these diagnostics require cold chain, laboratory services and expensive reagents. In resource-limited settings, these diagnostics are not yet used extensively (Mubanga et al., 2019). Rapid tests involving antibody and nucleic assays are under developing and showing promising results for resource-limited settings (Mubanga et al., 2019).

Health Sequelae and Economic Impact

Human infections, while seldom fatal, can cause pain, anaemia, weight loss, liver and gallbladder damage and may cause injury to other body systems in the case of ectopic migration of the flukes (Lotfy and Hillyer, 2003; Webb and Cabada, 2018). Infections in children can lead to developmental delays and reduced productivity and quality of life (Webb and Cabada, 2018). Furthermore, recent evidence suggests that human fascioliasis is more pathogenic than previously thought. A recent review found that fluke infections are significantly associational with cholelithiasis, hepatocellular carcinoma and cholangiocarcinoma (Xia et al., 2015). Additionally, human *F. gigantica* infections have been shown to occur more often than assumed and ectopic lesions occur more often with infection with *F. gigantica* than *F. hepatica* (Le et al., 2008; Lotfy and Hillyer, 2003; Marcos et al., 2009; Mas-Coma et al., 2014; Robinson and Dalton, 2009; Shahbazi et al., 2011).

Fascioliasis in animals causes chronic wasting and reduces herd productivity through inhibition of growth leading to reduced production and quality of wool, decreased fertility, diminished milk production, reduced carcass weight, confiscation of livers as well as mortality (Bernardo et al., 2011). It is thought that together, both species of *Fasciola* cause more than 3 billion USD in global economic losses (Mas-Coma et al., 2005; Robinson and Dalton, 2009), annually. During the period between 1990 and1999, Kenya estimated total economic losses due to *Fasciola* in cattle to be between 0.2-0.3

million USD per year (Kithuka, Maingi, Njeruh, & Ombui, 2002). An investigation at a Ugandan abattoir estimated abattoir losses at just over 92 million USD in 2014, 9.1% due to liver condemnation, 4.1% due to partial liver condemnation and 86.4% due to weight loss (Joan et al., 2015).

Treatment and Control

Triclabendazole is the treatment of choice for both humans and animals as it kills both immature and mature flukes, however, resistance in both humans and animals has been documented (Brennan et al., 2007; Keiser et al., 2005; Kelley et al., 2016). Albendazole is another form of treatment used in animals in many African countries, even with its numerous drawbacks (Elelu and Eisler, 2018; Greter et al., 2016; Keyyu et al., 2009; Kouadio et al., 2021; Nzalawahe et al., 2018). Albendazole is not effective against immature flukes and is also less efficacious than Triclabendazole (Aktaruzzaman et al., 2015; Keiser et al., 2005; Kelley et al., 2016). Control measures include treatment (individual or targeted MDA if highly endemic), snail control, and education concerning food hygiene and separation of livestock and humans (WHO, 2017b). Resistance to *F. gigantica* has been observed in sheep and goats. (Mas-Coma et al., 2009).

Other possible prevention and control measures include health education concerning safe food and water, treating domestic animals, and draining grazing lands containing snail intermediate hosts (Mas-Coma, 2005; WHO, 2015b). Uniform control measures are not recommended due to the heterogeneity of the parasite and the eco-epidemiological situation; each area must assess their individual situation and design the appropriate complement of measures to address their specific circumstances (Mas-Coma et al., 2009). Currently, there is no marketed vaccine for fascioliasis, although research is on-going (Molina-Hernández et al., 2015; Toet et al., 2014).

Molecular Methods

Molecular species identification is important for both *Fasciola* and *Schistosoma*. Molecular markers have been used for both genera since they became widely available, however in low resource setting they may not always be accessible. Ribosomal and mitochondrial markers such as *ITS1/2* and *cox1*, respectively are commonly used for species identification in both genera, however, due to the higher number and complexity of schistosome species, the *18S* maker should also be employed.
Ribosomal DNA (rDNA), located in the nuclear genome, codes for ribosomal RNA. As all cells need ribosomes to translate mRNA into protein, rDNA is found in every eukaryote species, often in high copy numbers. Ribosomal DNA is very valuable as a molecular marker as it has regions with different degrees of conservation, allowing determination of both close and distant relations. Plus, it is large enough to contain a lot of sequence information, but still small enough to be sequenced easily (Beebee and Rowe, 2008; Page and Holmes, 1998).

The rDNA array consists of a non-transcribed spacer (NTS), which is highly variable, followed by an external transcribed spacer (ETS) and then three coding regions that are separated by two internal transcribed spacers (*ITS1* and *ITS2*). This cluster is repeated several hundred times, forming a tandem array (Beebee and Rowe, 2008). These two internal transcribed spacers are useful in identifying *Fasciola* and *Schistosoma* species and their respective hybrid forms, as they exhibit a consistent level of sequence variation between the species (interspecific), and much less within the species (intraspecific). They have been used extensively for this purpose (Ai et al., 2010; Ali et al., 2008; Amer et al., 2011; Bazsalovicsová et al., 2010; Chen et al., 2015; Choe et al., 2011; Itagaki, et al., 2005; Liu et al., 2014)

The mitochondrion is an organelle that helps degrade fats and sugars in order to fuel the cell and is found in eukaryotes. The genome in the mitochondria is haploid and is maternally inherited. There are hundreds to thousands of mitochondria per cell and therefore multiple copies of the genome per cell (Tortora and Derrickson, 2012). The mitochondrial DNA (mtDNA) encodes only proteins that are required for its own essential functions; there is usually no non-coding DNA present. Mitochondrial DNA is a valuable tool for determining variability in closely related species, as it has a very low rate of recombination, a reduced effective population size and a relatively rapid rate of mutation. Haplotype frequencies can drift quickly, allowing there to be more genetic differences in less time. One of the main criticisms of using mitochondrial DNA, is that it is inherited only from the mother, and is only reflective of the maternal lineage (Beebee and Rowe, 2008; Page and Holmes, 1998; Rubinoff and Holland, 2005).The mitochondrial loci *cox1* and *nad* have been used frequently for genetic characterization of *Fasciola* and *Schistosoma* (Agatsuma et al., 2000; Ai et al., 2011; Amer et al., 2011; Angora et al, 2022; Blanton, 2019; Dar et al., 2012; Elliott et al., 2014; Farjallah et al., 2013; Ichikawa & Itagaki, 2012; Itagaki et al., 2009; Léger et al. 2020; Lotfy et al., 2008; Wannasan et al., 2014b; Webster et al., 2012).

Microsatellites are a commonly used marker for population genetics investigations to estimate population diversity and structure. Microsatellites, also known as short tandem repeats (STRs) are tandem arrays of usually 1-6 nucleotides, they are located in abundance all over the genome. They usually occur in non-coding regions, can have many alleles per locus and have a higher mutation rate (Blanton, 2019). Microsatellites are the most commonly used marker for population genetic studies of schistosomes due to their high information content, relatively low cost, high throughput and sufficient resolution for population genetics questions (Blanton, 2019).

Single nucleotide polymorphisms (SNPs) are more often used in studies designed to identify gene associated traits and diseases. SNPs are also very abundant in the genome, more so than microsatellites, therefore offering finer resolution, however their polymorphism tends to binary, and as a consequence offer less information content. They have been commonly used for *Schistosoma* species identification (Blanton, 2019).

‘One Health’

‘One Health’ is defined as “any added value in terms of health of humans or animals, financial savings or environmental services achievable by the cooperation of human and veterinary medicine when compared to the two medicines working separately” (Zinsstag et al., 2015). It values collaboration, participation and transdisciplinarity and has been shown to be an effective strategy for improving health care for mobile pastoralists in sub-Saharan Africa. A convincing illustration of this concept is combined vaccination campaigns for children and livestock, which provide both economic and health benefits (Greter et al., 2014).

One Health is a pragmatic approach for studying parasites such as *Fasciola* and *Schistosoma,* whose intermediate snail hosts release infectious cercariae into freshwater that both humans and animals may have contact with. This project is a continuation grant of a ‘One Health’ project in Sub-Saharan Africa that revealed a link between trematode prevalence in pastoralists and their cattle and the pastoralists’ ethnicity and lifestyle (Schelling et al., 2007).

Many factors influence transmission of these complex parasites; ecological, cultural and behavioural, to name a few. This project uses a unique combination of ‘One Health’ and population genetics methods to elucidate transmission dynamics of *Fasciola* and *Schistosoma* between livestock and humans, in an endeavour to improve control and elimination efforts.

We enquire about the potential of a closer cooperation between human and animal health systems to control schistosomiasis and fascioliasis in animals and humans in a better way. Both schistosomiasis and fascioliasis show that human and animal health alone are not sufficient to address the problem at its root. This has been recognized by extending the term “One Health” to “Health in Social-Ecological Systems” (HSES). HSES specifically considers the complex social-ecological issues driving the transmission of these parasites. The figure below (Fig. 1.9a), symbolize the extension of Schwabe’s General Medicine by adding a layer on human ecology. The second figure (Fig. 1.9b) symbolizes health as an outcome of complex social-ecological systems and the related disciplines.

In summary, for schistosomiasis, if open defecation of humans and animals in waterbodies cannot be stopped, transmission is continuously ongoing. Transmission cannot be interrupted by chemotherapy alone. Hence, necessary infrastructural changes like environmental sanitation for humans and safe watering places for domestic animals, together with behavioural changes like avoiding open defecation for humans are needed to reduce transmission significantly. Similarly, for *Fasciola*, the transmission is essentially linked to the habitat of the vector snail, which is adapted to swampy pastures. To reduce transmission of *Fasciola* effectively requires the drainage of swampy and flooded pastures, strategic chemotherapeutic control in livestock to avoid re-contamination and avoiding the consumption of fresh water related vegetables, such as watercress, in humans. This project used molecular methods to elucidate the transmission dynamics between humans, livestock and snails at a shared water source, their interface.

Whether these interventions are cost-effective depends on the magnitude of effort, additional benefits (i.e. reduced transmission of soil transmitted helminth) and success of behaviour change interventions.

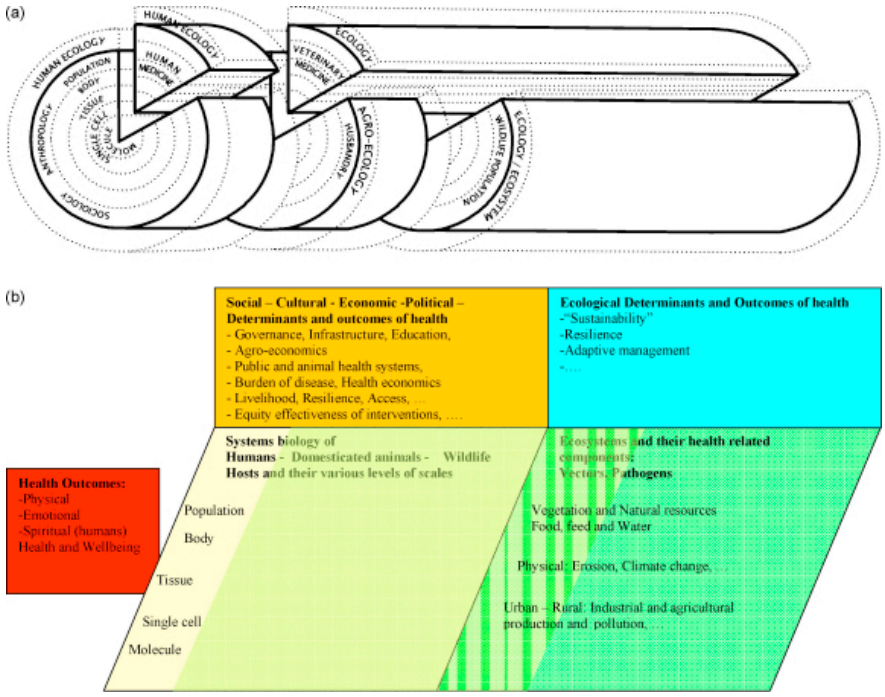

Figure 1.9. a) Generalized systems dynamic framework of health of humans and animals in social-ecological systems extending the original schematic by Schwabe (1984). b) Generalized systems dynamic framework of health of humans and animals extended from Ostrom (2007), Young et al. (2008) and Rock et al (2009) (Zinsstag et al., 2011)

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2. Study Rationale and Design

2.1 Identified Gaps

From a One Health perspective, very little is known about *Schistosoma* and *Fasciola* in Côte d'Ivoire. Prevalences of *Schistosoma* in school-aged children are well documented in Côte d'Ivoire, however, the epidemiological situation of *Schistosoma* and *Fasciola* in livestock in the South is largely unknown, as well as the extent to which *Fasciola* occurs in humans.

Limited research has been conducted in Côte d'Ivoire concerning parasite diversity or transmission dynamics between animals and humans. Population diversity and distribution of genotypes can reveal valuable information about population structure and transmission dynamics, e.g. if animals and humans are involved in the same transmission cycles. While some of these elements are of interest purely for interest's sake, the relationship between humans and animals in the transmission cycle can seriously affect the success of control and elimination programs because spill-overs are always a risk when animal reservoirs remain.

2.2 Aims, Objectives and Research Questions

This project is part of a large grant offered by Swiss National Science Foundation (SNSF) entitled: Transmission dynamics and hybridization of human and animal trematodes in sub-Saharan Africa. The objectives of this grant are as follows:

1. Generate whole-genome sequence data for *Fasciola* and *Schistosoma* species
2. Molecular identification of *Fasciola* and *Schistosoma* species and their hybrids
3. Population genetic analysis and modelling of *Fasciola* and *Schistosoma* transmission dynamics
4. Determine the prevalences of all parasite species (and hybrids) in multiple regions of Côte d'Ivoire.

This PhD project focuses on the transmission dynamics of schistosomes in Côte d'Ivoire but includes some genetic analysis and a clinical trial for *Fasciola* as well. It aims to determine the genetic diversity, population structure and transmission dynamics of *Schistosoma* and *Fasciola* species amongst livestock and humans in Côte d'Ivoire using a 'One Health' approach.

The three interconnected objectives are as follows:

1. to determine what species of *Schistosoma* and *Fasciola* are present in our sample,
2. to quantify genetic diversity and infer transmission dynamics of *Schistosoma* and *Fasciola* populations within one host and between host species in Côte d'Ivoire; and
3. to quantify genetic diversity and infer transmission dynamics of *Schistosoma* and *Fasciola* populations by geography in Côte d'Ivoire.

Objective 1: Trematode species in our sample

Background

Schistosomiasis in humans has been well documented in Côte d'Ivoire, although species determination has traditionally been extrapolated from the source of the egg (faeces = *S. mansoni*, urine = *S. haematobium*) and by egg morphology, which are both problematic in the presence of hybrids. *Fasciola* in humans is not well documented in Cote d'Ivoire, however, *Fasciola* in animals has been documented in the North and is considered common in livestock. Species identification in humans and livestock has been done by egg and fluke morphology, respectively, which can be imprecise due to overlapping dimensions between the two species. *Fasciola* hybrids further complicate this matter.

Research question 1

1. What species of *Schistosoma* and *Fasciola* are present in our sample?

Sub-questions

1.1 Are *F. hepatica* present in our sample?

1.2 Are within genus hybrids of *Fasciola* and *Schistosoma* present in our sample?

1.3 If *Schistosoma* hybrids are found, what mix of species are the hybrids (e.g. *S. haematobiu*m x *S. bovis*) and in which host species are they found? (i.e. are there hybrid forms consisting of livestock schistosomes found in humans and *vice versa*?)

Exploratory questions

1.4 Are human participants in our study infected with *Fasciola*?

Objectives 2: Genetic diversity and transmission dynamics of parasites within one host and between different host species

Background

Transmission of parasites between humans, livestock and snails impacts control and prevention strategies. If transmission dynamics link host species, parasite elimination in humans can never be assured, as risk of re-introduction from animal reservoirs is always a possibility. Furthermore, analysis of parasites from snails can increase understanding of transmission dynamics.

Understanding population structure of parasites is important to predict and understand the possible emergence and spread of alleles that would negatively affect control and prevention strategies, such as drug resistance. Highly structured populations between host individuals would increase the likelihood of recessive alleles, like drug resistance, accumulating in one individual, whereas high structure between geographical areas could reduce the flow of new genotypes, if they were to emerge (Gower et al., 2013).

Research question 2

2. How genetically distinct are parasite populations from different host species? Specifically, how much gene flow, and hence transmission, is there between different hosts?

Sub-questions

2.1 Do different host species (humans, cattle, sheep) contain different or similar genotypes of *Schistosoma* and *Fasciola*?

2.2 Do the snails located in the water source of farms contain the same parasite genotypes as the livestock on the farm?

Research question 3

3. How diverse are *Schistosoma* and *Fasciola* populations within an individual host? (How diverse are parasite populations (infra-populations) within one host individual?)

Sub-questions

3.1 Are parasite populations within one host diverse?

3.2 Does the majority of genetic variation of parasite populations exist within an individual host or between the hosts of the same species?

Objectives 3: Genetic diversity and transmission dynamics of parasites by geography

Background

While it is understood that swimming, fishing, washing dishes, food and laundry or playing in open water sources, drinking water from open water sources or wells and open urination and defecation do occur and are risk behaviours for acquiring or transmitting trematodes, data concerning the exact biological and/or behavioural mechanisms in hosts that are involved in parasite dispersal are lacking (Assaré et al., 2016; Krauth et al., 2015; Yapi et al., 2014). Population genetic studies from other West African countries revealed that genetic differentiation of *Schistosoma* is quite high between countries but not within countries (Gower et al., 2013; Yapi et al., 2014). This indicates that parasites disperse somewhat easily across a country, but struggle to cross political boarders. An investigation of *Fasciola* across Eastern Europe showed little structuring across the region, suggesting a panmictic population (Semyenova et al., 2006).

Research question 4

4. Are trematode populations genetically distinct by geography?

Sub-questions

4.1 Is there a genetic difference between parasite populations from different localities?

4.2 Are schistosome populations from the north and south (two different ecozones) genetically distinct?

2.3 Study Design and Field Activities

Study site

This cross-sectional, observational investigation took place in Côte d'Ivoire, a West African country of over 26 million people and an estimated 1.34 million cattle in 1988 (Fig. 2.1) (Food & Agriculture Organization of the United Nations and Livestock Information, Sector Analysis and Policy Branch, 2005; World Bank, 2020). Côte d'Ivoire is located on the coast of the Gulf of Guinea, with Liberia and Guinea to the west, Ghana to the east and Mali and Burkina Faso to the north. Côte d'Ivoire has three ecozones: the southern and western parts that have abundant rainfall with forests and, in the West, mountains, the northern region consisting of savannah and less rainfall and the northwestern part, also savannah, but with more rainfall than the north (Yapi et al., 2014). Four main rivers run through Côte d'Ivoire, the Cavally and Sassandra River in the west, the Bandama in the center and the Comoé in the East, (CILSS, 2016).

As Côte d'Ivoire is south of the Sahel, with rich grazing opportunities, it is a host country to transhumance herders (Alidou, 2016; Bassett, 2009). Transhumance, an animal production practice, entails the seasonal movement of livestock herds to better grazing areas by a few individuals of a pastoral group, while the other members remain at their permanent location (Alidou, 2016). In west African this typically occurs in January to May, with herds moving south during the dry season, and then back north. This leads to a large number of livestock migrating through Côte d'Ivoire and brining zoonoses with them (Oyetola et al., 2021).

Life-expectancy is 58 years and populations growth is 2.5%, as of 2020. GDP per capita was 2,325.70 in 2020 and, as of 2018, the poverty headcount ratio at $1.90 was 9.2% (the percentage of people living on less than $1.90 per day at 2011 international prices) (World Bank, 2022). Approximately 71% of the population is using at least basic water services (less than 15 minutes away and from an improved source) and 35% are using at least basic sanitation services (improved sanitation facilities that are not shared with other households) (World Bank, 2022).

Figure 2.1. Map of Côte d'Ivoire in West Africa (https://www.africageoportal.com).

Sampling

This investigation involved two sampling strategies, one to obtain samples from humans and livestock across Côte d'Ivoire and the second to obtain trematodes from all known intermediate and definitive hosts sharing a common water source. The field work (specimen collection) took place during multiple field trips carried out over two years (2017-2019).

In order to obtain samples from humans and livestock across Côte d'Ivoire, humans at five sites and cattle, sheep and goats at six sites across the country were sampled (Fig. 2.2). Three sites were in the Southeast region of the country, one site in the West and two sites in the North-central region. Feces and urine were collected from humans, while abattoirs sampling was conducted on livestock at these sites.

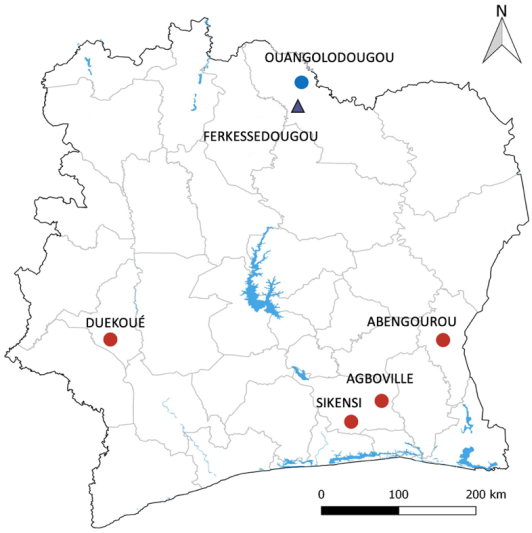

Figure 2.2. Map of Côte d'Ivoire, West Africa. Red circles indicate sites sampled for *Schistosoma* flukes from humans and slaughtered cattle in abattoirs. In Ouangolodougou (blue circle) schistosome flukes were collected from slaughtered cattle in abattoirs only. In Ferkessédougou (purple triangle) humans, slaughtered cattle from abattoirs, live cattle on farms and snails from the shared water source were sampled for schistosomes.

In Ferkessédougou, extensive sampling of all hosts known to share a common water source was performed. Fecal and urine samples from humans in villages and their livestock on farms were collected. Intermediate host snails of *Schistosoma* and *Fasciola* species know to infect definite hosts in the region, *Biomphalaria* and *Bulinus* genera and *Lymnaeidae* family, were collected at the common water source.

Specimen collection

Miracidia were collected from urine and fecal samples from humans and livestock on farms, respectively, while flukes were excised from slaughtered livestock and cercariae were collected from infected snails (Fig. 2.3).

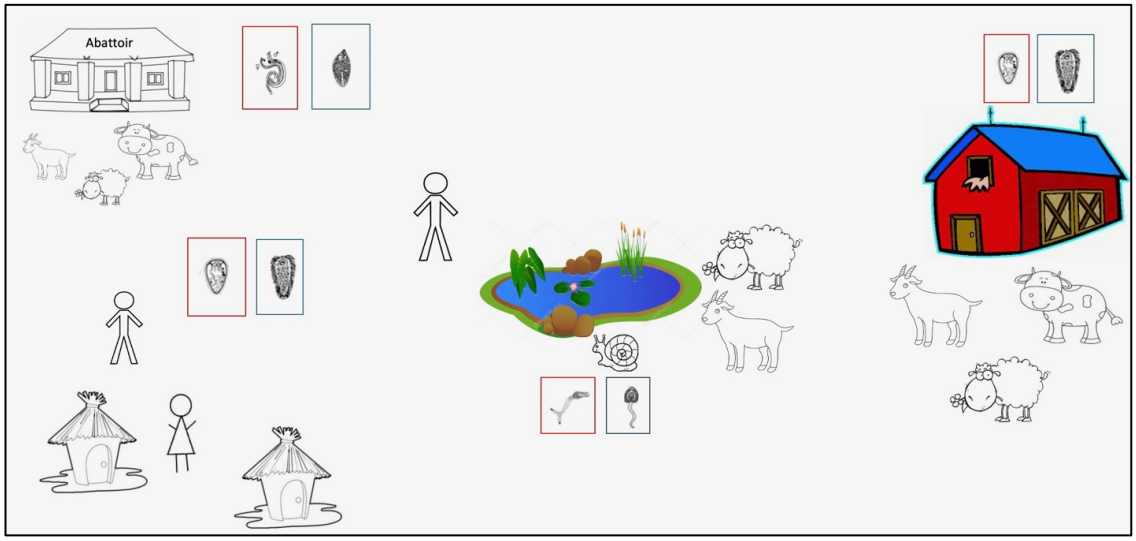

Fig. 2.3. Schematic of sampling strategy. *Schistosoma* samples are depicted by the red box and *Fasciola* by the blue box.

At all six abattoir sites, the mesenteric veins of slaughtered cattle, sheep and goats were examined for *Schistosoma* flukes, while livers and bile ducts were examined for *Fasciola* flukes. If present, flukes were excised from the tissue and stored in 95% ETOH or in 190 µl lysis solution (0.1 M Tris-HCl, 1% sodium dodecyl sulfate) and 10 µl of [20 µg/µl] proteinase K at room temperature before being shipped to Switzerland. For more details please see chapters 4a-4c.
On farms, feces were manually extracted from the rectums of cattle, sheep and goats, while in villages, fecal and urine specimens were collected from humans. All feces and urine were filtered to collect miracidia from hatched eggs, which were deposited on Whatman Filter paper. For more details please see chapters 6b-6d.

Laboratory Work
Briefly, DNA was extracted from specimens using different protocols, depending on the type of specimen; a phenol-chloroform protocol for *Fasciola* flukes, DNeasy blood and tissue column kits (Qiagen, Hilden, Germany) for *Schistosoma* flukes and Chelex® protocol (Bio-Rad Laboratories, Cressier, Switzerland) for miracidia (see chapter 6b-6d).
Polymerase chain reaction (PCR) was conducted to amplify genetic markers for species determination and population genetic analysis. A variety of different genetic makers were used based on the question being investigated: mitochondrial (*cox1*) and nuclear (*ITS*) markers were used for molecular species determination and microsatellite markers were used to investigate population genetics patterns.

2.4 Collaboration

This SNSF funded project (grant 31003A_1710113 “Transmission dynamics and hybridization of human and animal trematodes in sub-Saharan Africa”) was carried-out in collaboration with the Centre Suisse de Recherches Scientifiques (CSRS) and the University of Félix Houphouët-Boigny in Abidjan, Côte d’Ivoire. The SwissTPH and CSRS have enjoyed a close collaboration for many decades. The CSRS was established in 1951 and is supervised by the Ivorian Government and the Swiss Government, through the Swiss Topical and Public Health Institute (CSRS, 2022).
This project is a continuation of a previous SNSF-funded project entitled “Systems epidemiology of human schistosomiasis and livestock fascioliasis in sub-Saharan Africa” (grant no.320030_141246) conducted by the same applicants in Côte d’Ivoire and Chad.
A multidisciplinary project team of epidemiologists, ecologists, biologists, human and veterinary health professionals from Côte d’Ivoire and Switzerland were involved in the planning and execution of this project. Two PhD candidates were employed by the project, one from Côte d’Ivoire and one from Switzerland. Field activities were coordinated by the CSRS, in collaboration with local animal and human health officials and ministries, while laboratory work was coordinated and conducted at Swiss TPH.

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3. Outline of Thesis

This thesis consists of eight chapters written as manuscripts for peer-reviewed scientific publication, followed by a general discussion and conclusions. The eight chapters have been organized into three thematic groups:

1. Prevalence and diagnoses of trematodes
    - Chapter 4 – Prevalence and distribution of livestock schistosomiasis and fasciolosis in Côte d'Ivoire
    - Chapter 5 - Accuracy of the sedimentation and filtration methods for the diagnosis of schistosomiasis in cattle

2. Genetic characterization of schistosomes
    - Chapter 6a - A review of molecular methods for genetic structure and diversity of *Schistosoma*
    - Chapter 6b - Genetic characterization of *Schistosoma* species from cattle across Côte d'Ivoire
    - Chapter 6c - Genetic Characterization of *Schistosoma* from humans in Côte d'Ivoire
    - Chapter 6d - Host specificity and genetic exchange of *Schistosoma* species found in humans and cattle in Northern Côte d'Ivoire

3. *Fasciola* species and treatment
    - Chapter 7a - Molecular confirmation of a *Fasciola gigantic* X *Fasciola hepatica* hybrid in a Chadian bovine
    - Chapter 7b - Efficacy of triclabendazole against *Fasciola* infection in cattle in Côte d'Ivoire: a randomised blinded trial

Chapter 4

Prevalence and distribution of livestock schistosomiasis and fasciolosis in Côte d'Ivoire: results from a cross-sectional survey

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Prevalence and distribution of livestock schistosomiasis and fascioliasis in Côte d'Ivoire: results from a cross-sectional survey

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Abstract

Background: *Schistosoma* and *Fasciola* are zoonotic parasites of public health and veterinary importance. However, while the epidemiology of schistosomiasis in humans is well studied, little is known about fascioliasis and schistosomiasis in livestock in Côte d'Ivoire. This study aimed to determine the prevalence and the distribution of livestock schistosomiasis and fascioliasis across Côte d'Ivoire.

In 2018, we conducted a cross-sectional survey in abattoirs and farms in 13 departments of Côte d'Ivoire. In abattoirs, the mesenteric veins and livers of slaughtered cattle, sheep and goats were examined for adult *Schistosoma* and *Fasciola* flukes. Faeces from live cattle, goats and sheep were collected and examined for *Schistosoma* and *Fasciola* eggs using a sedimentation technique.

Results: A total of 386 cattle, 174 goats and 151 sheep from abattoirs and 435 cattle, 22 goats and 176 sheep from farms were sampled. The observed prevalence of schistosomiasis was higher in slaughtered animals. Fascioliasis was more prevalent in farm animals. The prevalence of schistosomiasis in slaughtered cattle varied between 5.9% (95% confidence interval (CI): 0.7–19.7%) and 53.3% (95% CI: 37.9–68.3%) with the highest prevalence observed in Ouangolodougou in the North. Cattle from farms had a relatively low prevalence of schistosomiasis, with the highest prevalence found in Ouangolodougou (2.4%, 95% CI: 0.7–6.1%). The prevalence of fascioliasis varied considerably from one department to another, ranging from nil (95% CI: 0.0–18.5%) to 50.8% (95% CI: 43.4–58.2%), with the highest prevalence found in farm cattle in Dikodougou in the North. Sheep and goats had a lower prevalence of schistosomiasis and fascioliasis than cattle. In slaughtered animals, cattle aged 4 years and older were at highest risk for schistosomiasis (odds ratio (OR): 2.4; 95% CI: 1.0–5.6) and fascioliasis (OR: 2.1; 95% CI: 1.1–3.9). In farm animals, male cattle had higher odds of being infected with *Schistosoma* (OR: 4.3; 95% CI: 0.7–26.9) than females.

(Continued on next page)

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(Continued from previous page)
Conclusions: Our study confirms that schistosomiasis and fascioliasis are endemic in livestock across Côte d'Ivoire. A strategic control programme should be considered, especially for cattle, including providing drinking water in troughs to reduce faecal contamination of water sources by cattle.

Keywords: Côte d'Ivoire, Cross-sectional survey, Epidemiology, *Fasciola*, Livestock, *Schistosoma*

Background

Schistosomiasis and fascioliasis are zoonotic diseases with a complex transmission cycle involving aquatic snails as intermediate hosts and mammalian definitive hosts [1, 2]. Schistosomiasis and fascioliasis are both caused by trematodes; the former by the genus *Schistosoma*, the latter by the genus *Fasciola* [3, 4]. Schistosomiasis and fascioliasis are widespread in tropical and subtropical regions of the world where climatic, ecological and hygienic conditions favour their transmission [3, 5]. Besides their considerable public health burden [6], schistosomiasis and fascioliasis are responsible for economic loss in livestock, mainly through reduced fertility and productivity, liver condemnation, stunted growth and premature death [7–9].

Schistosomiasis also exists in wild and domestic animals and a wide range of mammals are susceptible to schistosome infection, including buffaloes, camels, cattle, goats, horses, pigs and sheep [10–12]. *Schistosoma* species that affect mammals include *Schistosoma bovis*, *S. curassoni*, *S. hippopotami*,, *S. indicum*, *S. intercalatum*, *S. mattheei*, *S. nasalis*, *S. rohhaini* and *S. spindale* [13]. In many countries, *S. bovis* is one of the main species of veterinary and zoonotic importance [8, 14, 15]. In West Africa, *S. bovis* and *S. curassoni* are responsible for schistosomiasis in cattle and small ruminants (goat and sheep), respectively [16, 17]. The prevalence of bovine schistosomiasis varies greatly from 1.2% in Nigeria [18] to 21.7% in Ghana [19]. In Côte d'Ivoire, human schistosomiasis has been the subject of numerous epidemiological studies [20–22]. In contrast, livestock schistosomiasis, has received little attention [23], despite the economic importance of livestock in the country. Prevalence of livestock schistosomiasis has been reported at 35% in the northern part of the country [23]. Of note, recent studies have found that *S. bovis* is involved in the hybridisation of schistosomes in humans in Côte d'Ivoire [24, 25].

Fascioliasis is a chronic disease with a global distribution that mainly occurs in domestic ruminants [26, 27]. There are two *Fasciola* species; namely *Fasciola hepatica* and *F. gigantica*. The former is a cosmopolitan species adapted to temperate areas, while *F. gigantica* is responsible for fascioliasis in tropical and subtropical regions of Africa and Asia [28–30]. In areas where both species coexist, *F. hepatica* x *F. gigantica* hybrids have been found [31–33]. Recently, the presence of hybrids has also been confirmed in sub-Saharan Africa [34]. Fascioliasis is endemic in West Africa with the predominant species being *F. gigantica* [35, 36]. The disease has been reported in cattle with prevalence rates of 6.4–24.8% in Benin [37], 51.1% in Ghana [19], 7–50% in Mali [38] and 28–75% in Nigeria [18, 39–42]. Fascioliasis was also noted in North Côte d'Ivoire in 2003, with a prevalence of *F. gigantica* in cattle of 4% [23], however very little knowledge on the disease situation has been gained since then. Liver condemnation due to fascioliasis is frequent in abattoirs across the country.

The purpose of this study was to provide an overview of the epidemiology of schistosomiasis and fascioliasis in livestock by investigating the prevalence and the distribution of these diseases across Côte d'Ivoire. We designed a cross-sectional survey in abattoirs to collect adult flukes from slaughtered animals and surveyed animals on farms to identify parasite eggs in their faeces.

Results

Characteristics of the animal populations

A total of 711 slaughtered animals (386 cattle, 174 goats and 151 sheep) from abattoirs and 933 farm animals (735 cattle, 22 goats and 176 sheep) were sampled. Most animals were females, consisting of at least 61.4% in each group. Cattle breeds included "Taurin", "Zébu" and "Taurin x Zébu". "Zébu" were the most commonly slaughtered cattle (42.5%), while "Taurin x Zébu" were the most common breed found on the farms (79.9% of live cattle). Most of the cattle (79.6%) were at least 4 years old, both on farms and in abattoirs. As in cattle, more than 60% of the goats and sheep sampled were females on farms and in abattoirs. Goat breeds consisted of "Naine" and "Sahelien". The local breed "Naine" was the most common, at 98.9% and 100% of slaughtered and live goats, respectively. Sheep breeds included "Djallonké", "Sahelien" and "Djallonké x Sahelien". As with goats, the local breed "Djallonké" was the most common breed at 98.7% and 89.2% in abattoirs and on farms, respectively.

Prevalence of *Schistosoma* and *Fasciola*

Prevalence of Schistosoma and Fasciola infections in slaughtered animals

Post-mortem examination of livers and small intestines in slaughterhouses revealed the presence of *Schistosoma*

spp. and *F. gigantica* in 12.8% and 11.3%, respectively, of all slaughtered animals (cattle, goats and sheep). The mean prevalence of *Schistosoma* and *Fasciola* flukes in cattle across all departments was 22.5% and 19.7%, respectively (Table 1). Goats and sheep were considerably less often infected than cattle (Table 1). In goats, no *Fasciola* flukes were found, whereas *Schistosoma* flukes were found in 1.2% of the animals (Table 1). In sheep, *Schistosoma* and *Fasciola* flukes were found at a prevalence of 1.3% and 2.7%, respectively (Table 1). The highest prevalence of *S. bovis* and *F. gigantica* were found in slaughtered cattle, 53.3% and 40.6%, respectively.

The association between parasitic infection and sex, age and breed of slaughtered cattle are summarised in Table 2. Cattle aged 4 years and older showed the highest odds of *S. bovis* infection (multiple OR: 2.4; 95% CI: 1.0–5.6). There was no significant association with sex (multiple OR: 1.1; 95% CI: 0.6–1.8). The breeds "Taurin" (multiple OR: 0.9; 95% CI: 0.3–2.8) and "Zébu" (multiple OR: 0.6; 95% CI: 0.2–1.8) had lower odds of infection than "Taurin x Zébu".

In regards to fascioliasis, cattle aged 4 years and older had the highest odds of infection (multiple OR: 2.1; 95% CI: 1.1–3.9). Sex showed no association with fascioliasis (1.0; 95% CI: 0.6–1.6). The breeds "Taurin" (multiple OR: 0.4; 95% CI: 0.2–0.9) and "Zébu" (multiple OR: 0.7; 95% CI: 0.5–1.1) had lower odds of infection compared to "Taurin x Zébu".

Prevalence of Schistosoma and Fasciola infections in farm animals

Farm animals were found infected with *Schistosoma* spp. and *F. gigantica*, as determined by parasite eggs in faecal samples. In cattle, the overall prevalence of *S. bovis* and *F. gigantica* was 0.7% and 29.5%, respectively (Table 3). The prevalence of *Schistosoma* spp. was higher in goats than in sheep, while the contrary was observed for *F. gigantica*; but no significant difference was observed. The highest prevalence of *Schistosoma* (13.6%) and *Fasciola* (50.8%) were found in goats and cattle, respectively.

The association between parasitic infections and sex, age and breed of farm cattle are shown in Table 4. Males had higher odds of being infected with *S. bovis* (multiple OR: 4.3; 95% CI: 0.7–26.9). Cattle aged 4 years and older had lower odds of *Schistosoma* infection compared to cattle younger than 4 years (multiple OR: 0.6; 95% CI: 0.2–2.3), but these associations lacked statistical significance.

For fascioliasis, sex showed no strong association (multiple OR: 1.1; 95% CI: 0.8–1.7). Cattle aged 4 years and older had higher odds of infection compared to their younger counterparts (multiple OR: 1.7; 95% CI: 1.0–2.9). The breeds "Taurin" (multiple OR: 0.8; 95% CI: 0.3–2.5) and "Zébu" (multiple OR: 0.6; 95% CI: 0.3–1.0) had lower odds of infection compared to the "Taurin x Zébu", but the difference lacked statistical significance.

Spatial distribution of *Schistosoma* and *Fasciola* infection in livestock

The prevalence of schistosomiasis and fascioliasis varied by department and host species, with the highest prevalence observed in the northern part of Côte d'Ivoire (Fig. 1). As shown in Table 1, the highest prevalence of schistosomiasis in cattle sampled in slaughterhouses was found in Ouangolodougou (53.3%, 95% CI: 37.9–68.3%), while the highest prevalence of fascioliasis was observed in Katiola (40.6%, 95% CI: 23.7–59.4%). The lowest prevalence of schistosomiasis in slaughtered cattle was observed in Duekoué (5.9%, 95% CI: 0.7–19.7%). Fascioliasis was absent in slaughtered cattle in Niakaramadougou and Toumodi.

In farm cattle, *F. gigantica* was considerably more prevalent than *S. bovis* (Fig. 3b). As summarized in Table 3, the highest prevalence of *F. gigantica* in farm cattle was observed in Dikodougou (50.8%), whilst the highest prevalence of *S. bovis* was found in Ouangolodougou (2.4%).

In slaughtered sheep, *Schistosoma* spp. infection was present in Ferkessédougou and Sikensi with respective prevalences of 7.1% (95% CI: 0.2–33.9%) and 7.7% (95% CI: 0.2–36.0%). Slaughtered goats were also found infected with *Schistosoma* spp. in Ferkessédougou (6.9%; 95% CI: 0.0–9.7%). In Korhogo, sheep were infected with *F. gigantica* (2.8%; 95% CI: 0.1–14.5%), but not with *Schistosoma* and goats were not infected with either parasite. In slaughtered goats and sheep, the highest prevalence of *Fasciola* infection was recorded in sheep in Duekoué (8.0%; 95% CI: 1.0–26.0%). On farms, *Schistosoma* spp. were detected in sheep in Sinématiali (1.1%; 95% CI: 0.0–6.0%) and goats in Ferkessédougou (13.6%; 95% CI: 2.9–34.9%) (Table 3). Fascioliasis was found in farm sheep with a prevalence of 8.3% (95% CI: 2.8–18.4%) and 12.2% (95% CI: 6.3–20.8%) in Ferkessédougou and Sinématiali, respectively (Table 3).

Discussion

The prevalence and distribution of trematodes belonging to the genera *Schistosoma* and *Fasciola* in slaughtered and live livestock were assessed in the savannah (Centre and North) and forest (South and West) areas of Côte d'Ivoire. The results showed that schistosomiasis and fascioliasis are endemic in Côte d'Ivoire. *Schistosoma* and *Fasciola* infection were found at all the investigated sites. Cattle were infected more often with *Schistosoma* spp. and *F. gigantica* than goats and sheep, both in slaughterhouses and on farms. Sex was not associated with parasitic infections in slaughtered animals. However,

Table 1 Prevalence of *Schistosoma* and *Fasciola* infection in slaughtered cattle ($n = 386$), goats ($n = 174$) and sheep ($n = 151$) in 11 departments of Côte d'Ivoire in a cross-sectional survey conducted in March 2018

Department	Cattle					Goats					Sheep				
	N	*Schistosoma bovis*		*Fasciola gigantica*		N	*Schistosoma* spp.		*Fasciola gigantica*		N	*Schistosoma* spp.		*Fasciola gigantica*	
		n_{pos}	% (95% CI)	n_{pos}	% (95% CI)		n_{pos}	% (95% CI)	n_{pos}	% (95% CI)		n_{pos}	% (95% CI)	n_{pos}	% (95% CI)
Ouangolodougou	45	24	53.3 (37.9–68.3)	7	15.6 (6.5–29.5)	9	0	0.0 (0.0–33.6)	0	0.0 (0.0–0.3)	6	0	0.0 (0.0–45.9)	0	0.0 (0.0–45.9)
Ferkessédougou	57	14	24.6 (14.1–37.8)	6	10.5 (4.0–21.5)	29	2	6.9 (0.8–22.8)	0	0.0 (0.0–0.1)	14	1	7.1 (0.2–33.9)	0	0.0 (0.0–23.2)
Korhogo	52	18	34.6 (22.0–49.1)	14	26.9 (15.6–41.0)	36	0	0.0 (0.0–9.7)	0	0.0 (0.0–0.1)	36	0	0.0 (0.0–9.7)	1	2.8 (0.1–14.5)
Niakaramadougou	18	2	11.1 (1.4–34.7)	0	0.0 (0.0–18.5)	25	0	0.0 (0.0–13.7)	0	0.0 (0.0–0.1)	6	0	0.0 (0.0–45.9)	0	0.0 (0.0–45.9)
Katiola	32	3	9.4 (2.0–25.0)	13	40.6 (23.7–59.4)	6	0	0.0 (0.0–45.9)	0	0.0 (0.0–0.5)	–	–	–	–	–
Bouaké	50	6	12.0 (4.5–24.3)	10	20.0 (10.0–33.7)	7	0	0.0 (0.0–41.0)	0	0.0 (0.0–0.4)	9	0	0.0 (0.0–33.6)	0	0.0 (0.0–33.6)
Yamoussoukro	34	6	17.6 (6.8–34.5)	8	23.5 (10.7–41.2)	–	–	–	–	–	–	–	–	–	–
Toumodi	16	2	12.5 (1.6–38.3)	0	0.0 (0.0–20.6)	–	–	–	–	–	–	–	–	–	–
Agboville	27	5	18.5 (6.3–38.1)	4	14.8 (4.2–33.7)	20	0	0.0 (0.0–16.8)	0	0.0 (0.0–16.8)	42	0	0.0 (0.0–8.4)	0	0.0 (0.0–8.4)
Sikensi	21	5	23.8 (8.2–47.2)	6	28.6 (11.3–52.2)	2	0	0.0 (0.0–84.2)	0	0.0 (0.0–84.2)	13	1	7.7 (0.2–36.0)	1	7.7 (0.2–36.0)
Duekoué	34	2	5.9 (0.7–19.7)	8	23.5 (10.8–41.2)	40	0	0.0 (0.0–8.8)	0	0.0 (0.0–8.8)	25	0	0.0 (0.0–13.7)	2	8.0 (1.0–26.0)
Total	386	87	22.5 (18.5–27.0)	76	19.7 (15.8–24.0)	174	2	1.2 (0.1–4.1)	0	0.0 (0.0–2.1)	151	2	1.3 (0.2–4.7)	4	2.7 (0.7–6.6)

N Number of investigated animals per department, n_{pos} Number of infected animals, *CI* Confidence interval

Table 2 Multiple logistic GEE model analysis of variables associated with *Schistosoma bovis* and *Fasciola gigantica* infection among slaughtered cattle, adjusted for potential correlation within slaughterhouse in a cross-sectional survey conducted in Côte d'Ivoire in March 2018. Note that 82 animals from Agboville, Sikensi and Duekoué were not included in the analysis because of missing data on age

Trait	N	*Schistosoma bovis*			*Fasciola gigantica*		
		Infected n_{pos} (%)	Multiple OR	95% CI	Infected n_{pos} (%)	Multiple OR	95% CI
Sex							
Female	192	49 (25.5)			37 (19.3)		
Male	112	26 (23.2)	1.1	0.6–1.8	21 (18.8)	1.0	0.6–1.6
Age (years)							
1–3	62	9 (14.5)			7 (11.3)		
≥ 4	242	66 (27.3)	2.4	1.0–5.6	51 (21.1)	2.1	1.1–3.9
Breed							
Taurin x Zébu	107	31 (29.0)			26 (24.3)		
Taurin	77	20 (26.0)	0.9	0.3–2.8	8 (10.4)	0.4	0.2–0.9
Zébu	120	24 (20.0)	0.6	0.2–1.8	24 (20.0)	0.7	0.5–1.1

N Investigated animals, n_{pos} Number of infected animals, *CI* Confidence interval, *OR* Odds ratio

in farm cattle, males were infected more often with *Schistosoma* than females and the spatial distribution of infections showed that the highest prevalence was found in the northern part of the country.

The distribution of *Schistosoma* and *Fasciola* is governed by the presence of multipurpose dams and other freshwater bodies (e.g. rivers) and animal-water contact sites (for schistosomiasis) and consumption of aquatic plants (for fascioliasis). The river networks running North to South is of particular relevance, as it provides habitats for intermediate snail hosts. In addition, the livestock system itself may play an essential role in maintaining the transmission of schistosomiasis. In traditional livestock-systems, animals graze and drink water near and from rivers and dams, depositing faeces containing the parasite eggs, perpetuating the life cycle. A study carried out in the southern highlands of Tanzania demonstrated that livestock management systems influence the epidemiology of trematode infection. Indeed, it was found that the prevalence of trematodes was high in areas where traditional systems were practiced, moderate in large-scale dairy systems and lowest in small-scale dairy systems [44]. In Mali, another study showed that factors such as climatic conditions, presence of rivers and lakes, and livestock management practices influence the prevalence of trematode infections [38].

The prevalence of schistosomiasis and fascioliasis in goats and sheep was low compared to cattle. Cattle graze away from the villages and consume water from rivers and dams. Goats and sheep, in contrast, often graze around the villages and consume water that is provided by farmers. This likely reduces the contact with infested freshwater and reduces the risk of *Schistosoma* infection in goats and sheep. Our findings are in line with a previous study in the south-eastern Lake Chad area that reported a higher prevalence of adult liver flukes in cattle than sheep and goats [45]. Frequent grazing of cattle near the lake emerged as the key risk factor. In Iran, another study also reported a lower prevalence of *Fasciola* in goats and sheep compared to cattle. This observation was explained by the fact that goats consume leaves and heaths in elevated areas, sheep graze on open land, while cattle pasture near the springs and streams, and hence, cattle are at a higher risk of exposure to snail-infested freshwater [46].

The two trematode infections appear to be more prevalent in Côte d'Ivoire than elsewhere in sub-Saharan Africa. Indeed, the prevalence of 53.3% of *Schistosoma* is more than 2-fold higher than that recorded in South Ghana (21.7%) [19]. The prevalence of *Fasciola* (50.8%) in the current study is higher than previously recorded in the northern part of Côte d'Ivoire (4%) [23]. Our prevalence estimate is also higher compared to research conducted in Ethiopia (31.3%) [47]. However, other studies also showed prevalences above 50%; for instance in Chad (68%) [45], Ghana (51.1%) [19] and Zambia (53.9%) [48]. These high rates in Côte d'Ivoire may be due to the extensive farming systems with little or no veterinary input. The lack of a control programme against trematodes leads to livestock owners medicating their animals with commonly used anthelminthic drugs such as albendazole and nitroxynil without a prescription from a veterinary. Triclabendazole [49], the drug of choice against *Fasciola* is not available for use in Côte d'Ivoire. Praziquantel, which is highly effective against all visceral bovine schistosomiasis [50], is also not commercially available for livestock in the country. These factors likely contribute to the endemicity of livestock schistosomiasis and fascioliasis.

Table 3 Prevalence of *Schistosoma* and *Fasciola* in farm cattle ($n = 735$), goats ($n = 22$) and sheep ($n = 176$) in four departments of the northern part of Côte d'Ivoire in a cross-sectional survey carried out in August 2018

Department	Cattle					Goats				Sheep			
	N	*Schistosoma bovis*		*Fasciola gigantica*		N	*Schistosoma* spp.		*Fasciola gigantica*	N	*Schistosoma* spp.		*Fasciola gigantica*
		n_{pos}	% (95% CI)	n_{pos}	% (95% CI)		n_{pos}	% (95% CI)	n_{pos} % (95% CI)		n_{pos}	% (95% CI)	n_{pos} % (95% CI)
Ouangolodougou	166	4	2.4 (0.7–6.1)	37	22.3 (16.2–29.4)	–	–	–	– –	6	0	0.0 (0.0–45.9)	0 0.0 (0.0–45.9)
Ferkessédougou	199	1	0.5 (0.0–2.8)	33	16.6 (11.7–22.5)	22	3	13.6 (2.9–34.9)	0 0.0 (0.0–15.4)	60	0	0.0 (0.0–6.0)	5 8.3 (2.8–18.4)
Sinématiali	185	0	0.0 (0.0–2.0)	53	28.6 (22.3–35.7)	–	–	–	– –	90	1	1.1 (0.0–6.0)	11 12.2 (6.3–20.8)
Dikodougou	185	0	0.0 (0.0–2.0)	94	50.8 (43.4 58.2)	–	–	–	– –	20	0	0.0 (0.0–16.8)	0 0.0 (0.0–16.8)
Total	735	5	0.7 (0.2–1.6)	217	29.5 (26.3–33.0)	22	3	13.6 (2.9–34.9)	0 0.0 (0.0–15.4)	176	1	0.6 (0.0–3.1)	16 9.1 (5.3–14.3)

N Number of animals per department, n_{pos} Number of infected animals, *CI* Confidence interval

Table 4 Multiple logistic GEE model analysis of variables associated with *Schistosoma bovis* and *Fasciola gigantica* infection among farm cattle, adjusted for potential correlation within farms in a cross-sectional survey carried out in Côte d'Ivoire in August 2018

Trait	N	*Schistosoma bovis*			*Fasciola gigantica*		
		Infected n_{pos} (%)	Multiple OR	95% CI	Infected n_{pos} (%)	Multiple OR	95% CI
Sex							
Female	563	2 (0.4)			173 (30.7)		
Male	172	3 (1.7)	4.3	0.7–26.9	44 (25.6)	1.1	0.8–1.7
Age (years)							
1–3	150	2 (1.3)			33 (22.0)		
≥ 4	585	3 (0.5)	0.6	0.2–2.3	184 (31.5)	1.7	1.0–2.9
Breed							
Taurin x Zébu	587	4 (0.7)			183 (31.2)		
Taurin	37	0 (0.0)	ND	ND	10 (27.0)	0.8	0.3–2.5
Zébu	111	1 (0.9)	ND	ND	24 (21.6)	0.6	0.3–1.0

N Investigated animals, n_{pos} Number of infected animals, *CI* Confidence interval, *ND* Not determined because of low number of infected individuals in several categories, *OR* Odds ratio

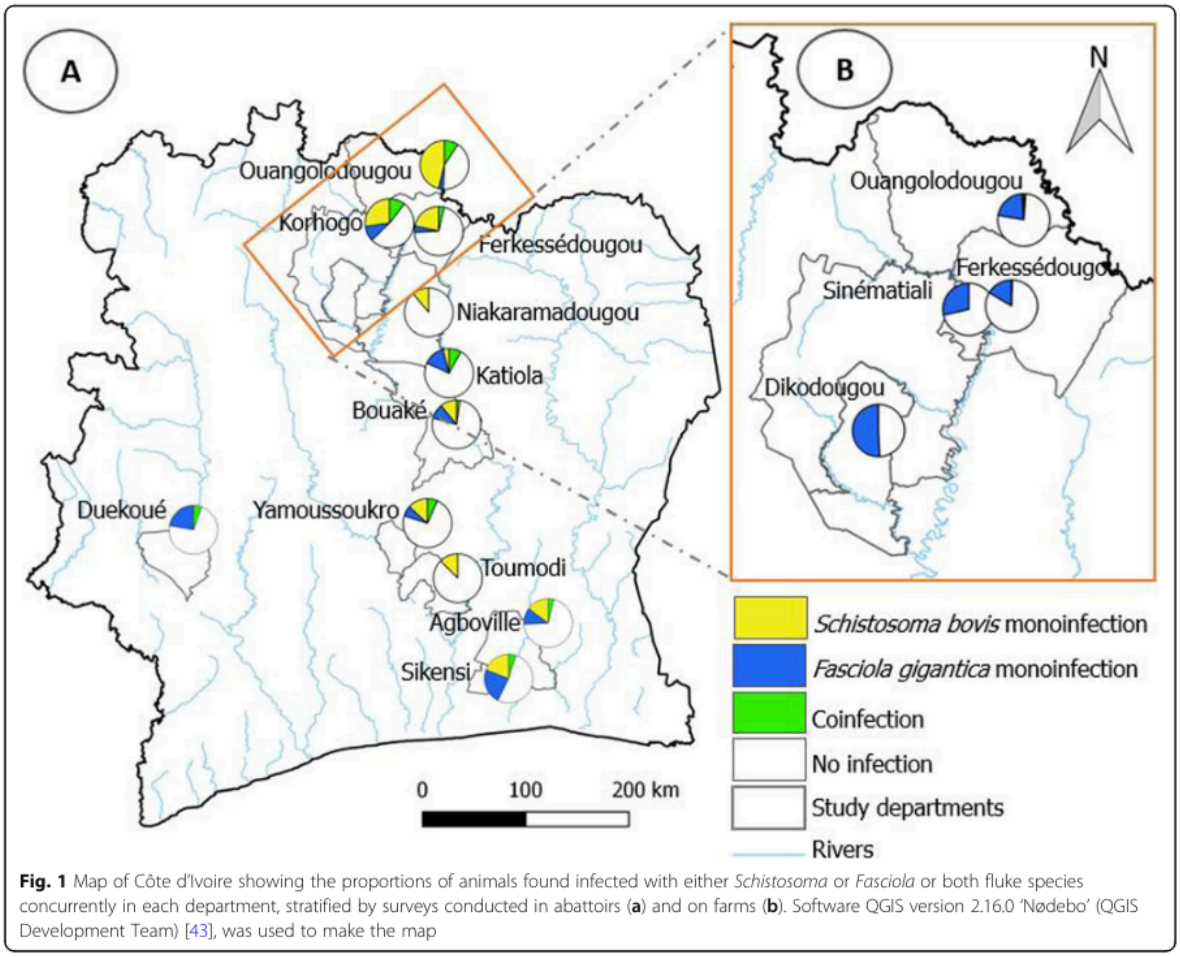

Fig. 1 Map of Côte d'Ivoire showing the proportions of animals found infected with either *Schistosoma* or *Fasciola* or both fluke species concurrently in each department, stratified by surveys conducted in abattoirs (**a**) and on farms (**b**). Software QGIS version 2.16.0 'Nødebo' (QGIS Development Team) [43], was used to make the map

Schistosoma infection was particularly high in male cattle on farms. A prior study from Nigeria showed that the prevalence of *Fasciola* was higher in male cattle than in females [51]. In that study, the authors speculated that the high infection rate in male cattle could be attributed to the fact that the males were more often slaughtered for consumption, while the females were left for milk production and breeding. This may have led the herdsmen to ensure that the females graze on clean pasture and drink clean water [51].

In slaughtered animals, sex was not associated with *Schistosoma* and *Fasciola* infection. In contrast to the aforementioned Nigerian abattoir survey [51], the prevalence of *Schistosoma* and *Fasciola* infection in farm cattle was higher in males than in females. Results concerning this issue are thus not consistent, as there are studies that report higher prevalence in male cattle [13, 52] and others that report higher prevalence in females [53, 54].

Cattle aged 4 years and older were infected more often by *S. bovis* and *F. gigantica* than their younger counterparts in slaughterhouses. This finding might be explained by the fact that farmers predominantly butcher older animals or animals that are ill to avoid the death of the animal on the farm, which would result in economic losses. It is conceivable that older animals are in contact with contaminated water for longer periods than younger animals that are kept on the farm for breeding. This is in agreement with studies that reported similar findings in Nigeria [16, 18] and Tanzania [55, 56]. Others studies from western Ethiopia [47] and in the Philippines [57] showed a statistically significant association of the age of the cattle with the prevalence of fascioliasis. In addition, when the cattle get older, their immunity against *Fasciola* might decrease [58]. However, studies found a higher prevalence rate of fascioliasis in young cattle than in adults [48, 59] and rationalised that older animals would develop acquired immunity that resulted in resistance [60].

The higher prevalence of *Schistosoma* and *Fasciola* found in the northern parts of Côte d'Ivoire compared to the South might be explained by the many small multipurpose dams built in the North in the 1970s and 1980s. Indeed, dams play a crucial role in maintaining the lifecycle of snail-borne infections, and some studies have shown the presence of intermediate snail hosts of *Schistosoma* and *Fasciola* in North Côte d'Ivoire [61, 62]. In Senegal, trematode infection increased after the construction of large dams across major rivers in the Senegal River basin [63]. The northern part of Côte d'Ivoire is also home to the largest livestock production in the country. The lower prevalence found in the central, southern and western regions could mean that there is a lower parasite pressure on animals compared to the North. The lowest prevalence of schistosomiasis in slaughtered cattle was observed in Duekoué, the western forested area of the country. Studies in humans in Western Côte d'Ivoire showed that *S. mansoni* is the predominant species [20, 64]. Unlike in cattle, the highest prevalence of fascioliasis in small ruminants was observed in sheep in the forested areas. However, the sample size was not large enough to draw firm conclusions.

The prevalence of *Schistosoma* in cattle was lower on farms and higher in slaughterhouses, while the opposite was observed for *Fasciola*. These observations should be interpreted with caution because the two populations of cattle differ in important ways, and the animals tested were not from the exact same places. Therefore, results from farms could not be compared to those from abattoirs.

The following considerations are offered for discussion. First, farm populations are more diverse in regards to age and include calves and young cattle that have had little time to be infected. Second, the cattle at slaughterhouses may be ill, their illness being the reason that they are brought for slaughter. Third, the prevalence of bovine schistosomiasis is often higher in wet season compared to the dry season [16]. As the farm sampling in this study was carried out in the rainy season in the North (August 2018), we expected the prevalence of schistosomiasis to be higher as seen in other investigations [65, 66]. Yet the prevalence was low there and the high prevalence of schistosomiasis was found in dry season (March 2018) in the abattoirs. In regards to the low schistosomiasis prevalences, sedimentation technique has been shown to have a low sensitivity, depending on the protocol. This may be due to factors that affect coprology in general, such as variation in the distribution of eggs within a single faeces specimen; daily fluctuations of faecal production and consistency in the host; and daily fluctuations related to oviposition patterns of the parasite [67–69]. The sensitivity is also influenced by the time samples are left to sediment. This is due to the rapid hatching of *Schistosoma* eggs, which may occur during the sedimentation process before the sediment is observed on the slide [70]. In fact, after exposure to water, *Schistosoma* eggs can hatch within 20 min [71]. In this study, the total time eggs were left to sediment in water was 45 min. It may also be because not all *Schistosoma* eggs are excreted in the faeces, many are left trapped the in tissue [52]. Lastly, the immune reaction of host organisms against schistosomes is not primarily directed towards the elimination of adult worms but rather towards the suppression of worm fecundity [72], also leading to reduced egg output. However, with post-mortem examination, adult flukes can be easily detected in the mesenteric vein or liver.

The higher prevalence of *Fasciola* observed in farm cattle might be due to the higher endemicity of *Fasciola* in the breeding areas. In addition, faecal examination appears to be more sensitive for *Fasciola* egg detection.

Corroborating this finding, a study in South Ethiopia found a higher prevalence of *Fasciola* in farm cattle [73]. The higher prevalence of fascioliasis in this study could be due to the fact that sampling was done during the rainy season in the northern part of Côte d'Ivoire. In fact, fascioliasis prevalence is known to vary depending on the season, with an increase in prevalence during the wet season [41, 74, 75]. In contrast, an abattoir study in Southwestern Nigeria reported a higher prevalence of fascioliasis in the dry season compared to the rainy season [76]. To investigate further the epidemiology of trematode infections in livestock in Côte d'Ivoire, a seasonal study of both infections is needed.

Our study has some limitations. First, we did not count the eggs of parasites in the faeces of farm animals; we only determined the prevalence but not the intensity of infection. Second, the sample sizes at some abattoirs were small especially for goats and sheep; in addition, farms from central, southern and western parts of Côte d'Ivoire were less sampled than farms in the North, as livestock populations are mainly concentrated in the northern parts of Côte d'Ivoire. Third, we did not record the history of the treatments that might influence the prevalence of trematodes on farms. However, this study highlights the importance of veterinary trematodes in Côte d'Ivoire, as well as provides prevalence information for regions not previously sampled and updates the existing literature on livestock schistosomiasis and fascioliasis in West Africa, particularly in Côte d'Ivoire.

Conclusions

Animal schistosomiasis and fascioliasis are prevalent in abattoirs and on farms across Côte d'Ivoire, with the highest prevalence found in the North. Our study revealed that *Fasciola* and *Schistosoma* infection are both associated with older age cattle and male sex. In view of our findings, a strategic control programme for cattle trematodes is warranted. An important component of such a control programme is to water cattle in troughs rather than to let them freely consume water from ponds, in order to reduce faecal contamination.

Methods

Study area

The study was conducted in 13 departments located in the northern, central, southern and western parts of Côte d'Ivoire: Ouangolodougou, Ferkessédougou, Sinématiali, Korhogo, Dikodougou, Niakaramadougou, Katiola, Bouaké, Yamoussoukro, Toumodi, Agboville, Sikensi and Duekoué (Fig. 2). The areas in the North and the Centre are characterised by a tropical climate and savannah vegetation with some forested areas. The South and West are in the tropical rainforest zone. Rainfall is higher in the southern and western parts, compared to the central and northern parts. The South and West also have less grass for livestock than the central and northern parts. The hydrography is composed of rivers running from North to South and several small multipurpose dams, mainly used for cattle breeding in the North [77]. The rivers and dams provide suitable habitats for intermediate host snails of schistosomiasis and fascioliasis [61] and the savannah with its grass is favourable for cattle breeding.

Sampling and study design

Two cross-sectional surveys were carried out in March and August of 2018 to determine the prevalence of schistosomiasis and fascioliasis in cattle, goats and sheep. The first survey was conducted to obtain an overview of the prevalence of schistosomiasis and fascioliasis in slaughtered animals, in 11 of the 13 departments across the full geographical range. At least 3 days were spent at each local abattoir, where the livers and small intestines from slaughtered livestock were excised during the routine meat inspection. The samples were stored and transferred to a nearby laboratory of the "Direction Départementale du Ministère des Ressources Animales et Halieutiques". There, livers and small intestines were examined for *Fasciola* and *Schistosoma* flukes. Demographic information such as sex, age, breed and origin were recorded. The animals in the abattoirs came from the local villages or in the northern part of the country, in particular for central, southern and western abattoirs. Of course, there were transhumant animals from the northern border countries, Mali and Burkina Faso, but these animals were not included in the study.

The second cross-sectional survey aimed to determine the prevalence of schistosomiasis and fascioliasis in farm animals and was restricted to the departments of Ouangolodougou, Ferkessédougou, Sinématiali and Dikodougou. These sites were chosen due to the high prevalences of infection detected there in the first cross-section study, in combination with the presence of dams and water bodies frequented by both humans and animals, in the area. A total of 16 farms (in villages) were randomly selected, four farms from each department. Rectal sampling was conducted under license as mentioned in the ethics approval and consent to participate section. After consent from the farmers, veterinary technicians collected faecal samples from the rectums of cattle, goats and sheep, as well as demographic information for each animal. Both abattoir and farm samplings were carried out in the dry season (March) and rainy season (August), respectively. The average monthly temperature and precipitation for the study months sampled are shown in the supplemental material (Additional file 1).

The sample size was determined based on the expected prevalence (p) of bovine fascioliasis and the desired absolute precision (d) and 95% confidence interval (CI) by

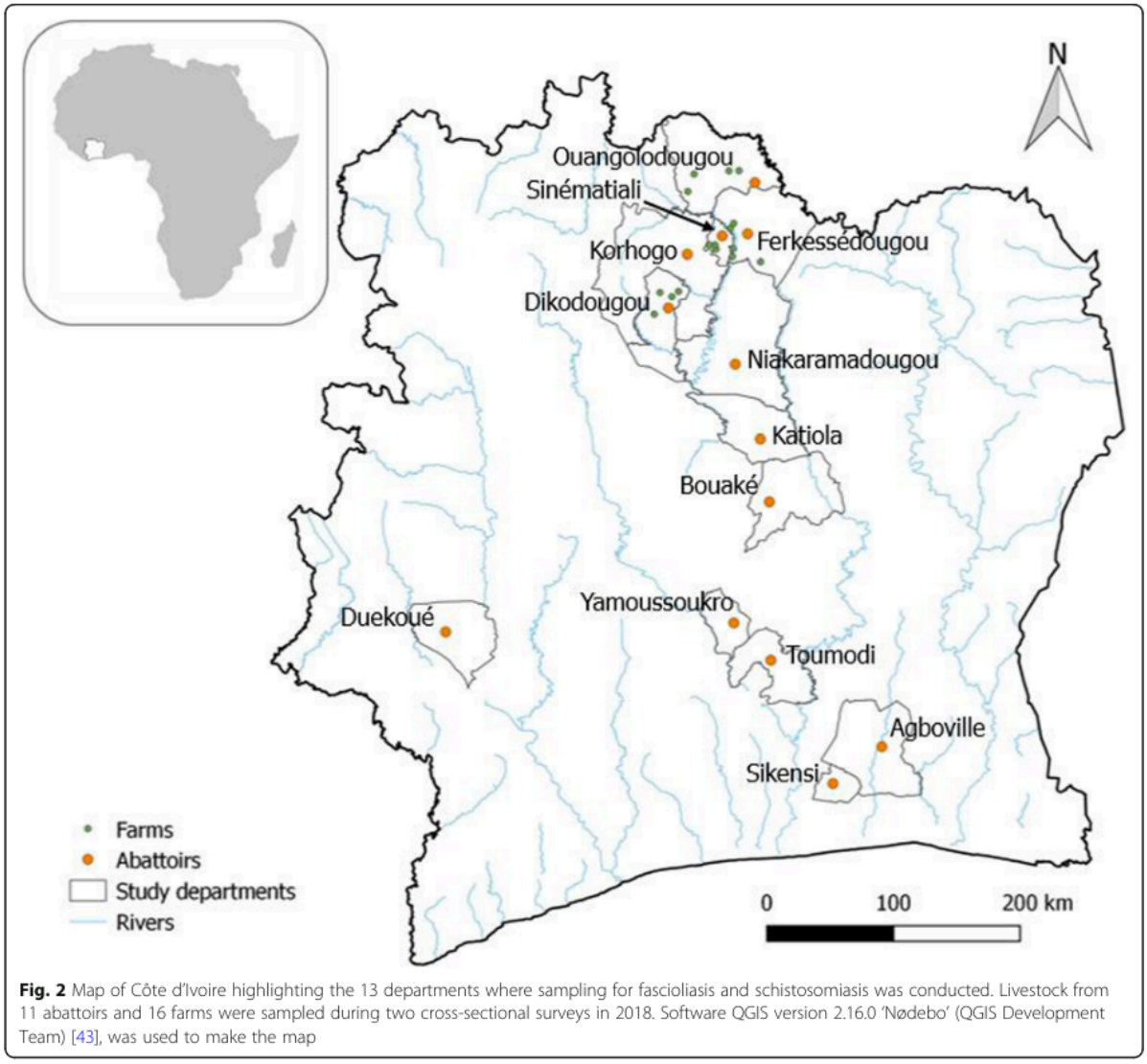

Fig. 2 Map of Côte d'Ivoire highlighting the 13 departments where sampling for fascioliasis and schistosomiasis was conducted. Livestock from 11 abattoirs and 16 farms were sampled during two cross-sectional surveys in 2018. Software QGIS version 2.16.0 'Nødebo' (QGIS Development Team) [43], was used to make the map

using the formula $n = (Z^2 * p * (1\text{-}p)) / d^2$, where Z = 1.96, $p = 60\%$ and $d = 5\%$ [78]. Thus, a sample size of 369 animals was ascertained for each group of animals, from either slaughterhouses or farms.

Laboratory procedures

Macroscopic inspection of the small intestine and liver of animals for adult flukes was carried out in the laboratory. The mesenteric veins of the small intestine (Fig. 3a) and hepatic veins were examined for schistosomes. *Fasciola* were recovered from the biliary duct and the parenchyma of the liver (Fig. 3b) [45].

Faecal samples from farm animals were processed by a sedimentation method [79]. In brief, approximately 5 g of faeces was placed in a cup and mixed with 30 ml of tap water. The faecal suspension was sieved through a 400 μm sieve into a 500 ml conical beaker. Tap water was added until the beaker was almost full. The conical beaker sat undisturbed for 15 min. The supernatant was decanted, and the sediment re-suspended in tap water. The process of suspension and decanting was repeated three times. Finally, using a pipette, a drop of the sediment was transferred onto a slide, stained with methylene blue (1%) and examined under a microscope for parasite eggs.

Statistical analysis

Data were double entered into EpiInfo version 3.5.4 (Centers for Disease Control and Prevention; Atlanta, USA) and checked for internal consistency. R software version 3.5.2 [80] was used to calculate proportions and

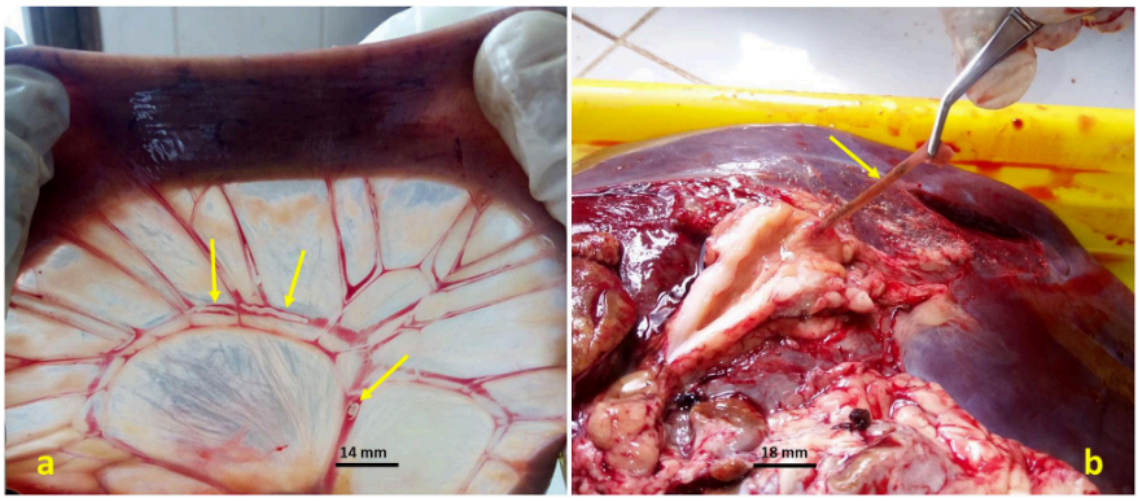

Fig. 3 *Schistosoma* flukes in mesenteric veins of the small intestine (**a**) and a *Fasciola* fluke in a cattle liver (**b**)

95% CIs. Associations between parasitic infections and risk factors (sex, age and breed) were assessed using generalized estimating equation (GEE) analysis for binary outcomes with farms and slaughterhouses as clusters. Animals from Agboville, Sikensi and Duekoué were not included in the GEE analysis due to missing data on animal traits in these areas.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-020-02667-y.

Additional file 1. Climate diagram of the northern (a), central (b), southern (c) and western (d) areas of Côte d'Ivoire in 2018.

Abbreviations

CI: Confidence interval; OR: Odds ratio; *S*: *Schistosoma*; *F*: *Fasciola*; *p*: Prevalence; *d*: Desired absolute precision; GEE: Generalized estimating equation

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Authors' contributions

JNK, JGE, LYA, DF, MO, KDS, BB, JH, JU, JZ, OB and EKN conceived of and designed the study. JNK, JGE, LYA and DF conducted the study. JNK and JH analysed and interpreted the data. JNK wrote the first draft. JGE, LYA, DF, MO, KDS, BB, JU, JZ, OB and EKN revised the manuscript. All authors read and approved the final version of the manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from Dr. Oliver Balmer on reasonable request.

Ethics approval and consent to participate

The study was a part of a project entitled "Transmission dynamics and hybridization of human and animal trematodes in Côte d'Ivoire" that was approved by the National Ethics Committees of Côte d'Ivoire (reference no.: 035/MSH /CNER-kp) and Switzerland (UBE-2016-00707). In addition, the "Direction des Services Vétérinaires" of the "Ministère des Ressources Animales et Halieutiques de Côte d'Ivoire" authorized the study. The farmers signed informed consent forms for the sampling of their animals. International and national guidelines for the care and use of animals were followed.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Publisher's Note

Chapter 5

Accuracy of the sedimentation and filtration methods for the diagnosis of schistosomiasis in cattle

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HELMINTHOLOGY - SHORT COMMUNICATION

Accuracy of the sedimentation and filtration methods for the diagnosis of schistosomiasis in cattle

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Abstract
Infection with blood flukes of the genus *Schistosoma* causes considerable human and animal morbidity, mortality and economic loss to the livestock industry. Current diagnostic tools have limitations. In this study, we compared the sedimentation and filtration methods for the diagnosis of schistosomiasis in livestock. A total of 196 faecal samples from cattle in Côte d'Ivoire were subjected to sedimentation and filtration for the diagnosis of schistosomiasis and other intestinal parasite infections. *Schistosoma* eggs or miracidia were discovered in 32 samples: 15 by filtration only, seven by sedimentation only, six concurrently by both methods and four by observing miracidia swimming on the sedimentation slide. The sensitivity of sedimentation and filtration was 41% and 66%, respectively. Cases with no *Schistosoma* eggs identified in the sediment but miracidia swimming on the slide indicate that eggs had hatched before microscopy. More accurate diagnostic are required for livestock schistosomiasis, in order to better understand the epidemiology and inform control and elimination efforts in livestock and human populations.

Keywords Côte d'Ivoire · Diagnostic accuracy · Filtration · Livestock · Schistosomiasis · Sedimentation

Introduction

Schistosomiasis, caused by a zoonotic parasite infection, is responsible for considerable morbidity, mortality and burden in humans and livestock (GBD 2017 DALYs and HALE Collaborators 2018; McManus et al. 2018). Indeed, it is estimated that schistosomiasis affects more than 250 million people, particularly school-aged children in sub-Saharan Africa (Hotez et al. 2014) and 165 million cattle (De Bont and Vercruysse 1997). Its impacts are felt in the livestock industry in terms of long-term negative effects on growth and productivity of cattle and other domestic animals (MSD 2016). An important epidemiological feature of schistosomiasis is its focality, which poses a challenge to the control and elimination of the disease.

There are 21 *Schistosoma* species known to infect mammals, eight of which are of veterinary relevance and six of medical concern (Standley et al. 2012; MSD 2016). Recently, hybrids of human and livestock *Schistosoma* have been found in humans in different parts of the world (Leger and Webster 2017), including Côte d'Ivoire (Angora et al. 2019; Tian-Bi et al. 2019). This is concerning as many resources are devoted to the control and elimination of schistosomiasis in the human population by mass drug campaigns using praziquantel (Tian-Bi et al. 2018).

Schistosoma bovis (Bilharz, 1852) and *S. curassoni* (Weinland, 1858) are the two species found in West African livestock (De Bont and Vercruysse 1998). In Côte d'Ivoire, 35% of cattle were found to be infected with *S. bovis* in the

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northern Savannah region (Achi et al. 2003). The prevalence in other regions of Côte d'Ivoire was, until recently, unknown and thought to be low (Achi et al. 2003). However, an epidemiological investigation in 10 departments of Côte d'Ivoire in 2018 revealed prevalences in slaughtered cattle ranging from 9 to 53% in the northern, 12 to 18% in the central, 6% in the western and 19 to 24% in the southern parts of the country (Kouadio and colleagues, unpublished data).

Methods for the diagnosis of schistosomiasis in animals have received little attention and mainly rely on stool microscopy using the sedimentation technique (Sirois 2017). Sedimentation involves the identification of parasite eggs on a slide. The method, however, does not lend itself well to the detection of *Schistosoma* eggs due to the biology of the parasite. Indeed, *Schistosoma* eggs might hatch in as little as 20 min when exposed to freshwater at ambient temperatures (Sturrock 1993; Jones et al. 2008; Schmidt and Roberts 2013). It is also important to note that the sedimentation method may vary from one laboratory to another, though a common feature is that faeces are allowed to sediment in water for a total of 15–90 min (Sirois 2017). It follows that eggs may hatch before the sediment is examined under a microscope, which could result in false negative results, since eggs are not easily identifiable after they have hatched (Fig. 1).

The purpose of this study was to determine the accuracy of sedimentation compared to filtration for the diagnosis of schistosomiasis in cattle. The study was embedded in a larger project that aimed to determine the prevalence and transmission dynamics of *Schistosoma* in Côte d'Ivoire. Due to the exposure of *Schistosoma* eggs to water during sedimentation, we hypothesised that the sensitivity of sedimentation was low.

Methods

Overall frame and ethics

Cattle faeces were sedimented in order to diagnose *Schistosoma* and other intestinal parasites for prevalence estimates. Miracidia were collected from faeces, which was achieved by filtering faeces through a Pitchford filter (Visser and Pitchford 1972) (Fig. 2).

During a first spell of fieldwork in August 2018, it was observed that the standard sedimentation method failed to detect all cases of schistosomiasis. Hence, the method was adjusted for a second round of fieldwork pursued in June 2019. All procedures were done in accordance with Ivorian ethical regulations. Local authorities provided permission for the investigation and written informed consent was obtained from cattle owners prior to stool sampling.

Sampling

Faeces were manually extracted from the rectums of cattle on farms in the department of Ferkessédougou in the northern part of Côte d'Ivoire on two separate occasions. In August 2018, farms from four villages were sampled and faecal specimens transferred to the Laboratoire National d'Appui au Développement Agricole (LANADA) in Korhogo for diagnostic work-up.

The farms in one of the aforementioned villages were resampled in June 2019 and faecal samples analysed at a laboratory in Ferkessédougou by LANADA technicians from Bingerville, Bouaké and Korhogo laboratories. All cattle aged

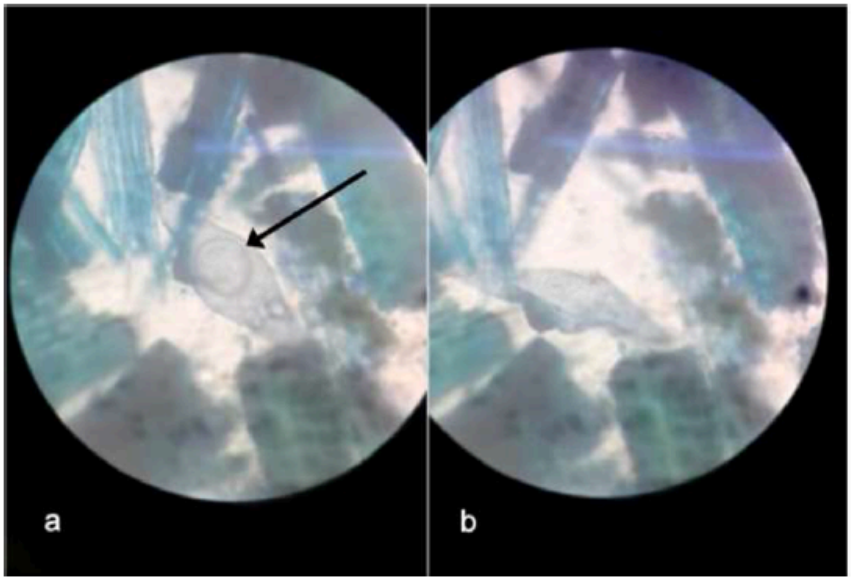

Fig. 1 An intact *Schistosoma bovis* egg with the miracidium inside (arrow) under a microscope at × 400 magnification (**a**) and a *S. bovis* egg after the miracidium has exited (**b**)

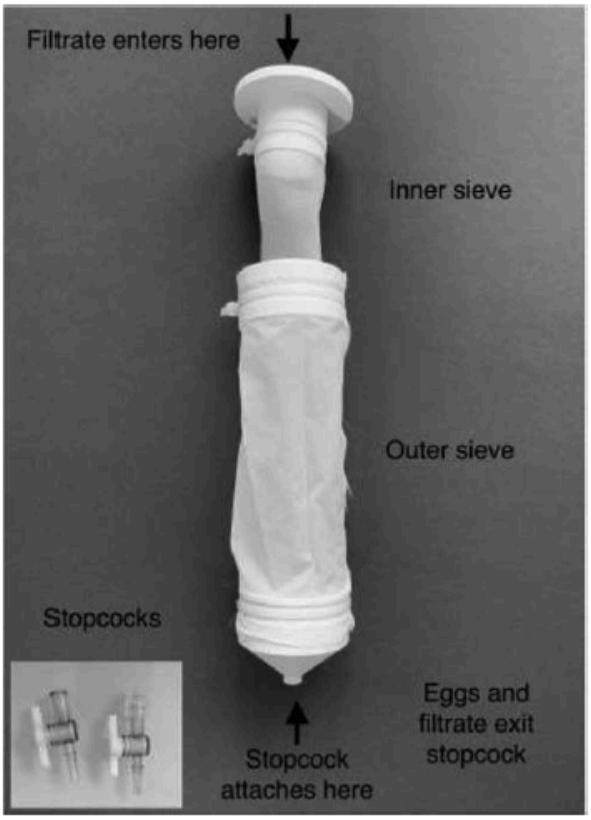

Fig. 2 Pitchford filter, stopcocks without and with Luer lock (Fisher Scientific AG, Reinach, Switzerland, product numbers 11772643 and 13457438)

1 year and older that could be captured on the day of sampling were included. Faecal samples were subjected to the sedimentation and filtration methods.

Sedimentation

During the initial fieldwork in August 2018, 5 g of faeces was mixed with 30 ml of tap water and homogenised with a mortar and pestle. This mixture was poured through a tea strainer into a 500 ml conical beaker and topped up with tap water to 490 ml. The faeces were allowed to sediment for 15 min, after which the supernatant was decanted. The sedimenting steps were performed three times, for a total of 45 min of exposure to water. Methylene blue was added to the sediment. Experienced veterinary technicians from LANADA examined the entire sediment under a microscope on slides at 100-fold magnification. Samples were considered positive if at least one *Schistosoma* egg was identified on the slides.

Of note, the protocol was slightly changed in the subsequent fieldwork done in June 2019, in an effort to increase the sensitivity of the method. In brief, 3 g of faeces was sedimented and in order to reduce the amount of time eggs were exposed to water, samples were sedimented twice for 3 min only. Additionally, homogenisation of faeces was performed with a wooden spatula and faeces were sedimented in 100 ml conical beakers.

Filtration

The Pitchford filter mesh size was adjusted, as compared to the originally published version (Visser and Pitchford 1972) to accommodate for the typical size of *S. bovis* eggs, which are longer and more slender than human *Schistosoma* eggs (i.e. inner sieve mesh was 300 µm, outer sieve mesh was 30 µm). A walnut size portion of faeces was placed onto a 400-µm sieve, washed with locally purchased bottled water and scraped with a wooden spatula until only large debris remained on the sieve. The filtrate was poured through a Pitchford filter (Fig. 2), where the inner sieve retained debris and the outer sieve retained parasite eggs, but allowed excess water and smaller debris to pass through.

The filtrate from the Pitchford filter was drained into a Petri dish and left to incubate in a semi-sunny area for 4 h. After 1 h, and every hour thereafter, the Petri dishes were examined for miracidia using a stereomicroscope at × 10 magnification. Samples were considered positive if at least one *Schistosoma* miracidium was detected in the Petri dish.

Results and discussion

During the initial fieldwork in August 2018, four cases of *Schistosoma* infection were detected in the 45 faecal samples. Sedimentation (three times 15 min) identified one of the four cases, whereas the Pitchford filter detected all four cases.

In the more extensive fieldwork conducted in June 2019, faecal samples from 196 cattle subjected concurrently to the sedimentation and filtration methods revealed 32 positive results (Fig. 3). There were 13 *Schistosoma* infections detected by the sedimentation method with *Schistosoma* eggs diagnosed under a microscope. Of these 13 infections, seven were detected only by sedimentation. Fifteen cases were identified as miracidia swimming on the slide with only two of these cases characterised by the presence of both eggs and miracidia. Considering only those cases with confirmed eggs under a microscope, the sedimentation revealed a sensitivity of 41%. The sensitivity increased to 81% when considering both eggs and miracidia swimming on the slide as positive results.

Overall, *Schistosoma* miracidia were detected in 21 of the filtrations, owing to a sensitivity of this method for the diagnosis of schistosomiasis in cattle of 66%. Six of these samples were found positive only by filtration, while in six samples both filtration and sedimentation identified *Schistosoma* miracidia or eggs, respectively. Two samples were positive by filtration, sedimentation and presence of miracidia on the slides.

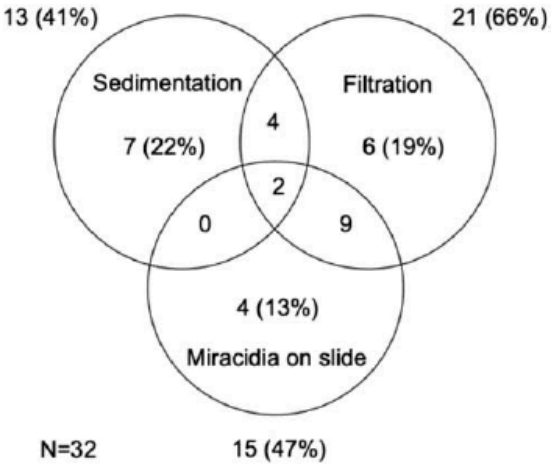

Fig. 3 Venn diagram of schistosomiasis cases found positive for eggs detected under a microscope using either sedimentation or filtration, or the presence of miracidia on sedimentation slides in 196 faecal samples obtained from cattle in the northern part of Côte d'Ivoire in June 2019

Coprological methods, such as filtration and especially sedimentation, are the mainstays for the diagnosis of *Schistosoma* and other intestinal parasite infections in livestock (Sirois 2017). However, coprological methods lack sensitivity for the diagnosis of *Schistosoma* eggs, which might be explained by the biology of the parasite. Indeed, adult schistosome worm pairs produce considerably fewer eggs compared to other helminth species. Additionally, from the eggs produced, only about one-third make it through the intestinal wall to be excreted in the faeces (Schmidt and Roberts 2013). Low infection intensity, day-to-day variation in egg output and uneven distribution of eggs in the faeces further contribute to the low sensitivity of these methods (Weerakoon et al. 2015). It should also be noted that coprological methods are dependent on the proficiency and expertise of the laboratory technicians operating light microscopy.

The sedimentation method, while very useful in identifying some trematodes (e.g. *Paramphistomum*, *Dicrocoelium* and *Fasciola*), has a couple of shortcomings for the identification of *Schistosoma* eggs. Schistosomes are unique in that their eggs hatch rapidly when exposed to freshwater. Since the sedimentation method relies on identifying eggs, it is critical that the eggs have not hatched before examination of the faeces under a microscope. The faecal samples obtained in the first round of fieldwork were allowed to sediment for a total of 45 min. Compared to filtration, only one of four positive samples was detected. Even after reducing the total sedimentation time to 6 min, eggs still hatched before microscopy in 41% of the cases (miracidia were identified on the slide, but no eggs) and, in 59% of the cases, eggs were absent on the slide.

There were 15 (47%) *Schistosoma* infections detected by filtration that did not have eggs on the sedimentation slides. Of these, six cases had only three or fewer miracidia collected from the Petri dish, five had 4–9 miracidia and the remaining four had 10–19 miracidia. Our observations suggest that sedimentation is related to eggs hatching during the sedimentation process, rather than low infection intensity, as 60% of the cases that did not have eggs on the slide had ≥4 miracidia collected from Petri dishes.

Of note, six cases (19%) were missed by sedimentation, either by the absence of eggs or the absence of hatched miracidia on the slide. There are three possible explanations for this observation. First, eggs hatched during sedimentation and all the miracidia were poured off with the supernatant. Second, the sedimentation time was too short and eggs stayed in suspension and were poured off. Third, the relatively small amount of faeces (3 g) subjected to the sedimentation method did not contain any eggs.

The sensitivity of the sedimentation method can be improved by adjusting the protocol to prevent eggs from hatching during the process. *Schistosoma* eggs begin to hatch when they are exposed to the lower osmolality of freshwater at ambient temperatures (Sturrock 1993; Schmidt and Roberts 2013). Hence, altering these conditions during the sedimentation process could, in principle, delay hatching. Reducing the time during which eggs were exposed to water, from 45 to 6 min, increased the proportion of positive cases detected from 25 to 41%. Further adjustments might include the use of salt water for sedimentation to keep osmolality high, refrigerating samples during sedimentation and pending diagnosis under a microscope or using cold water for sedimentation. Moreover, considering the presence of miracidia as positive cases considerably improved the sensitivity of sedimentation. Taken together, identifying eggs and miracidia on sedimentation slides identified 88% of the cases, which is more than the 66% identified by filtration. Currently available institutional protocols may not consider identifying miracidia on the slide as a positive diagnosis. We therefore call upon the scientific community to carefully review the existing protocols and, should need arise, amend the protocols accordingly.

Various filtration methods have been utilised for the diagnosis of schistosomiasis in livestock. Although there is a paucity of reports in the literature of filtering animal faeces and subsequent examination under a light microscope, this approach has been successfully used with human samples (De Bont and Vercruysse 1998; Gower et al. 2013).

The main limitation encountered with filtration in the current study was the amount of debris in the Petri dishes, which jeopardised detecting miracidia. Cattle faeces contain a substantial amount of debris when compared to human faeces. The change in sieve sizes exacerbated this issue. The Pitchford filter sieve sizes were changed to accommodate the long and slender *S. bovis* eggs. The inner sieve was larger, allowing larger debris inside the filter and the outer sieve was smaller, retaining smaller debris. Filtration did not identify

22% of cases that were positive by sedimentation and another 13% of cases positive by identifying miracidia on the slide. Of the seven cases identified by sedimentation, six had only one egg in the 3 g of faeces examined and the other case had only two eggs. As there were so few eggs per gram of faeces, it is conceivable that there were no eggs present in the faeces used for filtration or that none of the eggs present was viable. It is also conceivable that the one or two miracidia could not be detected amongst all the debris in the Petri dish.

Another consideration for filtration is that it does not permit the identification of the infecting *Schistosoma* species, as this cannot be determined by morphology of the miracidia alone. However, this can be addressed at an increased cost by isolating and genetically characterising the miracidia.

While sedimentation is quite a time-consuming endeavour, slight modifications may improve the sensitivity without drastically increasing the processing time. For example, large quantities of faeces can be filtered using this method, which increases the probability of detecting parasites in general, but particularly in light infections (De Bont and Vercruysse 1998).

Our study has several limitations. First, sedimentation proceeded faster than filtration and this resulted in technicians sometimes knowing that the samples they filtered had been deemed positive by sedimentation. This might have led to a bias in the filtration method, as knowing the sample was positive may have resulted in perseverance to find miracidia. Second, there is a lack of a 'true' diagnostic gold standard. Combined with the low infection intensity, this means that the prevalence may be underestimated, which would affect our results.

Diagnosing schistosomiasis in livestock is challenging, including capturing cattle for collection of faeces, transferring the samples to adequately equipped laboratories and, ultimately, examination under a microscope by experienced staff. Without new techniques, practitioners are left with the traditional method of sedimentation. Our study highlights the challenges of diagnosing schistosomiasis in livestock and reveals that coprological methods can produce a high proportion of false negatives and, hence, underestimate the true prevalence. With the recent discovery of *S. bovis* and *S. haematobium* hybrids in the human population of Côte d'Ivoire (Angora et al. 2019), it becomes essential to have an accurate understanding of the epidemiology of livestock schistosomiasis, not only for the economic well-being of the livestock industry but also for public health.

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Compliance with ethical standards

All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

Conflict of interest The authors declare that they have no conflict of interest.

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Chapter 6a

A review of molecular methods for genetic structure and diversity of *Schistosoma*

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Working Draft

Abstract

Highly sensitive PCR-based assays have been developed for the detection of schistosome DNA to confirm infection, infecting species and to genetically characterize schistosome populations. Despite the enormous number of people infected with *Schistosoma haematobium*, the vast majority of population genetics studies of *S. haematobium* are from Asia, with only a few from Africa. A review of the methods used and genetic patterns found in populations of *S. haematobium* is needed. We systematically reviewed the scientific literature pertaining to the methods used to study population genetic structuring and diversity of *S. haematobium* across Africa. Available electronic databases (i.e. MEDLINE/PubMed, Science Direct, Web of Science and Google Scholar) were searched and published articles from 1997 to 2020 of any language, which focused on population genetics of *S. haematobium* were included. The titles and abstracts of studies were screened for eligibility and relevant articles were read in full for inclusion in the review. Seventeen peer-reviewed publications were considered for the final analysis. These papers reported four methods used for determining population genetic structuring and diversity of *S. haematobium*, these include restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), DNA barcoding and microsatellites genotyping. Variable genetic diversity and population structure were observed within and across countries.

The current research suggests that *S. haematobium* has variable genetic diversity and structure within and across countries. Microsatellite analysis seems to be the suitable approach to assess population genetics of *S. haematobium*.

Keywords: Africa, Genetic structure, Diversity, Molecular methods, *S. haematobium*

Introduction

Helminth infections caused by nematodes, cestodes and trematodes are a significant contributor to the global burden of disease, particularly in populations from the tropics and subtropics (Hotez et al. 2008). Schistosomiasis, a neglected tropical disease according to World Health Organization, is a water-associated, chronic parasitic infection caused by a trematode worm of *Schistosoma* genus. *Schistosoma* species infect humans, wildlife and livestock (definitive hosts) as well as snails (intermediate hosts). Schistosomiasis affects more than 250 million people in the world and is estimated to cause at least 11,000 deaths per year (Knowles et al. 2017). The disease remains a great public health and socioeconomic problem in several tropical and subtropical countries (Jordan 2000; Southgate et al. 2005; Gryseels et al. 2006). According to the Global Burden of Disease (GBD) study, 1.4 million Disability-Adjusted Life Years (DALYs) were lost due to schistosomiasis in 2017 and the number of cases in the world is considered to be greater than 250 million, of which most occur in Africa (Hotez et al. 2014; GBD 2017 DALYs and HALE Collaborators 2018). Six species of schistosomes are known to infect humans: *Schistosoma mansoni*, *S. haematobium*, *S. intercalatum* and *S. guineensis* in Africa, and *S. mekongi*, as well as *S. japonicum* in Asia (Rollinson et al. 1997; Colley et al. 2014). *Schistosoma haematobium* causes the urogenital form of the disease and is endemic in sub-Saharan Africa and the Middle East. It is the most prevalent species in sub-Saharan Africa. *Schistosoma* eggs that become lodged in the tissues cause granulomatous inflammation, ulceration and squamous-cell carcinoma of the bladder, or pseudopolyposis of the vesical and ureterine walls. The common early signs include dysuria, pollakisuria, proteinuria, and especially haematuria. Chronic lesions can evolve to fibrosis or calcification of the bladder and lower ureters (van der Werf et al. 2003; Gryseels et al. 2006; Akinwale et al. 2008; Gray et al. 2011; IARC 2012).

Laboratory methods are necessary for diagnosis and treatment of schistosomiasis and are commonly used to identify eggs in feces or urine, depending on the infecting species and where the parasite resides. In the case of urogenital schistosomiasis, microscopic examination of urine samples by filtration remains the gold standard for the diagnosis (Colley et al. 2014). Molecular characterization using specific and highly sensitive PCR-based assays have been developed for the detection of schistosome DNA to confirm infection (Wichmann et al. 2009; Gomes et al. 2010; Oliveira et al. 2010; Mulero et al. 2019; Alzaylaee et al. 2020). This approach has the potential to diagnose schistosomiasis at all phases of clinical disease, including the acute (Katayama syndrome) and chronic stages of disease, and for the evaluation of treatment (Gray et al. 2011).

Two molecular markers are frequently used to identify parasites species: a nuclear marker (*ITS2*, ribosomal internal transcribed spacer 2), which is diploid, and a mitochondrial marker (*COI* or *Cox1*, cytochrome oxidase subunit I) which is haploid and is maternally inherited (Huyse et al. 2009; Boissier et al. 2016). Mitochondrial markers have a higher mutation rate than nuclear markers, and are often used for phylogenetic analyses within species. These methods and markers are now frequently used to genetically characterization parasites (Littlewood et al. 2006; Huyse et al. 2009). Molecular

methods are also commonly used to identify the *Schistosoma* species of adult worms, cercariae, eggs and miracidia (Barber et al. 2000; Huyse et al. 2013; Boissier et al. 2016). Maternal and nuclear markers are used in combination for this purpose. Prior to the advent of molecular methods, *Schistosoma* species could only be determined by egg morphology and unusual egg phenotypes led researchers to suspected that hybrids between schistosome species existed as far back the 1940s (Leger and Webster 2017). The use of molecular markers has confirmed these suspicions, with genetically verified schistosome hybrids found across sub-Saharan Africa (Leger and Webster 2017; Boon et al. 2018; Webster et al. 2019).

Despite the considerable burden of *S. haematobium*, research on the genetic diversity of this schistosome species is sparse, particularly in Africa (Gower et al. 2011). There is a need to review the methods used and genetic patterns found in populations of *S. haematobium*. Therefore, a literature review was conducted to summarize the methods used to assess genetic structure and diversity of *S. haematobium* and patterns found in Africa.

Methods

Search strategy

This systematic literature review was performed to identify all scientific studies investigating genetic structure and diversity of *S. haematobium* in Africa. The research was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al. 2009).

The following electronic databases were systematically searched: MEDLINE/PubMed, Science Direct, Web of Science and Google Scholar. The search strategy included the keywords related to schistosomiasis, genetics, variability, molecular methods and Africa. The full search strategy outlined used a combination of terms including at least the concept "schistosome" **(Table 1)** and the PRISMA checklist is provided in **Additional file 1**.

Table 1: Concepts, search terms and combinations of key words used in search strategy.

#	Concepts	Search strategy
1	Schistosome	Schistosom* OR Bilharz*
2	Genetic	Gen* OR Genom* OR Single nucleo*
3	Diversity	Divers* OR Varia* OR Polymorph* OR Distrib*
4	Technique	Techni* OR Method* OR Diagnos* OR Molecular* OR Labo*
5	Africa	Afri*
		Combinations
		1+5; 1+2+3; 1+2+5; 1+3+5; 1+2+4; 1+3+4

Publications were screened first by title and then by relevance of the abstracts. All manuscripts that met the selection criteria were included for analysis. A subsequent snowball search of the reference

lists of all full text articles that met inclusion criteria was conducted to find further relevant sources. All articles published from 1997 to 2020 were eligible for inclusion without language restrictions. Conference abstracts or book chapters detected through these databases were also considered. Studies that were conducted outside Africa, but with African samples were included, however publications focusing on non-African samples or other schistosome species were excluded. Laboratory studies without field samples were excluded, as well.

Data from the full text articles were extracted using a standardized spreadsheet. Items obtained included study date, country, type of samples, method of determining genetic structure or diversity for *S. haematobium* and major findings of genetic analyses.

Results

Search results

The literature search yielded 392 published studies, with an additional four articles from other sources (snowballing) for a set of 396 articles **(Fig. 1)**. After removing 12 duplicates, 384 articles were evaluated in more detail and from that, 337 studies were further excluded based on the relevance of the titles and abstracts.

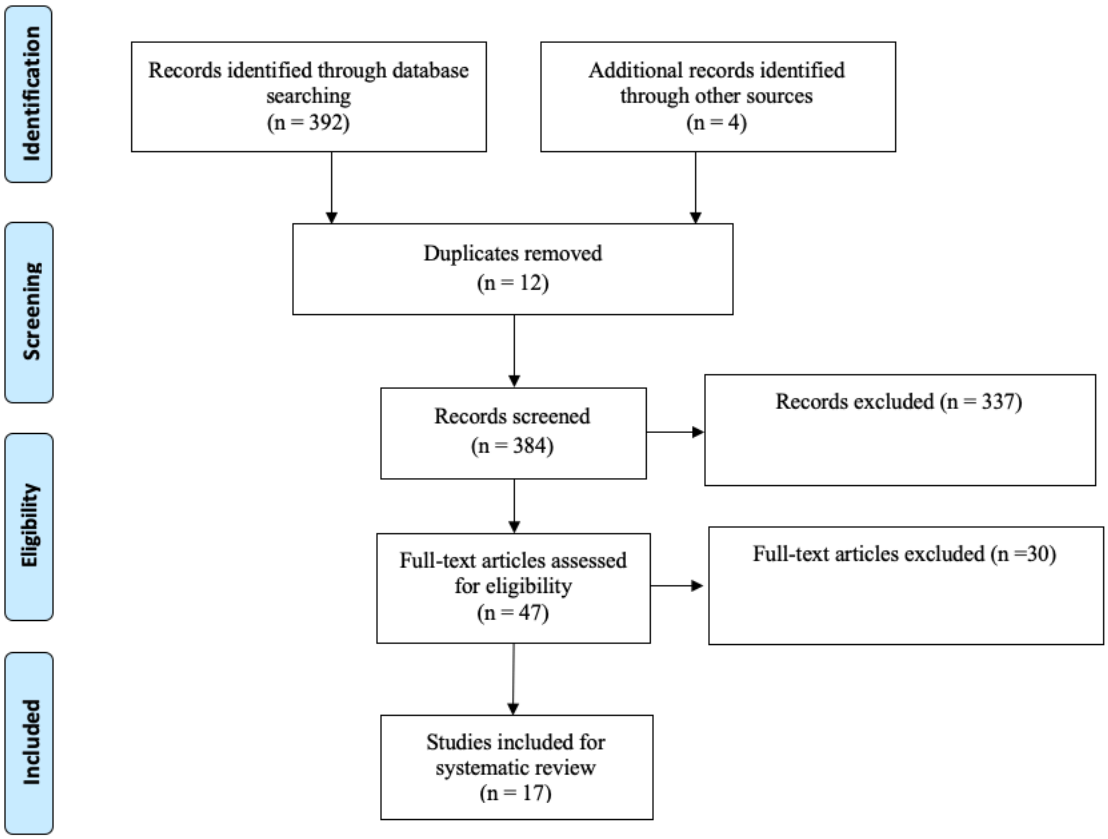

Fig. 1 PRISMA Flow Diagram of the studies reviewed.

We performed the analysis of full text on the remaining 47 studies and 30 articles were excluded because their scope did not include population genetics of *S. haematobium* in Africa, leaving 17 articles that were included in the review **(Table 2)**.

Table 2: Study locations, type and sample size, and main findings of 17 studies investigating genetic variability and population structure of *Schistosoma haematobium* across Africa, sorted by molecular methods.

Molecular methods	Reference	Country	Samples	Sample size	Main findings
Microsatellite genotyping	Boon et al. (2019)	Senegal	Miracidia, cercariae, adult worms	606 samples (529 miracidia, 13 cercariae; 64 adult worms)	*S. haematobium* was spatially more differentiated than *S. mansoni*. Strong species boundary between human and cattle schistosome was observed, despite the opportunity for hybridization.
	Ezeh et al. (2015)	Nigeria, Mali	Eggs	245	High level of genetic variability of *S. haematobium* was found between Mali and Nigeria suggesting that geographical population differentiation may occur in the regions.
	Webster et al. (2015)	Zanzibar	Miracidia and cercariae	26 samples (10 miracidia and 16 cercariae)	Higher genetic diversity was observed in Zanzibar compared to that from Niger.
	Gower et al. (2013)	Cameroun, Kenya, Niger, Mali, Tanzania, Uganda	Miracidia	2737	High level of genetic diversity of *S. haematobium* populations was found.
	Gower et al. (2011)	Mali	Miracidia, adult worms	884 samples (862 miracidia and 22 adult worms)	High genetic diversity of parasite populations was found within schools. Low genetic structure was observed.
Randomly amplified polymorphic DNA (RAPD)	Afifi et al. (2016)	Egypt, Zimbabwe and South Africa	Eggs	Eggs from three urine samples	*S. haematobium* from the three locations are genetically distinct with the possible existence of two phylogenetic groups.
	Gasmelseed et al. (2014)	Sudan	Eggs	83 urine samples	No association between genetic diversity of *S. haematobium* and the severity of the disease.
	Glenn et al. (2013)	Malawi, Nigeria, Senegal, Zanzibar	Cercariae	3703 cercariae of which 72 individual were genotyped using 15 microsatellites identified after sequencing	High genetic diversity was observed and variation between countries. Strong population genetic structure between countries was found.
	Brouwer et al. (2003)	Zimbabwe	Cercariae	133	Genetic diversity of *S. haematobium* does not influence the severity of urinary schistosomiasis in children
	Brouwer et al. (2001)	Zimbabwe	Cercariae	133	Genetic differentiation of *S. haematobium* was observed between children.
	Shiff et al. (2000)	Zimbabwe	Cercariae	1000	High degree of genetic diversity was found in *S. haematobium* from Zimbabwe
	Dabo et al. (1997)	Mali	Adult worms	414	Low genetic variation among populations compared to within populations.
Restriction fragment length polymorphism (RFLP)	Quan et al. (2015)	Sudan	Eggs	13	No genetic diversity was observed in *S. haematobium* isolated from Sudan compare to those collected in Kenya.
DNA barcoding	Angora et al. (2020)	Côte d'Ivoire	Miracidia	549	Low genetic variation of *S. haematobium* compared to *S. bovis* using *Cox1* gene
	Tian-Bi et al. (2019)	Côte d'Ivoire	Cercariae	75	Molecular data from schistosome cercariae clearly show the sympatric distribution of *S. haematobium* and *S. bovis*.
	Webster et al. (2013)	Zanzibar	Adult worms	214	High levels of parasite diversity were found across Zanzibar
	Webster et al. (2012)	East, central and west Africa	Miracidia, cercariae, adult worms	1978 samples (1869 miracidia, 46 cercariae and 241 adult worms)	Low level of diversity within *S. haematobium* across most of mainland Africa

Samples and study sites

The selected publications investigated samples from many African countries **(Fig. 2)** and employed different methods to collect samples. Six studies collected *Schistosoma* eggs (Afifi et al. 2016; Ezeh et

al. 2015; Gasmelseed et al. 2014; Quan et al. 2015) or miracidia (Angora et al. 2020; Gower et al. 2013) from urine samples, depositing and storing them on Whatman-FTA® cards (GE Healthcare Life Sciences; Amersham, UK).

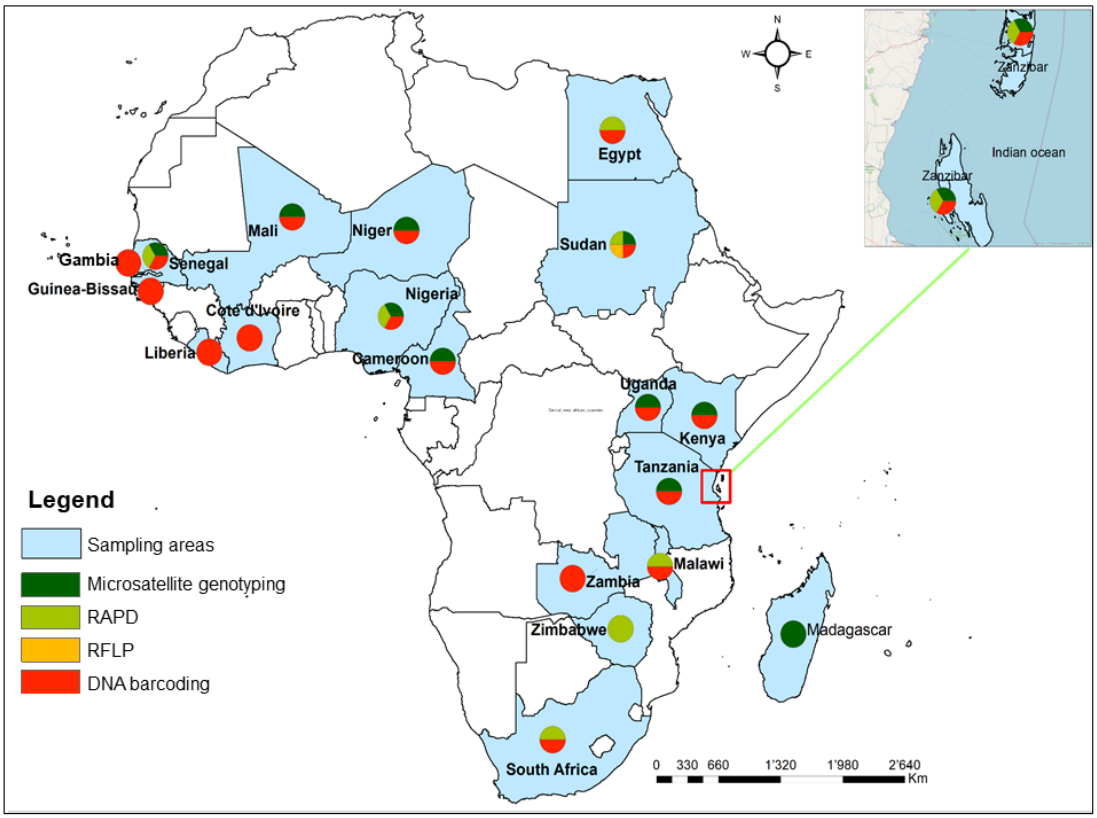

Fig. 2 Map of sampling areas of the publication included in the review and molecular methods applied. RAPD, randomly amplified polymorphic DNA; RFLP, restriction fragment length polymorphism.

Another six publications collected cercariae from snails that were infected with miracidia in the laboratory (Shiff et al. 2000; Brouwer et al. 2001, 2003; Gower et al. 2011; Webster et al. 2012; Glenn et al. 2013; Boon et al. 2019). Some studies performed genetic analyses directly on the cercariae (Shiff et al. 2000; Brouwer et al. 2001; Tian-Bi et al. 2019), while others used the cercariae to infect laboratory hamsters. Adult schistosome worms were used for genetic analysis after being collected from the mesenteric vein system of the hamsters by means of perfusion and dissection (Dabo et al. 1997; Gower et al. 2011; Webster et al. 2012, 2013; Boon et al. 2019).

Molecular methods used

Four molecular methods were used to assess the genetic diversity and population structure of *S. haematobium* in the studies included in our review: microsatellite genotyping, randomly amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and DNA barcoding (**Table 2**).

Microsatellite analyses used sets of four to eighteen markers to assess genetic diversity and population structure of *S. haematobium* within Senegal, Zanzibar and Mali (Boon et al. 2019; Webster et al. 2015; Gower et al. 2011) and across Kenya, Tanzania, Uganda, Cameroon, Niger, Mali and Nigeria (Ezeh et

al. 2015; Gower et al. 2013). This method showed high resolution in terms of genetic analysis and fingerprints of genetic patterns in the genome. A variable level of genetic diversity and population structure of *S. haematobium* were reported within and across countries.

Randomly amplified polymorphic DNA (RAPD) was the most commonly used method to analyze the genetic diversity of *S. haematobium* within Sudan, Zimbabwe, Mali (Gasmelseed et al. 2014; Brouwer et al. 2003, 2001; Shiff et al. 2000; Dabo et al. 1997) and across Egypt, Nigeria, Senegal, Zimbabwe, South Africa, Malawi and Zanzibar (Afifi et al. 2016; Glenn et al. 2013). The number of primers used for the PCR varied from four to 32 and produced at least 15 high-quality unambiguous loci. A variable genetic diversity was observed with a mean number of 32.7 genotypes. In the study of Glenn et al. (2013), 15 high quality, unambiguous microsatellite loci were identified. High genetic diversity and population genetic structure were observed across countries using this method.

Restriction fragment length polymorphism (RFLP) was employed to characterize the genetic diversity of *S. haematobium* among human hosts in Sudan. To select samples for the RFLP analysis, a multiplex PCR was performed using genomic DNA isolated from patients with microscopically confirmed urinary schistosomiasis using primers specific for two microsatellite markers of *S. haematobium* (Quan et al. 2015). In this study, RFLP used the digestion enzyme Sau3A1 that cut the internal transcribed spacer 2 region (ITS2) of *S. haematobium* in three places, producing fragments at 237, 98, 83, and 50 bp. No genetic difference was observed in *S. haematobium* isolated from Sudan compared to those collected in Kenya.

DNA barcoding was employed in four studies within Côte d'Ivoire and Zanzibar (Angora et al. 2020; Tian-Bi et al. 2019; Webster et al. 2013), and across several African countries (Webster et al. 2012). The mitochondrial cytochrome oxidase subunit I (*Cox1*), was the most commonly used marker, however the partial region of the mitochondrial NADH-dehydrogenase subunit 1 (nad1) gene was also utilized occasionally (Webster et al. 2012). In one study, molecular analysis was performed using cercariae collected from naturally infected *Bulinus* snails (Tian-Bi et al. 2019). Ninety-six *S. haematobium Cox1* haplotypes were identified in the four studies. In several countries, more than one molecular method was employed to analysis genetic patterns of *S. haematobium*. **Fig. 2** shows sampling areas and molecular methods used for analysis.

Discussion

This review aimed to provide an overview of the methods used for analysis of genetic variability and population structure of *S. haematobium* in humans across Africa and of the patterns emerging from these studies. To our knowledge, this is the first systematic literature review on this topic. Four molecular methods were used (DNA barcoding, RAPD, RFLP and microsatellite genotyping). Taken together, the available studies suggest that *S. haematobium* is genetically diverse within and across countries. A population genetic structure was consistently observed in the studies investigating patterns using microsatellite genotyping.

Microsatellite genotyping

Microsatellites or short tandem repeats (STRs) are repetitive DNA motifs ranging in length from two to six base pairs, that are tandemly repeated 5-50 times and occur at thousands of locations within the genome (Richard et al. 2008; Gulcher 2012). They have a higher mutation rate than most other genome regions, leading to high genetic diversity (Brinkmann et al. 1998). They are found in prokaryotes and eukaryotes and are used for a variety of purposes, including forensics, paternity testing, population genetics, genetic mapping, and phylogeography (Vieira et al. 2016). Five studies included in this review have employed microsatellite genotyping to assess the genetic variation and structure of *S. haematobium*, using between four to eighteen loci (Boon et al. 2019; Ezeh et al. 2015; Webster et al. 2015; Gower et al. 2013, 2011). These markers have also been used to differentiate *S. haematobium* from *S. bovis* (Djuikwo-Teukeng et al. 2019).

Variable genetic diversity and population genetic structure were observed within and across countries. Boon et al. (2019) reported significant genetic diversity indices between *S. haematobium* from humans and *S. bovis* from cattle, suggesting species boundary between human and cattle schistosomes, despite the evidence of hybridization.

Similarly, significant genetic variation of *S. haematobium* was found between parasite populations (Ezeh et al. 2015; Webster et al. 2015; Gower et al. 2011). The population structure analysis showed clustering of parasite populations within countries (Gower et al. 2013). All these findings revealed that microsatellite analyses provide opportunities to investigate many important topics. The most relevant being the relationships between parasite genetics and disease burden and consequences of various control activities on parasite populations (Norton et al. 2010). Furthermore, the potential for the circulation of strains adapted to human and reservoir hosts (Lu et al. 2010) and the likely evolution and spread of drug resistance (Webster et al. 2008) could be investigated with this method. Although the development of high-throughput single nucleotide polymorphism (SNP) platforms for genome analysis is accelerating, microsatellite markers remain highly informative measures of genomic variation for linkage and association assessment (Ott et al. 2015). Up until now, SNPs have not been developed for schistosomes, but would be a very helpful tool. They would likely also reveal further markers to distinguish between species like the known polymorphisms in the nuclear *ITS2* region that differentiate between *S. haematobium* and *S. bovis* (Angora et al. 2020; Webster et al. 2013; Huyse et al. 2009). Microsatellite genotyping remains the principal method for population genetics related to high information content, low cost, high throughput and sufficient resolution for population structure.

Randomly amplified polymorphic DNA (RAPD)

The genetic diversity analyses using this method are related to the number of unambiguous loci recovered after PCR. This method allows differentiation of both inter- and intraspecific relationships. This method requires a low amount of DNA, which is a critical issue when analyzing schistosome

miracidia or cercariae (Brouwer et al. 2001; Shiff et al. 2000). It require neither a DNA probe nor information about the genome of the organism. However, the main drawback is the segregation of dominant markers with heterozygous and homozygous dominant individuals having the same banding pattern at a locus while homozygous recessive individuals having no band at that locus (Williams et al. 1990). Limitations of the RAPD method due to a notorious inconsistency of patterns have been improved by running duplicate reactions, increasing the quantity of DNA and using high quality reagents (Shiff et al. 2000). To enhance genotype banding patterns produced by RAPD-PCR, Brouwer et al. (2003) have added an additional procedure to estimate the related groupings of the population. This provides a picture of the genetic relationships among individuals. In five studies, the extent of genetic diversity of *S. haematobium* infections was assessed by using RAPD on parasite samples to produce unambiguous loci (Afifi et al. 2016; Gasmelseed et al. 2014; Brouwer et al. 2003, 2001; Dabo et al. 1997). Glenn et al. (2013) used this method to produce microsatellite markers and compare results between populations in Senegal, Zanzibar, Malawi, Mauritius, Nigeria and South Africa. The microsatellite analysis in this study showed strong significant structuring between all sites (F_{ST} = 0.16 – 0.54, P=0.001), but especially Senegal and South Africa (F_{ST} = 0.65, P=0.001). There were no significant deviations from Hardy-Weinberg Equilibrium or significant signs of inbreeding, although samples sizes were small (between N = 10-12). Expected heterozygosity was quite high for all sites but South Africa (0.51-0.70 and 0.06, relatively). From this RAPD analyses, a variable genetic diversity of *S. haematobium* populations was reported with levels ranging from low (Dabo et al. 1997) to high (Afifi et al. 2016; Glenn et al. 2013; Brouwer et al. 2001; Shiff et al. 2000). Randomly amplified polymorphic DNA has also been used to assess the association between genetic diversity of *S. haematobium* and the severity of the disease in which no correlation was observed (Gasmelseed et al. 2014; Brouwer et al. 2003).

Restriction fragment length polymorphism (RFLP)

This method uses differences in the length of a fragment of DNA between restrictions sites to quantify genetic diversity in populations. The polymorphisms in homologous DNA causes the difference in fragment length and are used to distinguish individuals, populations, or species (Barber et al. 2000). Quan et al. (2015), used this method in combination to characterize the genetic diversity of *S. haematobium* in Sudan using the *ITS2* region. The multiplex PCR results showed that most of the samples did not produce the expected PCR band patterns for *S. haematobium* (Quan et al. 2015). The genetic patterns of *S. haematobium* were observed in Sudan similar to those from Kenya (Quan et al. 2015). This could be explained by the possible inflow of a Kenyan strain into Sudan in spite of the geographical distance and likely the lack of sensitivity of this method.

DNA barcoding

DNA barcoding is a method that identifies organisms by matching a standardized genomic DNA fragments from a reference sequence library (Kress et al. 2015). Four publications in our review used this approach to document the genetic variation of *S. haematobium* from several geographical areas using historical collections from across Africa (Angora et al. 2020; Tian-Bi et al. 2019; Webster et al. 2012) and from Zanzibar Island (Webster et al. 2013). Different gene regions are used to identify the different species. The most commonly used barcode regions are the cytochrome C oxidase subunit 1 (abbreviated *COI* or *Cox1*) and the NADH-dehydrogenase subunit 1 (*Nad1*), both found in mitochondrial DNA (Standley et al. 2010). The nuclear internal transcribed spacer (*ITS*) is another gene region suitable for DNA barcoding (Kress et al. 2015). The haplotype diversity analysis showed that *S. haematobium* was less polymorphic than *S. bovis* (Angora et al. 2020; Webster et al. 2013). This variation in mitochondrial DNA (mtDNA) is commonly used in systematics to infer species phylogenies and sub-species ancestry (Webster et al. 2006). The DNA barcoding approach is useful to access haplotype diversity and to infer species identification and phylogeny. This would be feasible by identifying genetic distance limit values within two individuals belonging to the same species. However, the strong limitations of this method reside particularly in defining species boundaries (Witt et al. 2006).

Conclusions

The current systematic review elucidated that *S. haematobium* has variable genetic diversity and population structure within and across African countries using molecular analyses. Microsatellite analysis seems to be the suitable approach to assess population genetics of *S. haematobium*. From the perspective of schistosomiasis elimination in Africa, it is important to better identify and understand the challenges that actually affect control interventions. However, the deployment of any intervention must be accompanied by diagnostic activities involving the use of molecular methods to monitor the success of the intervention.

Abbreviations

DALYs: disability-adjusted life years; GBD: Global Burden of Disease; PCR: Polymerase chain reaction; RAPD: Randomly amplified polymorphism DNA; RFLP: Restriction fragment length polymorphism; SNP: Single nucleotide polymorphism.

Declarations

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Conflict of interest: The authors declare that they have no conflict of interest.

Ethics approval: not applicable

Consent to participate: not applicable

Consent for publication: not applicable

Availability of data and material: not applicable

Code availability: not applicable

Authors' contributions: EKA conducted the systematic review of the literature, extracted and analyzed data, and drafted the first version of the manuscript. HM, JB, JU and OB revised the manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflicts of Interest

The authors declare that they have no competing interests.

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Supplementary file 1: Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) checklist.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3, 4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5, 6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	N/A
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	N/A
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	N/A
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	N/A
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	7, 8
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	N/A
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	N/A
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	N/A
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	N/A
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	N/A
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	9
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	9 to 12
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	13
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	N/A

Chapter 6b

Genetic characterization of *Schistosoma* species from cattle across Côte d'Ivoire

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Working Draft

Abstract

Schistosomiasis is a parasitic disease that affects both humans and livestock. Considerable resources are dedicated to the surveillance, disease mapping, control and prevention of human schistosomiasis in endemic areas, including Africa. However, this is not the case for livestock schistosomiasis. There is a lack of data concerning the species present, prevalence, and population genetic diversity and structuring in livestock and a general indifference or neglect for prevention and control of livestock schistosomiasis. We aimed to identify schistosome species found in cattle across Côte d'Ivoire using molecular methods and to describe population diversity and structuring using microsatellites. We collected 400 flukes from slaughtered cattle at six sites across Côte d'Ivoire and 114 miracidia from live cattle at one site, Ferkessédougou. All flukes and miracidia had *Schistosoma bovis* or *S. curassoni* genotypes in the mitochondrial *cox1* gene. Of the 101 flukes and 4 miracidia further subjected to nuclear *ITS1/2* sequencing, all were consistent with the pattern for *S. bovis*. Genetic diversity indices revealed a deficiency of heterozygotes and signals of inbreeding across all sites, while structure analyses displayed little geographical structuring and differentiation. Cattle in Côte d'Ivoire appear to be infected with only *S. bovis.* Hybrids of *S. haematobium* x *S. bovis* seem not to be present in Ivorian cattle, despite their presence in humans. Cattle schistosomes appear to be panmictic across the country. The WHO neglected tropical diseases (NTD) roadmap identifies a One Health approach as an important strategy for controlling NTDs, including schistosomiasis, in humans. These results contribute to our understanding of schistosome populations in Ivorian cattle and emphasize the need for surveillance, prevention and control programmes for livestock schistosomiasis.

Introduction

Schistosoma, a genus of trematodal parasites, causes the neglected tropical disease (NTD) schistosomiasis. There are over 20 known species of schistosomes, some of which are zoonotic, infecting a wide range of definitive hosts from livestock and wildlife to humans (Standley et al. 2012b). The occurrence of specific species is focal and depends on the presence of their respective definitive and intermediate hosts.

There are two species endemic in humans in Africa - *S. haematobium* (urogenital) and *S. mansoni* (intestinal) (Weerakoon et al. 2015) and three occurring in livestock, *S. bovis*, *S. curassoni*, *S. mattheii* (all intestinal) (Standley et al. 2012a; Leger and Webster 2017). Contrary to the situation in humans, the exact infecting species of cattle are usually not known or are merely presumed based on egg morphology or geography. However, this can be imprecise, especially in the presence of hybrids of *Schistosoma* species. The available data indicates that *S. bovis* occurs throughout Africa, but mostly north of 10 degrees south. South of this latitude it is replaced by *S. mattheei,* while *S. curassoni* is located sympatrically with *S. bovis* in some parts of West Africa (Standley et al. 2012a). Natural hybridization within and between human and animal schistosomes has been suspected since the 1940s and more recently evidence of this phenomenon has been well documented in the literature (Léger et

al. 2016). Recently, hybrids of the livestock species *S. bovis* and the human species *S. haematobium* have been documented in Africa.

Ninety percent of all human schistosomiasis cases occur in sub-Saharan Africa, and thanks to a focus on control and prevention by the WHO, is well mapped epidemiologically (WHO, 2017). Contrarily, few resources are dedicated to the epidemiology, control and prevention of livestock and wildlife schistosomiasis, although it is thought to be common in African cattle, and therefore, little is known. Available data estimates that prevalences in animals ranges between 31 and 81% in Africa (De Bont and Vercruysse 1997). More recent data from Cameroon found schistosomiasis in 19.5% of slaughtered cattle (Djuikwo-Teukeng et al. 2019). In Senegal, Léger et al. (2020) found *S. bovis* (confirmed by molecular methods) in 92% of cattle, 14% of sheep and 15% of goats. In the same investigation *S. curassoni* prevalences were found to be 8% in cattle, 73% in sheep and 84% in goats. The average prevalence of schistosomiasis across Côte d'Ivoire in slaughtered cattle, sheep and goats was estimated to be 22.5%, 1.3% and 1.2%, respectively (Kouadio et al. 2020). Despite high prevalences in livestock and the identification of zoonotic disease management as part of the prevention strategy in the current NTD roadmap, there are currently no schistosomiasis control programs for livestock in Africa (Gower et al. 2017; WHO 2020).

Hybrids between a variety of *Schistosoma* species have been reported in humans, snails and wildlife in Cameroon, Côte d'Ivoire, Corsica, Kenya, Mali, Niger, Senegal, South Africa, Tanzania and Zambia (Leger and Webster 2017; Boon et al. 2019; Tian-Bi et al. 2019; Angora et al. 2020). A recent outbreak of schistosomiasis in Europe (Corsica, France), where schistosomiasis had not been reported before, involved patients who were infected with *S. haematobium*, *S. haematobium* x *S. bovis* and *S. bovis* of Senegalese origin (Boissier et al. 2016). This highlights the mobility and potential for geographical expansion of these parasites. Furthermore, a case of human infection with a hybridized *S. bovis* x *S. curassoni* miracidium in Niger has been reported (Léger et al. 2016).

Hybridization of animal and human schistosomes further threaten human schistosomiasis control programs.

There is a paucity of data concerning schistosome hybrids in livestock, especially when compared to humans. This may be due to the fact that livestock schistosomiasis is often overlooked by global health institutions. Epidemiological investigations that use molecular species identification (necessary for identifying hybrids) of livestock schistosomiasis are not often carried out. Nevertheless, hybrids in cattle have been investigated in Benin, Cameroon and Senegal. In Cameroon (Djuikwo-Teukeng et al. 2019) and Senegal (Boon et al. 2019; Léger et al. 2020) no hybrids were found, but there is a report of a *S. bovis* x *S. haematobium* hybrid in Benin (Savassi et al. 2020).

Hybrids are concerning for a number of reasons. They complicate the diagnosis of schistosomiasis in two ways: i) diagnosis is often made by means of coprology or uroscopy. Given that different schistosome species inhabit different anatomical sites of the host, hybrids may reside at sites not commonly occupied by schistosomes in those hosts, and therefore have the potential to be missed or

assigned to the wrong species and ii) hybridization affects egg morphology, making species identification challenging (Rollinson et al. 1990; Savassi et al. 2020). Lastly, some hybrids have been shown in experiments to demonstrate hybrid vigor, based on newly acquired advantageous traits such as increased virulence or transmission and host range expansion (Leger and Webster 2017).

The lack of understanding concerning the epidemiology of wildlife and livestock schistosomiasis and the role of zoonotic interactions in the human schistosomiasis transmission cycle is troubling. Zoonotic reservoirs are an important component of the transmission cycle as they can harbor parasites and perpetuate transmission cycles, threatening control efforts and costly gains made by human schistosomiasis control programs (Léger et al. 2016; Webster et al. 2016). Furthermore, zoonotic reservoirs can influence parasite evolution by providing opportunities for host switching or genetic exchange which can lead to novel genetic combinations (Webster et al. 2016). To ignore them is to ignore a significant factor of the transmission equation.

Genetic diversity, differentiation and structuring can reveal transmission dynamics and patterns that could impact the spread of advantageous gene variants and improve our understanding of biology and options for preventive interventions. Genetic diversity and structuring have been investigated in *S. mansoni* and *S. haematobium* (Gower et al. 2013), but we are aware of only two investigations into *S. bovis* population genetics in Africa to date (Boon et al. 2019; Djuikwo-Teukeng et al. 2019*)* and none on *S. curassoni*.

This study aimed to clarify basic population genetic patterns in *Schistosoma* species from cattle in Côte d'Ivoire. In particular, we used a *cox1*-based diagnostic PCR to unequivocally determine the *Schistosoma* species and microsatellite analysis to investigate the geographical population structure and genetic differentiation between male and female *Schistosoma* flukes across Côte d'Ivoire. Microsatellites were used as they have many alleles and a high mutation rate leading to high levels of informative variation that result in high resolution (Griffiths et al. 2015). Microsatellite analysis was further used on samples from one focal region (Ferkessédougou) to assess in detail how genetic diversity across the host population compares to that within individual hosts and if flukes from slaughtered cattle yield the same patterns as miracidia collected from live animals.

Materials & Methods

Study sites

This investigation took place in Côte d'Ivoire, a West African country of almost 25 million people and an estimated 1.34 million cattle in 1988 (Food & Agriculture Organization of the United Nations and Livestock Information, Sector Analysis and Policy Branch 2005; World Bank 2019). Côte d'Ivoire is located on the coast of the Gulf of Guinea and has three ecozones: south and west parts with abundant rainfall and forest, the northern region with savannah and less rainfall and the northwestern part with savannah with more rainfall than the north (Yapi et al. 2014).

This study involved sampling cattle at six sites across Côte d'Ivoire in August 2018 and July 2019 (Fig. 1). Three sites were located in the South-East region of the country, one site in the West and two sites in the North-Central region. Sampling of adult parasite flukes was performed from slaughtered cattle in abattoirs at all sites. Additionally, in Ferkessédougou parasite miracidia were sampled from live cattle on farms to compare population genetic patterns revealed by sampling of the different host populations and parasite live-stages.

Figure 1. Map of Côte d'Ivoire, West Africa. Red circles indicate abattoir sites sampled for *Schistosoma* flukes from slaughtered cattle. In Ferkessédougou (purple triangle) both slaughtered cattle from abattoirs and live cattle on farms were sampled for flukes and miracidia, respectively.

Specimen collection and storage

At all six abattoir sites, the mesenteric veins of slaughtered cattle were examined for *Schistosoma* flukes. If present, flukes were excised from the mesenteric veins, rinsed in phosphate buffered saline (PBS), the male and female flukes were separated and stored individually in 95% EtOH or in 190 µl lysis solution (0.1 M Tris-HCl, 1% sodium dodecyl sulfate) and 10 µl of [20 µg/µl] proteinase K at room temperature before being shipped to Switzerland. After flukes in lysis solution appeared completely lysed (approximately 2-3 days), 200 µl of TE buffer (10 mM Tris and 1 mM EDTA) was added.

On farms, feces were manually extracted from the rectums of cattle during two fieldwork expeditions in August 2018 and July 2019 and transported to the Laboratoire National d'Appui au Développement Agricole (LANADA) laboratory in Korhogo or the laboratory of the Ministère des Ressources Animales et Halieutiques (MIRAH) in Ferkessédougou for filtration. A walnut-size portion of feces

was washed, filtered and scraped with a wooden spatula through a drum filter (400 µm mesh size) with locally purchased bottled water. The filtrate was further filtered through a Pitchford filter (Visser and Pitchford 1972) with an inner sieve mesh of 300 µm and an outer sieve mesh of 30 µm as described elsewhere (Giovanoli Evack et al. 2020). The filtrate was drained into a Petri dish which was left in a semi-shady area for 1 hour to encourage *Schistosoma* eggs to hatch. Then Petri dishes were examined under 10x magnification for miracidia and again every hour for 4 hours. Miracidia were individually collected with a pipette in 5 µl of water, deposited on a Whatman Indicating FTA card (GE Healthcare Life Sciences, Buckinghamshire, UK) and left to dry for one hour.
Additionally, at the Ferkessédougou abattoir, five bladders were collected from the carcasses of cattle because hybrids with other species could be excreted via urine. The urogenital veins were inspected for *Schistosoma* flukes and each urine was filtered through a 30 µm filter with locally purchased bottled water into a Petri dish. The Petri-dishes were treated as described above for the collection of miracidia.

DNA extraction

In Switzerland, flukes stored in 95% EtOH were rinsed in PBS, and then deposited in 190 µl of lysis solution with 10 µl of [20 µg/µl] Proteinase K, as described above, and incubated at 56 °C with agitation for one hour. DNeasy blood and tissue column kits (Qiagen, Hilden, Germany) were used for DNA extraction of all fluke samples. The manufacture's protocol was followed with the exception of the lysis solution.
DNA of miracidia was extracted using a Chelex® protocol (Bio-Rad Laboratories, Cressier, Switzerland). A 3 mm Harris Uni-core micro punch was used to cut out the indicated spot where the miracidia was deposited on the FTA card. The punch was placed in a 1.5 ml Eppendorf tube with 100 µl of ultra-pure water and left to sit at room temperature for 10 minutes. The water was then removed with a pipette and the punch rinsed once more as just described. Afterwards, 80 µl of 5% Chelex solution was added (Chelex solution was mixed on a magnetic agitator to allow distribution of Chelex beads) and incubated at 65 °C with agitation for at least 30 minutes. The temperature was increased to 99 °C for a final eight minutes, after which the solution was centrifuged at 13,000 rotations per minute (rpm) for 2 minutes. Finally, 60 µl of the DNA-containing supernatant was pipetted into a new Eppendorf with 5 µl of Low TE buffer (10 mM Tris and 0.1 mM EDTA) and stored at -20 °C.

PCR species determination

A multiplex *cox1* polymerase chain reaction (PCR) was used to determine species by band length (*S. haematobium* 120 base pairs (bp)*; S. mansoni* 215 bp; *S. bovis* and *S. curassoni* 260 bp). Partial *cox1* was amplified in a 10 µl PCR containing 0.5 µl DNA, 5.0 µl of Qiagen Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 3.5 µl distilled water, 1µl of primer mix (4 µl of each primer (100 µM) in

84 µl double-distilled water; three forward primers: Sh.F 5'-GGTCTCGTGTATGAGATCCTATAGTTTG -3'; Sm.F 5'- CTTTGATTCGTTAACTGGAGTG -3'; Sb.F 5'- GTTTAGGTAGTGTAGTTTGGGCTCAC -3' and one reverse primer: Shmb.R R5'-CAAGTATCATGAAAYARTATRTCTAA -3'). The PCR was initiated by 15 min at 95 °C; followed by 45 cycles of 94 °C for 10 secs, 52 °C for 30 secs and 72 °C for 10 secs and a final extension of 72 °C for 2 mins.

The PCR products were loaded onto a 2% agarose gel (Promega, Madison, Wisconsin) with 0.5X Tris-borate-EDTA (TBE) (Thermo Scientific, Waltham, Massachusetts) and run for 25 mins at 75 V. Agarose gels were soaked in GelRed (Merck KGaA, Darmstadt, Germany) for at least 30 minutes before being visualized.

A random selection of specimens was further selected for PCR and sequencing of the *ITS1/2* locus. Sequences from the *ITS1/2* marker were used to confirm species by using five variable sites known to distinguish *S. haematobium* from *S. bovis*, one of which also distinguishes *S. bovis* from *S. curassoni* (Huyse et al. 2009; Webster et al. 2013).

The *ITS1/2* locus was amplified in a total volume of 10 µl consisting of 1 µl BD 10X buffer (Solis BioDyne, Tartu, Estonia), 1.3 µl of 25mM $MgCl_2$, 0.8 µl of 10mM dNTP mix (2.5 mM of each dNTP), 0.1 µl of 5U/ml FirePol® Taq polymerase (Solis BioDyne, Tartu, Estonia), 0.5 µl DNA, 5.3 µl water and 0.5 µl of each forward (ITS5: 5'- GGAAGTAAAAGTCGTAACAAG -3') and reverse primers (ITS4: 5'- TCCTCCGCTTATTGATATGC -3'). The PCR protocol was initiated with 4 mins at 94 °C, followed by 40 cycles of 30 secs at 94 °C, 30 secs at 54 °C and 30 secs at 72 °C and a final extension of 2 mins at 72 °C. PCR products were sent to Microsynth (Balgach, Switzerland) for uni-directional Sanger sequencing using the reverse primer.

ITS1/2 sequences were compared to other sequences in GenBank using the Basic Local Alignment Search Tool (BLAST) and then aligned and analyzed in CodonCode Aligner version 6.0.2 (CodonCode Corporation, Centerville Massachusetts). *ITS1/2* trace files were inspected to determine heterozygosity at the five variable sites.

Microsatellite analysis

A sub-selection of specimens (Tab S1) from all sites were analyzed using two panels of microsatellites (Webster et al. 2015). These microsatellites were developed for *S. haematobium*, a sister species of *S. bovis*, and therefore some loci produced poor results (C102, Shae_14, C131, Shae_07, Shae_10, Shae_08) and were removed from the analysis (Tab 1). Loci Shae_05 and Shae_15 were further omitted from analyses that included miracidia, because they consistently produced poor results with miracidia DNA.

The protocol for these two multiplex PCRs were the same: 5 µl Qiagen Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 1 µl DNA, 3 µl double-distilled water and 1 µl of 10 µM primer mix. The

PCR protocol was initiated by 15 mins at 95 °C, followed by 45 cycles of 94 °C for 30 seconds, 56 degrees Celsius for 1.5 mins and 72 °C for 1 min and a final extension of 72 °C for 5 minutes. PCR products were sent to Microsynth (Balgach, Switzerland) for fragment length analysis using the Applied Biosystems 3730xl DNA Analyzer with size standard GS500LIZ.

The trace files were imported into Genemapper (v. 6.0, ThermoFisher Scientific), automatic bin calling was established based on the samples, and all automatically called peaks were confirmed at least twice by visual inspection. We experienced some difficulties distinguishing the fluorescent dyes as there was leakage between green, blue and yellow, which proved difficult for peak calling when allele ranges between colors overlapped. Any peaks that were questionable were thus amplified and sequenced again to confirm the call.

Table 1. *Schistosoma* microsatellite panels, loci used, allele size ranges (base pairs) and fluorescent dye. Loci Shae_05 and Shae_15 were omitted from analyses including miracidia.

Panel	Locus	Allele range	Fluorescent dye
Panel 1	C111	185-209	ATTO565 (red)
	Shae_01	240-282	HEX (green)
	Shae_03	291-375	FAM (blue)
	Shae_06	305-331	ATTO550 (yellow)
	Shae_09	190-241	FAM (blue)
Panel 2	Shae_02	152-224	ATTO550 (yellow)
	Shae_04	264-313	FAM (blue)
	Shae_05	261-291	ATTO550 (yellow)
	Shae_11	172-209	HEX (green)
	Shae_12	241-262	ATTO565 (red)
	Shae_13	163-229	FAM (blue)
	Shae_15	270-297	HEX (green)

Genetic diversity measures

Rarified allelic richness (A_r) and fixation index (F_{IS}) with 95%-confidence intervals (bootstrapped with 999 repetitions) were calculated using the function *basicStats* from the package diveRsity, version 1.9.90 (Keenan et al. 2013) in R v. 4.0.2 (R Core Team 2013). Observed (H_o) and expected heterozygosity (H_e) with 95%-confidence intervals (bootstrapped with 1000 repetitions) as well as mean, minimum, median, and maximum number of alleles (A) across loci were calculated using functions *popgen* and *bootstrapHet* from the package PopGenKit, version 1.0 (Rioux Paquette 2012) in R v. 4.0.2 (R Core Team 2013). Where not otherwise stated, statistical significance was determined by comparing the 95% confidence intervals, deeming any overlap of confidence intervals arms of 50% or less as significant as suggested by (Cumming et al. 2007). Probability of deviation from Hardy-Weinberg equilibrium (P_{HWE}) was calculated in GENEPOP v. 4.7.5 (Rousset 2008) using option 1, suboption 3.

Genotypic differentiation

Differentiation was measured by F_{ST} and R_{ST} calculated in RStudio with the package GENEPOP, v.1.1.7 (Rousset 2008). Due to the very low values of F_{ST} and R_{ST} in all analyses we did not calculate and report statistical significance values.

Population structure

Population structure analysis was completed in STRUCTURE v. 2.3.4 (Pritchard et al. 2000) using the automation implemented in STRAUTO v. 1 (Chhatre and Emerson 2017). All analyses were run with a burn-in of 50,000 and 500,000 MCMC iterations after burn-in. Each K from 1 to 16 was run 10 times, and then the best K determined by the Evanno method in STRUCTURE HARVESTER v. 0.6.93 (Earl and vonHoldt 2012). The results of the 10 runs at the best K were averaged in CLUMPP V. 1.1.2 (Jakobsson and Rosenberg 2007) and used to produce plots visualizing the contribution of each genetic cluster to the genome make-up of each individual parasite. Discriminant analysis of principal components (DAPC) was performed, and results plotted in RSTUDIO using the package adegenet v. 2.1.3 (Jombart 2008; R Core Team 2013). Lastly, principal components analysis (PCA) was performed using GENETIX v. 4.05.2(Standley et al., 2012) (Belkhir et al. 1996) and results plotted using STATA v. 15.1 (StataCorp LLC, Texas, USA).

Resampling strategy to eliminate potential biases

Using multiple parasite samples from one host individual may introduce biases due to non-independence of the samples. This is especially the case for miracidia because numerous offspring (siblings) from the same couple may be sampled, which are clearly not independent from one another. Therefore, in some analyses, a sub-sample of one *Schistosoma* couple (flukes) or of one miracidium from each host was randomly selected and the genetic diversity indices were calculated on this set of fewer samples. This random selection of a sub-sample was performed 100 times and indices averaged over the 100 runs. This approach results in less power due to reduced sample size but removes one (unavoidable) potential source of bias and was applied to verify the findings of the analysis with the full data set.

Analysis 1: Molecular Schistosoma *species determination*

First, the species of all parasites sampled was determined by molecular tools. All 514 samples (400 flukes and 114 miracidia) used in this study were included in this analysis.

Analysis 2: Geographical population structure of flukes across Côte d'Ivoire

To compare genetic diversity and investigate geographical differentiation across the country we analyzed microsatellite data from a subsample of the 400 flukes. To maximize data independence, we

maximized the number of hosts analyzed per sampling site but analyzed only one couple (i.e. paired male/female) per host individual.

Analysis 3: Genetic differences between male and female schistosome flukes

Our sampling scheme allowed for a highly controlled comparison of the genetic diversity and of population genetic patterns of male and female parasites across the country. This is important for theoretical reasons because in many species the sexes' ecologies differ, although this is not to be expected for *Schistosoma* species because of their very limited possibilities to modulate their dispersal. It is also important for practical reasons because an absence of differences means that male and female parasites can be freely combined in population genetic analyses. For this analysis, parasites from all locations were used that were collected either as couples or (in few cases) as the only parasite from one host. Since the focus of this analysis was the comparison between sexes, we included more than one couple per host individual where available, resulting in a higher sample size compared to analysis 2. Couples were split at the time of collection.

Analysis 4: Comparison of within and between-host genetic diversity in flukes

The fourth analysis compared within-host to between-host genetic diversity using flukes from slaughtered cattle in Ferkessédougou to assess if analyses based on many parasites from a few hosts are representative for the population across host individuals. This is important to know because it is often much easier (and therefore often applied) to sample many parasites from a few host individuals but this risks to miss important components of diversity that are visible only across the host population. Within-host diversity was assessed in the "within group" consisting of three slaughtered cattle (named "W1", "W2", "W3"), from which 19, 20 and 22 adult flukes were analyzed, respectively. Most flukes were coupled males and females that were separated for the analysis, the remainder were uncoupled flukes. Between-host diversity was assessed in one "between group" (named "B"), which contained few parasites per host but from a large number of cattle. Specifically, the "between group" consisted of 84 flukes from 39 cows: four singleton flukes (one per host) from four slaughtered cattle, 33 couples (66 flukes total) from 33 slaughtered cattle (one couple per host) and another 14 flukes from two slaughtered cattle (8 flukes from one and 6 from the other). For statistical analysis, ten rounds of random subsampling were performed. In each subsampling round, two groups of 10 flukes were randomly drawn without replacement from each of the three "within" cattle and four groups of 10 flukes were drawn from the "between group" following these rules: in the "within" cattle, the flukes were randomly assigned to two groups of ten. In the cow with 22 flukes, two were randomly excluded at the start of any of the 10 rounds. In the cow with 19 flukes, one fluke was randomly drawn twice. In the "between group", in every round, first four flukes were randomly excluded (3 from the cow with 8 flukes, 1 from the cow with 6 flukes) and then the remaining 40

flukes were randomly assigned to 4 groups of 10 flukes. In the end, this procedure resulted in 20 randomly drawn groups of 10 flukes for each of the three "within-cattle" and in 40 randomly drawn groups of 10 flukes for the "between group". Diversity measures were calculated for each group of 10 flukes and differences between the "between group" and the three cattle of the "within group" were assessed by a linear model using function *lm* in R v. 4.0.2 (R Core Team 2013).

Analysis 5: Genetic differentiation between flukes and miracidia in Ferkessédougou

Lastly, genetic diversity and population genetic patterns were compared between flukes and miracidia to assess if analysis of the two life-stages yields comparable (and combinable) results and if one can be substituted for the other. This analysis was performed in Ferkessédougou in two ways. In the first analysis genetic indices and population structure of 30 miracidia obtained from 30 live cattle (one miracidium per host) were compared to 80 flukes obtained from 42 slaughtered cattle (one couple or one singleton from different hosts) to assure complete independence of the parasite samples. In the second analysis, we investigated the amount of genetic diversity occurring within hosts, specifically if within-host diversity differed between slaughtered cattle (flukes) and live cattle (miracidia). For this analysis, samples from much fewer host individuals, but more samples per host (22, 20, and 19 flukes from three slaughtered hosts and 17, 16, 11, 10, and 10 miracidia from five live cattle), were used. The purpose of comparing these two approaches was to assess if samples obtained from only one or a few host animals (which are much easier to obtain in the field) give results comparable to a sampling across many hosts (which gives more independent samples but is difficult in the field) and can thus be used to represent the overall local parasite population. In this analysis, we used the mean index values for each host individual to determine significant differences between the two approaches using linear models, i.e. function *lm* in R v. 4.0.2 (R Core Team 2013).

Ethical considerations

This study was part of larger project that investigated transmission dynamics of trematodes in humans and livestock in Côte d'Ivoire. Ethical approval was obtained from the National Ethics and Research Committees of Côte d'Ivoire (reference no. 035/MSH /CNER-kp) and Switzerland (reference no. UBE-2016-00707). The "Direction des Services Vétérinaires" of the "Ministère des Ressources Animales et Halieutiques en Côte d'Ivoire" gave authorization to conduct the research. The objectives and methods of the study were explained to farmers, who then signed an informed consent to have their livestock sampled.

Results

Molecular Schistosoma *species determination*

A total of 400 flukes from slaughtered cattle across the six sites (Fig. 1) and 114 miracidia from live cattle in Ferkessédougou were collected and their DNA extracted (Tab S1). Three hundred and seventy-one flukes (93%) and 101 miracidia (89%) produced bands in the multiplex *cox1* PCR, all of which were 260 bp in length, indicating that all flukes and miracidia had mt DNA of *S. bovis* or *S. curassoni*.

Of the 107 flukes subjected to *IT*S1/2 PCR, 101 (94%) produced sequences (GenBank accession numbers: xxxx). All were consistent with the pattern for *S. bovis* at the variable sites and no heterozygotes were found. Of the 34 miracidia from cattle that were subjected to the same PCR, only four produced sequences and they too were consistent with the pattern for *S. bovis*. In particular, at the first variable site, which can distinguish *S. bovis* from *S. curassoni*, all flukes and miracidia were consistent with the pattern expected for *S. bovis*.

None of the bladders or urine collected from slaughtered cattle in Ferkessédougou harbored *Schistosoma* flukes or eggs.

Geographical population structure of flukes across Côte d'Ivoire

The geographical analysis explored genetic diversity at different locations and structuring between the locations. A total of 146 flukes from 76 hosts across the six sites were included in the analysis (Tab. 2).

All sites showed high levels of genetic variability as measured by A_r, H_o and H_e. Values were comparable across all sites, with the exception of Abengourou, but this was likely due to low sample size at this site. H_e exhibited highly skewed confidence intervals because it approached or reached 1.0 in several loci in all sites, preventing a meaningful interpretation of the statistical significance of differences between the groups. Significant deviation from Hardy-Weinberg equilibrium and high levels of inbreeding were apparent at all six sites, but again with little variation between sites.

Table 2. Genetic diversity indices for *Schistosoma* flukes from cattle at six locations across Côte d'Ivoire based on 12 microsatellite loci. N, number of fluke samples (maximally two per host individual); N_H, number of host individuals from which flukes were collected; A, average (minimum, maximum) number of alleles across loci; A_r, mean (+95% CI) allelic richness across loci; H_o, mean (+95% CI) observed heterozygosity across loci; H_e, mean (+95% CI) estimated heterozygosity across loci; P_{HWE}, probability of deviation from Hardy-Weinberg Equilibrium; F_{IS}, mean (+95% CI) fixation index across loci.

Location	N	N_H	A	A_r	H_o	H_e	P_{HWE}	F_{IS}
Ouangolodougou	21	11	9.92 (3, 18)	4.78 (3.75-5.58)	0.556 (0.484-0.619)	0.754 (0.679-0.773)	$2.7*10^{-12}$	0.238 (0.129-0.308)
Ferkessédougou	80	42	11.25 (4, 22)	5.00 (4.33-5.58)	0.574 (0.543-0.598)	0.725 (0.703-0.73)	$1.2*10^{-15}$	0.222 (0.178-0.255)
Abengourou	6	3	4.92 (2, 11)	3.88 (3.00-4.58)	0.528 (0.444-0.611)	0.644 (0.468-0.650)	$1.9*10^{-2}$	0.185 (-0.042-0.224)
Duekoué	14	7	7.25 (2, 14)	4.46 (3.33-5.42)	0.527 (0.468-0.577)	0.679 (0.599-0.687)	$1.1*10^{-8}$	0.248 (0.135-0.292)
Agboville	14	7	8.50 (3, 14)	4.97 (3.67-5.92)	0.600 (0.542-0.652)	0.744 (0.671-0.737)	$2.1*10^{-7}$	0.199 (0.055-0.248)
Sikensi	11	6	6.00 (2, 11)	4.15 (3.33-4.83)	0.542 (0.472-0.601)	0.674 (0.578-0.677)	$1.8*10^{-6}$	0.24 (0.054-0.295)

All F_{ST} and R_{ST} values between geographical locations were close to zero (highest F_{ST}, 0.022; highest R_{ST}, 0.055), indicating no or very little differentiation between populations (not shown). Likewise, STRUCTURE analysis revealed no structuring between the six locations (Fig. 2). Evanno's method suggested three genetic clusters (K = 3) (Fig. S1) with very similar relative representation in the parasites' genomes in all six sites. The only sites that could be said to show a somewhat different genetic make-up were Agboville and Sikensi, which may have, respectively, a weaker or stronger contribution of the genetic cluster depicted in blue in Fig. 2.

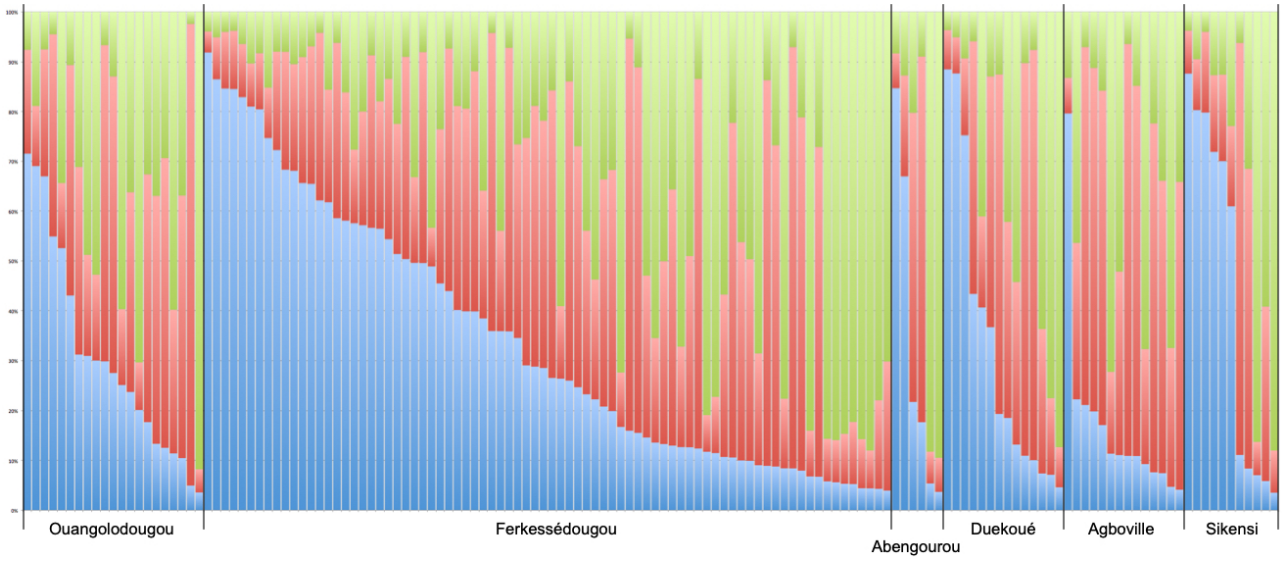

Figure 2. Plot of STRUCTURE analysis of schistosomes flukes from cattle across Côte d'Ivoire. Each column represents one fluke with the colors indicating the relative contribution of the three genetic clusters (K=3) to the parasite's genome.

The DAPC detected no differentiation between locations (Fig. 3a). PCA indicated some degree of differentiation between some schistosomes from Agboville, Duekoué and Ouangolodougou, while populations from Ferkessédougou, Abengourou and Sikensi appeared to be indistinguishable (Fig. 3b).

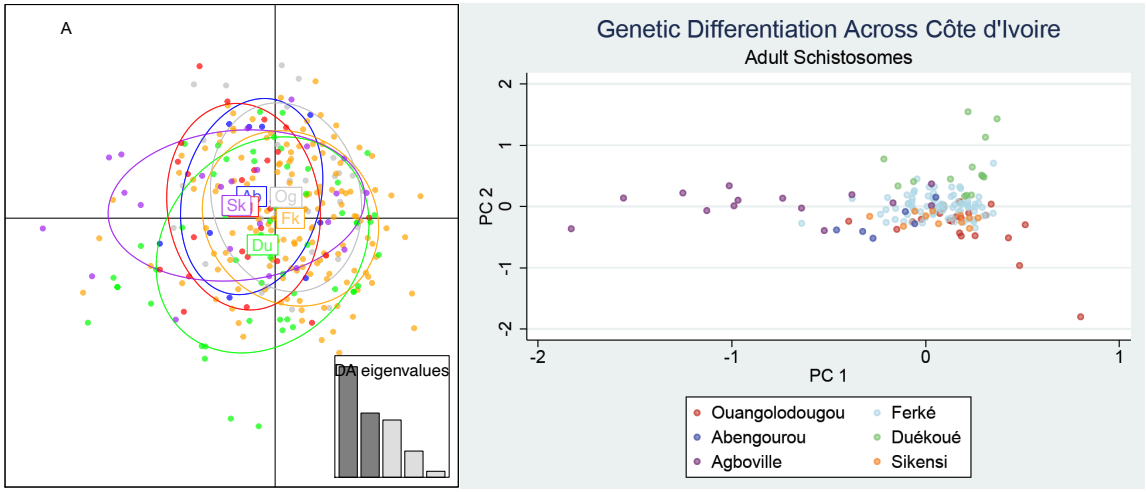

Figure 3. Discriminant analysis of principal components (DAPC) (top) and principal component analysis (PCA) of populations of flukes from cattle, based on geography.

Genetic differences between male and female schistosome flukes

To determine if there are genetic differences between male and female schistosomes, 295 flukes, mostly from couples, were analyzed. A_r and H_e were comparable in both sexes, but the sexes significantly differed in H_o. There was significant deviation from Hardy-Weinberg equilibrium and high levels of inbreeding in both sexes, with higher levels in females than males (Tab. 3).

Table 3. Genetic diversity indices of male and female *Schistosoma*, from slaughtered cattle across Côte d'Ivoire based on 12 microsatellite loci. N, number of fluke samples; N_H, number of host individuals from which flukes were collected; A, average (minimum, maximum) number of alleles across all loci; A_r, mean (+95% CI) allelic richness across all loci; H_o, mean (+95% CI) observed heterozygosity across all loci; H_e, mean (+95% CI) estimated heterozygosity across all loci; P_{HWE}, probability of deviation from Hardy-Weinberg Equilibrium; F_{IS}, mean (+95% CI) fixation index across all loci.

Fluke sex	**N**	**A**	**A_r**	**H_o**	**H_e**	**P_{HWE}**	**F_{IS}**
Female	134	12.25 (5, 23)	11.46 (11.00 -11.83)	0.536 (0.516-0.552)	0.725 (0.707-0.729)	$2.3*10^{-15}$	0.270 (0.237-0.298)
Male	161	12.75 (6, 25)	11.77 (11.25 -12.25)	0.594 (0.571-0.608)	0.736 (0.721-0.739)	$3.2*10^{-20}$	0.222 (0.190-0.247)

There appeared to be very little genotypic differentiation between males and females, with F_{ST} and R_{ST} values of 0.002 and 0.015, respectively. This was confirmed by the STRUCTURE analysis where Evanno's method suggested four genetic cluster, but no population structuring was evident between the two sexes (Fig. 4).

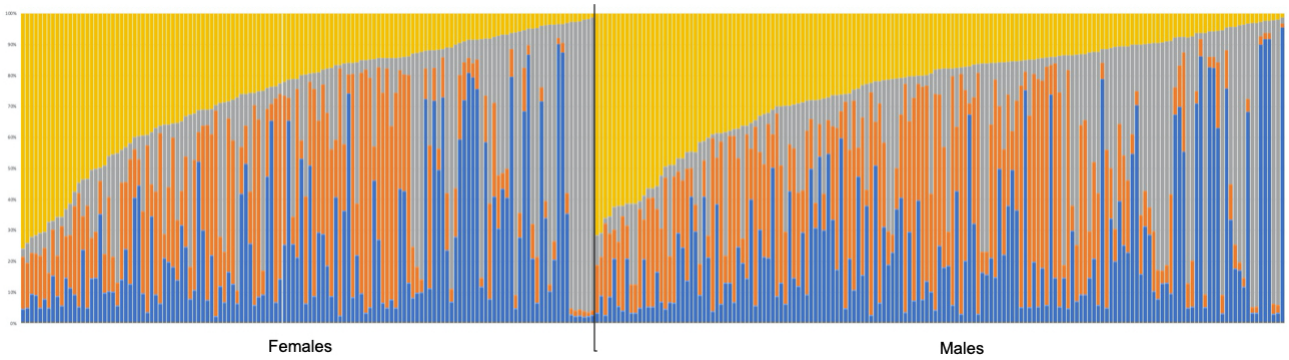

Figure 4. STRUCTURE analysis of male and female Schistosomes, from slaughtered cattle across Côte d'Ivoire (K=4) (see Fig. S4).]

PCA revealed a slight separation of males and females (Fig. 5). Omission of the two outliers in Fig. 5 did not change this outcome.

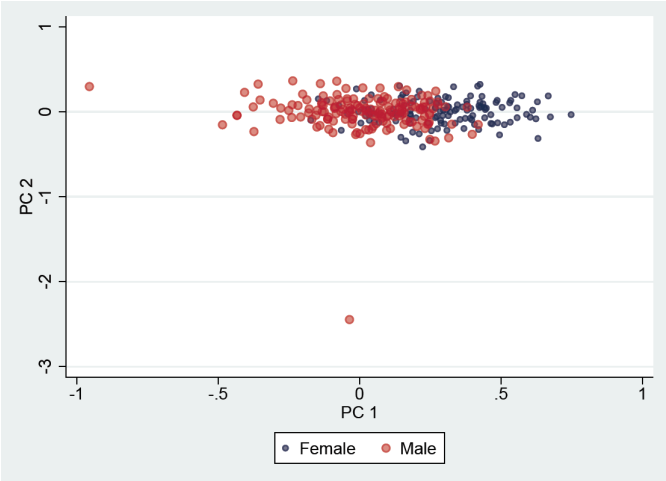

Figure 5. Principal component analysis (PCA) of male and female schistosomes from slaughtered cattle across Côte d'Ivoire

Comparison of within and between-host genetic diversity in flukes

The comparison of diversity measures within individual host animals to that across all host animals revealed statistically significant but moderate differences (Fig. 6, Tab. S2). Genetic diversity within the three cow individuals for which 19 to 22 flukes were analyzed was lower than across the entire population for H_e, A_r and F_{IS}, while H_o was the same or even higher. However, in all measures the differences were slight and for every measure one or two of the assessed cows were indistinguishable from the overall population.

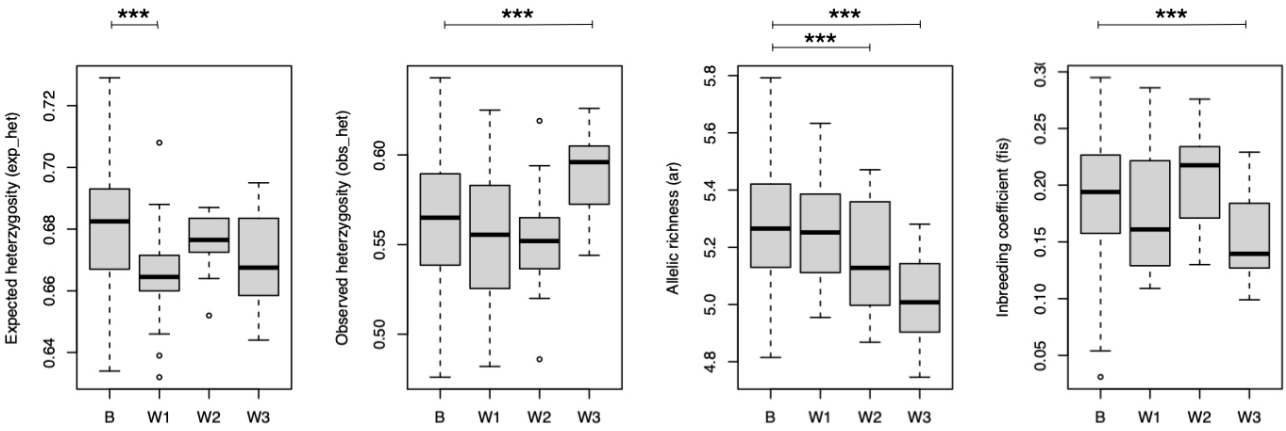

Fig. 6. Boxplots of diversity measures from randomly drawn flukes across all cattle hosts (between, "B") and within three individual cattle hosts (within, "W") in Ferkessédougou. ***, significant pairwise difference at $p < 0.001$. See Tab. S2 for detailed statistical values.

Genetic differentiation between flukes and miracidia in Ferkessédougou

It was first assessed how patterns compared between flukes and miracidia when only one to two parasites were sampled per host. All diversity values were lower in miracidia than flukes, although this difference was statistically significant only in H_o (Tab. 4). F_{IS} was strikingly higher in miracidia than in flukes and anywhere else in this study and deviation from Hardy-Weinberg equilibrium was apparent in both populations.

Table 4. Genetic diversity indices for *Schistosoma* from live farm cattle and slaughtered cattle from abattoirs in Ferkessédougou, Côte d'Ivoire based on 10 microsatellite loci. N, number of fluke samples (maximally two per host individual); N_H, number of host individuals from which flukes were collected; A, average (minimum, maximum) number of alleles across loci; A_r, mean (+95% CI) allelic richness across loci; H_o, mean (+95% CI) observed heterozygosity across loci; H_e, mean (+95% CI) estimated heterozygosity across loci; P_{HWE}, probability of deviation from Hardy-Weinberg Equilibrium (stars highlight significance at <0.05); F_{IS}, mean (+95% CI) fixation index across loci.

Life stage	**N**	**N_H**	**A**	**A_r**	**H_o**	**H_e**	**P_{HWE}**	**F_{IS}**
Flukes	80	42	12.7 (5, 22)	9.83 (9.10-10.60)	0.652 (0.62-0.676)	0.787 (0.766-0.79)	$5.4*10^{-13}$	0.188 (0.140-0.221)
Miracidia	29	29	9.9 (3, 15)	8.50 (7.60 -9.20)	0.509 (0.444-0.536)	0.772 (0.701-0.770)	$1.6*10^{-22}$	0.379 (0.302-0.425)

In contrast, very low genotypic differentiation was apparent between the populations ($F_{ST} = 0.011$ and $R_{ST} = 0.025$). Likewise, STRUCTURE analysis showed no structuring between the two life-stages. The analysis of Evanno's K suggests that the genetic backgrounds of the schistosomes investigated was best explained by three genetic clusters (Fig. S2). The assignments of each parasite's genome to these clusters was not markedly different between the life-stages (Fig. 7).

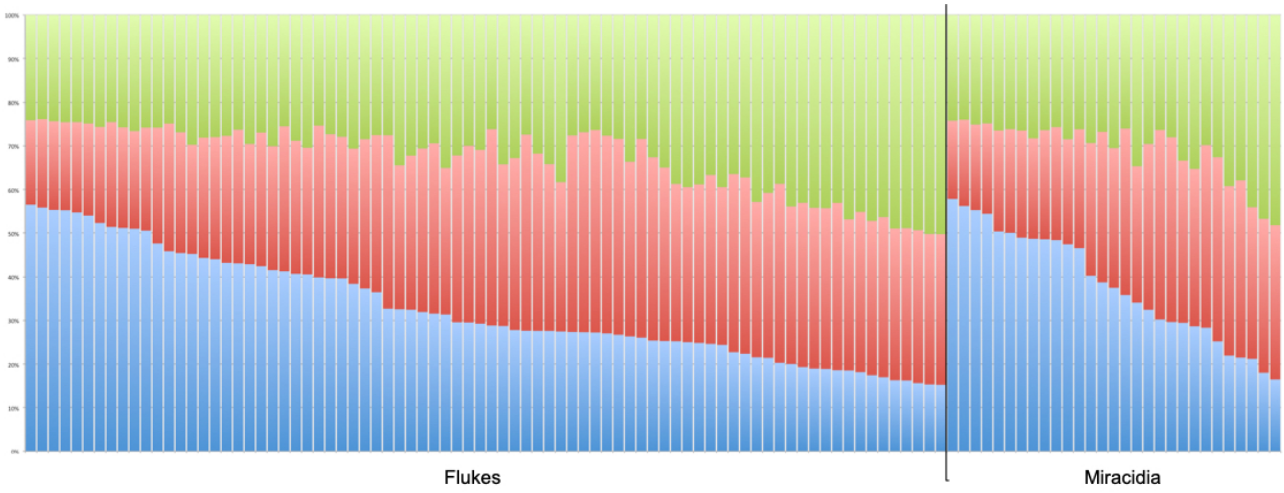

Figure 7. STRUCTURE analysis of *Schistosoma* miracidia from live cattle and flukes from slaughtered cattle, in Ferkessédougou. (K=3).

PCA showed clear differentiation between flukes and miracidia (Fig. 8).

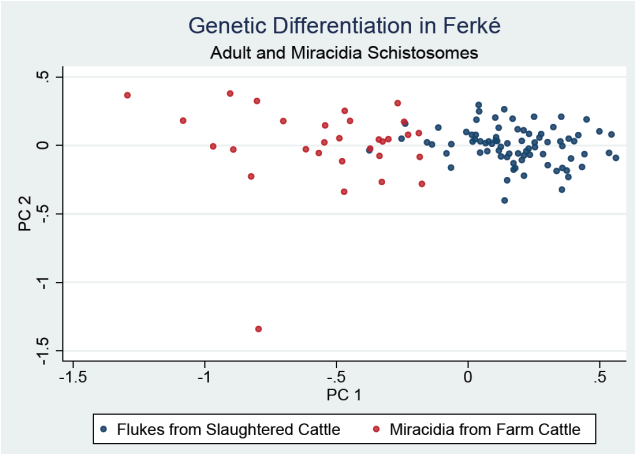

Figure 8. Principal component analysis (PCA) of *Schistosoma* miracidia from live cattle and flukes from slaughtered cattle, in Ferkessédougou.

To assess if within-host genetic diversity differed between flukes and miracidia, genetic diversity was compared, and differentiation assessed between the parasite populations within three slaughtered cattle and five live cattle (Tab. 6). One couple from each slaughtered host and one miracidium from each live host were already included in the analysis before (Tab. 5).

Deficiency of heterozygotes and statistically significant deviation from HWE was apparent in all hosts (Tab. 5). Re-iterating the result of the first analysis (Tab. 4), A_r and H_e were generally higher in flukes than miracidia and F_{IS} was higher in miracidia in every host individual. When taking the mean values per host individual as entries for linear models, the models showed significant differences between flukes and miracidia in A_r ($F = 7.9$, $P = 0.031$), H_o ($F = 15.3$, $P = 0.008$) and F_{IS} ($F = 8.8$, $p = 0.025$) but not H_e ($F = 2.9$, $p = 0.138$). A was not assessed because it is sensitive to sample size.

Table 5. Genetic diversity indices for *Schistosoma* from within individual cattle hosts based on 10 microsatellite loci. Flukes from slaughtered cattle Cfr20, Cfr78 and Cfr84 and miracidia from live cattle 195, 232, 236, BV34, BV04 on farms in Ferkessédougou, Côte d'Ivoire. Host, host ID; N, number of parasite samples; A, average (minimum, maximum) number of alleles across loci; A_r, mean (+95% CI) allelic richness across loci; H_o, mean (+95% CI) observed heterozygosity across loci; H_e, mean (+95% CI) estimated heterozygosity across loci; P_{HWE}, probability of deviation from Hardy-Weinberg Equilibrium; F_{IS}, mean (+95% CI) fixation index across loci.

Life stage	Host	N	A	A_r	H_o	H_e	P_{HWE}	F_{IS}
Flukes	Cfr20	20	9.7 (3, 17)	6.54 (5.50-7.40)	0.628 (0.567-0.685)	0.753 (0.699-0.758)	$2.5*10^{-6}$	0.181 (0.058-0.256)
Flukes	Cfr78	18	8.8 (3, 15)	6.30 (5.20-7.40)	0.627 (0.558-0.687)	0.75 (0.681-0.757)	$1.3*10^{-6}$	0.177 (0.059-0.236)
Flukes	Cfr84	22	8.7 (3, 14)	6.13 (5.10-7.00)	0.664 (0.617-0.707)	0.751 (0.704-0.755)	$1.1*10^{-7}$	0.139 (0.043-0.189)
Miracidia	195	17	7.6 (2, 13)	5.70 (4.70-6.60)	0.585 (0.494-0.654)	0.741 (0.666-0.732)	$2.8*10^{-7}$	0.229 (0.072-0.333)
Miracidia	232	10	5.8 (3, 10)	4.75 (3.80-5.50)	0.490 (0.455-0.525)	0.642 (0.531-0.650)	$3.1*10^{-5}$	0.239 (0.063-0.282)
Miracidia	236	11	6.3 (2, 12)	5.07 (4.20-5.80)	0.560 (0.486-0.625)	0.677 (0.566-0.693)	$2.2*10^{-4}$	0.235 (0.087-0.269)
Miracidia	BV34	16	8.3 (3, 14)	6.08 (5.10-6.91)	0.523 (0.460-0.564)	0.765 (0.67-0.763)	$5.2*10^{-15}$	0.363 (0.254-0.405)
Miracidia	BV04	10	5.6 (2, 8)	4.61 (3.60-5.40)	0.475 (0.370-0.57)	0.643 (0.517-0.657)	$2.3*10^{-7}$	0.325 (0.011-0.444)

Genotypic differentiation between the life-stages as measured by F_{ST} and R_{ST} was weak with the highest F_{ST} and R_{ST} values being, 0.048 and 0.055, respectively (Tab. S3). Values between life-stage were not significantly different from those within life stages (linear models, $F = 1.06$, $p = 0.31$ and $F = 0.75$, $p = 0.39$ for F_{ST} and R_{ST}, respectively).

Likewise, STRUCTURE analysis revealed two genetic clusters (Fig. S2), but no consistent differentiation between flukes and miracidia (Fig. 9). Three of the five miracidia populations from live cattle (232, 236, BV4) appeared to be different from flukes in slaughtered cattle but the other two live cattle populations were indistinguishable.

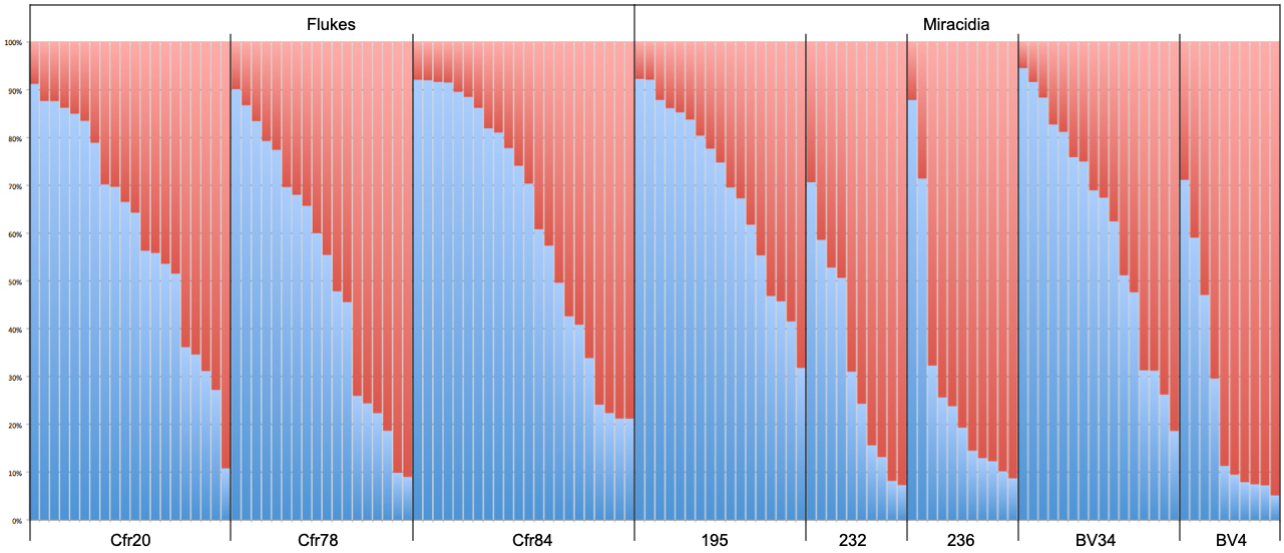

Figure 9. STRUCTURE analysis of *Schistosoma* miracidia and flukes from within live and slaughtered cattle, respectively, in Ferkessédougou (K=2).

DAPC showed weak differentiation between populations (Fig. 10a). BV4 and BV34, both live cattle, were the hosts with least overlap of their parasite populations. However, PCA indicated that the flukes from all slaughtered cattle cluster together, away from most miracidia (Fig. 10b). Among miracidia, individuals from hosts BV4 and 195 were most distinct. BV4 also showed the highest (but still very moderate) pairwise F_{ST} values with the other hosts (Tab. 7).

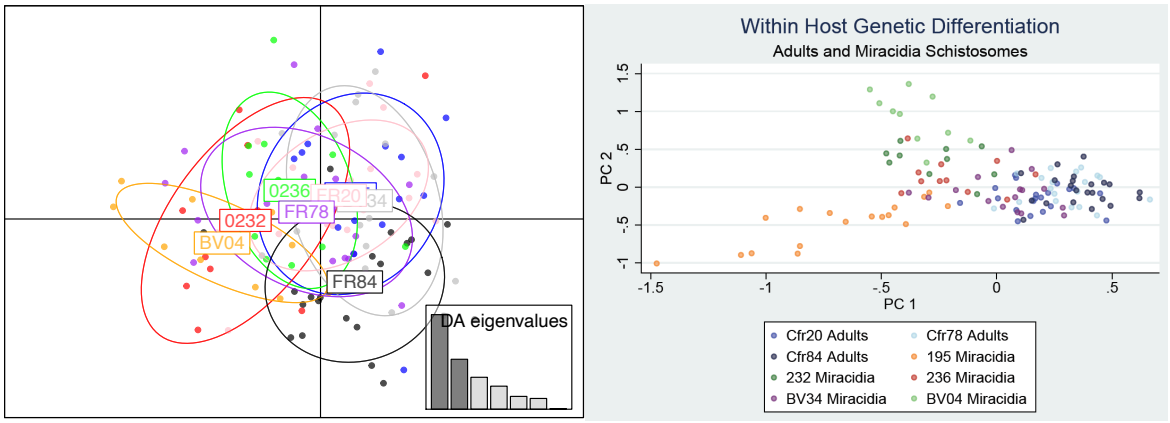

Figure 10. Discriminant analysis of principal components (DAPC) and principal component analysis (PCA) and of schistosomes from within slaughtered and live cattle hosts.

Discussion

In our counting, this is only the sixth investigation using molecular species identification of *Schistosoma* in African cattle (Benin, Cameroon and Senegal) (Webster et al. 2013; Boon et al. 2019; Djuikwo-Teukeng et al. 2019; Léger et al. 2020; Savassi et al. 2020), and the third population genetics

investigation, all published within the last two years (Cameroon and Senegal) (Boon et al. 2019; Djuikwo-Teukeng et al. 2019).

All flukes and miracidia from cattle investigated in this study had the typical *S. bovis* sequences both at the mitochondrial *cox1* and the nuclear *ITS1/2* genes. There were no indications of *S. haematobium*, *S. bovis* x *S. haematobium* hybrids (i.e. hybrids of livestock and humans schistosomes) or *S. curassoni* at any of the six locations investigated across Côte d'Ivoire in our cattle samples. Our results thus mirror findings from Cameroon and some results from Senegal, where cattle were also found to harbor only pure *S. bovis* (Boon et al. 2019; Djuikwo-Teukeng et al. 2019).

Distinguishing *S. curassoni* from *S. haematobium* and *S. bovis* using the *cox1* PCR and *ITS* variable sites is not straight forward. *S. curassoni* can present as either *S. bovis* or with two bands (*S. bovis* and *S. haematobium*) in the *cox1* multiplex PCR (Webster et al. 2013) and the *ITS1/2* sequence has only one variable site that distinguished *S. bovis* from *S. curassoni*, where *S. curassoni* displays the same nucleotide as *S. haematobium*. *S. curassoni* have been reported in Mauritania (Gretillet et al. 1962), Mali, Senegal (enzymatic and morphological analysis) (Rollinson et al. 1990) and Niger (egg morphology) (Mouchet et al. 1989) and hybrids of *S. bovis* x *S. curassoni* have been reported in livestock in Senegal and Mali (Rollinson et al., 1990) and in a child in Niger (Léger et al. 2016). The absence of *S. curassoni* in our sample may be due to the demonstrated lack of their preferred intermediate host snail, *B. umbilicatus,* in Côte d'Ivoire (Krauth et al. 2017; Tian-Bi et al. 2019). Given the presence of *S. curassoni* in livestock in West Africa (Mouchet et al. 1989; Rollinson et al. 1990; Léger et al. 2016) and the threat of livestock x human schistosome hybrids, better molecular methods to distinguish *S. curassoni* should be developed.

S. haematobium are normally found in the plexus of the urinary bladder in humans, as opposed to *S. bovis, S. curassoni* and *S. mansoni*, which are found in the mesenteric veins of cattle and humans, respectively. This leads one to ask the question: could hybrids involving S. *haematobium* be found in the bladder and urine of cattle? We examined five bladders and urine samples from slaughtered cattle, but no schistosome flukes or eggs were found. We attempted to collect urine samples from live cattle, however these samples were difficult to collect as obtaining urine from live cattle is dangerous in a field setting without restraining equipment. Furthermore, the samples are often contaminated with feces during the collection. However, our limited results are in line with what Léger at al. (2020) found after examining vesical blood vessels and urine from 60 slaughtered cattle and 69 live cattle; no eggs or miracidia, with the exception of one cow which did have flukes in the vesical blood vessels in Senegal.

To date there has been only one report of *S. bovis* x *S. haematobium* hybrids in cattle determined by cercarial shedding and molecular markers (*cox1* and *ITS1/2*) from eggs in stool samples from Benin (Savassi et al. 2020). Hybrids of *S. bovis* x *S. haematobium* have, however, been reported in humans in Senegal and Niger (Brémond et al. 1993; Huyse et al. 2009; Webster et al. 2013; Boon et al. 2019). It has previously been thought that pure *S. bovis* or other animal schistosomes cannot mature in humans,

however, what appears to be pure *S. bovis* has been reported in humans in Corsica and Côte d'Ivoire (Boissier et al. 2016; Angora et al. 2020) and a *S. bovis* x *S. curassoni* hybrid was found in a child in Niger (Leger 2016). We have also identified *S. bovis* x *S. haematobium* hybrids in humans at the same sites where cattle were sampled in the present study (Giovanoli et al. unpublished data), yet only *S. bovis* was present in cattle.

Our results support the recently proposed multi-host, multi-parasite transmission cycle for the *S. haematobium* group in West Africa (Léger et al. 2020). In this revised cycle, transmission of *S. haematobium* and *S. haematobium* x *S. bovis* hybrids occurs exclusively in humans with occasional spill-over of *S. bovis* from livestock. The barrier between humans and animal transmission remains mostly intact but is occasionally punctured by animal parasites infecting humans. This lifecycle is supported by evidence from other countries where *S. haematobium* and *S. haematobium* x *S. bovis* are found in humans only, such as Corsica (Oleaga et al. 2019) and Senegal (Léger et al. 2020). In Malawi a similar new model has been suggested for transmission cycles involving humans, cattle, *S. mansoni*, *S. mattheii* and *S. mansoni* x *S. mattheii* hybrids (Stothard et al. 2020).

Schistosomes from cattle across the six sites in Côte d'Ivoire appear to be panmictic, which has been found across Senegal and Cameroon in cattle as well (Boon et al. 2019; Djuikwo-Teukeng et al. 2019). There is generally an excess of homozygotes, high levels of inbreeding and little structuring or differentiation across all analyses performed (location, life stage, between hosts and by parasite sex). There was also virtually no difference or structuring between male and female populations of schistosomes, which is congruent with their mode of transmission. Male and female schistosomes occupy the spaces and therefore, have equal chances to infect intermediate and definitive hosts. Curiously, for both analyses, the PCA showed some differentiation while the DAPC and STRUCTURE did not, a pattern we do not know from any other study and for which we have no convincing interpretation.

Our analysis suggests that the genetic diversity of adult flukes inside a host individual is comparable to that found across the host population. The slightly lower diversity we found in the few cattle individuals in which we analyzed a large number of flukes can be expected due to sampling imbalance. Our between values were based on a sample double the size of that of the individual hosts. This will lead to the availability of more diversity, even if our resampling scheme assured equal group sizes in every resampling round. The key results is, however, that for all measures we analyzed some individual cows harbored as much diversity as the overall population. This finding is in contrast to patterns found in *Schistosoma* miracidia in urine of school children in the South of Côte d'Ivoire, where many children harbored parasites that were genetically very similar and much less diverse than across hosts (Angora et al. 2022). The biological reason for this discrepancy may be that flukes enter the host individually, i.e. they are relatively independent at the time of infection, whereas eggs are produced inside the host by these fluke couples and may therefore be highly related, at least in some cases. Our data thus suggest that - in cattle at least - the definitive host or its behavior does not reduce

the schistosome genetic diversity present in the environment at the time of infection. They also suggest that many flukes from few individual cattle can represent the local parasite population in population genetic analyses, since they do not appear to be more closely related than across hosts. However, we would like to caution that theoretical considerations and the findings by other researchers nonetheless advocate for using as broad a host sampling as possible. And it must be kept in mind that the same is not necessarily true for miracidia, i.e. for studies using live hosts.

It appears that miracidia and flukes from farms and abattoirs, respectively, in the same region harbor indistinguishable genotypes. Especially high levels of inbreeding were seen in the miracidia population compared to flukes. This trend was visible in the within host analysis as well when comparing within live cattle to slaughtered cattle. It thus appears that while flukes and miracidia are part of the same gene pool there are processes that lead to different genetic diversity and inbreeding and that the two life-stages cannot simply be combined in analyses, even in the same region.

Mobile pastoralism occurs across West Africa, including Côte d'Ivoire, where pastoralists come from Mali and Burkina Faso through the department of Ferkessédougou in the dry season to graze their animals in the Savannah. They move south and sometimes sell their animals for slaughter along the way, allowing their livestock to release and pick-up schistosomes in Côte d'Ivoire (Bassett 2009). This may be one reason for the lack of genetic structuring amongst schistosomes from cattle in Côte d'Ivoire. We expected the schistosomes from the site in the West (Duekoué) to show some distinction from the other sites, as it is not on the cattle migration route and the schistosomes in humans from Duekoué are distinct from the other sites (Angora et al., 2022, Giovanoli et al., in preparation). However, no such geographical structuring was apparent in cattle parasites. We see two reasonable explanations for this observation. First, parasite mixing in the cattle population may be too intense across the entire strongly interconnected economic zone from Mali and Burkina Faso to the coastal harbors in Côte d'Ivoire for genetic structuring to arise. Or second, a methodological problem may blur patterns even if they were present. It is often very difficult in field settings to reliably verify the origin of animals in slaughterhouses and on farms. A portion of cattle may thus not really be from the area where they were sampled.

Our study faces a number of limitations. First, transmission dynamics are best studied by collecting parasites from all definitive and intermediate hosts. Yet the prevalences in sheep, goats and snails were very low across the study sites and we were not able to obtain enough samples to analyze schistosomes from these other hosts. Secondly, our sampling does not include schistosomes from the Central, North-east and North-west parts of the country, partly also due to very low prevalences and a lack of samples. Thirdly, schistosomes from farm and abattoir cattle populations may differ in terms of origin. Besides blurring geographical patterns, this may also be problematic in the analysis comparing miracidia from farm cattle and flukes from slaughtered cattle. As mentioned above transhumance practices occur in Ferkessédougou and during this migration livestock are often sold and slaughtered at abattoirs along the route. Often, information about the origin of the animals in the abattoirs is not

very reliable. Animals on farms were almost exclusively born on the farm. According to the declaration by the farmers selling their cattle to the slaughterhouses, animals slaughtered in Ferkessédougou were also raised there. But some uncertainty remains. According to the declaration by the farmers selling their cattle to the slaughterhouses, animals slaughtered in Ferkessédougou were also raised there. But some uncertainty remains. This potential difference may have distorted our results.

This investigation together with concurrent studies on human patients shows the benefits of a One Health approach, as promoted by WHO for the control of NTDs. Schistosomiasis prevalences in cattle are high in Côte d'Ivoire, yet there is very little surveillance and no control program for livestock, which could threaten progress made in human schistosomiasis control programs. Our data suggest that at this point there is little transmission between Ivorian cattle and humans, but further research should be undertaken to confirm and monitor this situation. Epidemiological data from livestock provides invaluable information that can improve the success of prevention and control programs, as zoonotic reservoirs are considered.

Despite the presence of human and bovine hybrid schistosomes in Ivorian humans, there appears to be no hybrids and no *S. curassoni* in cattle in Côte d'Ivoire**.** Schistosome populations in cattle from the North and South appear to be panmictic, perhaps as they are on the migration route**.** The WHO NTD roadmap (WHO 2020) advocates for a One Health approach as one of the strategies for controlling NTDs including schistosomiasis. The combination of the presence of hybrids between human and livestock schistosome species in humans and the panmictic population of livestock schistosomes suggests a need for livestock schistosome control programme.

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Supplemental Material

Table S1. ID, life stage, location of collection and results of genotyping and sequencing of all *Schistosoma* samples analyzed in this study. Flukes were collected in abattoirs, miracidia from live animals on farms. n/a, did not amplify; -, not done.

Sample ID	Life stage	Location	Multiplex *cox1* species	*ITS* species	*cox1* haplotype
SA036	Fluke	Ouangolodougou	*S. bovis*	*S. bovis*	-
SA038	Fluke	Ouangolodougou	*S. bovis*	*S. bovis*	-
SA039	Fluke	Ouangolodougou	*S. bovis*	*S. bovis*	-
SA154	Fluke	Ouangolodougou	*S. bovis*	*S. bovis*	-
SA155	Fluke	Ouangolodougou	*S. bovis*	-	-
SA156	Fluke	Ouangolodougou	*S. bovis*	*S. bovis*	-
SA157	Fluke	Ouangolodougou	*S. bovis*	-	-
SA158	Fluke	Ouangolodougou	*S. bovis*	*S. bovis*	-
SA159	Fluke	Ouangolodougou	n/a	*S. bovis*	-
SA160	Fluke	Ouangolodougou	*S. bovis*	*S. bovis*	-
SA161	Fluke	Ouangolodougou	*S. bovis*	*S. bovis*	-
SA279	Fluke	Ouangolodougou	*S. bovis*	-	-
SA280	Fluke	Ouangolodougou	*S. bovis*	-	-
SA281	Fluke	Ouangolodougou	*S. bovis*	-	-
SA282	Fluke	Ouangolodougou	n/a	-	-
SA283	Fluke	Ouangolodougou	*S. bovis*	-	-
SA284	Fluke	Ouangolodougou	*S. bovis*	-	-
SA285	Fluke	Ouangolodougou	*S. bovis*	-	-
SA286	Fluke	Ouangolodougou	*S. bovis*	-	-

SA287	Fluke	Ouangolodougou	n/a	-	-
SA288	Fluke	Ouangolodougou	*S. bovis*	-	-
SA289	Fluke	Ouangolodougou	*S. bovis*	-	-
SA290	Fluke	Ouangolodougou	*S. bovis*	-	-
SA291	Fluke	Ouangolodougou	*S. bovis*	-	-
SA292	Fluke	Ouangolodougou	*S. bovis*	-	-
SA294	Fluke	Ouangolodougou	*S. bovis*	-	-
SA390	Fluke	Ouangolodougou	*S. bovis*	-	-
SA391	Fluke	Ouangolodougou	*S. bovis*	-	-
SA392	Fluke	Ouangolodougou	*S. bovis*	-	-
SA393	Fluke	Ouangolodougou	*S. bovis*	-	-
SA394	Fluke	Ouangolodougou	*S. bovis*	-	-
SA395	Fluke	Ouangolodougou	*S. bovis*	-	-
SA398	Fluke	Ouangolodougou	*S. bovis*	-	-
SA399	Fluke	Ouangolodougou	*S. bovis*	-	-
SA400	Fluke	Ouangolodougou	*S. bovis*	-	-
SA003	Fluke	Ferkessédougou	*S. bovis*	-	-
SA004	Fluke	Ferkessédougou	*S. bovis*	-	-
SA148	Fluke	Ferkessédougou	*S. bovis*	*S. bovis*	-
SA149	Fluke	Ferkessédougou	*S. bovis*	*S. bovis*	-
SA152	Fluke	Ferkessédougou	*S. bovis*	*S. bovis*	-
SA164	Fluke	Ferkessédougou	*S. bovis*	*S. bovis*	-
SA165	Fluke	Ferkessédougou	*S. bovis*	*S. bovis*	-
SA166	Fluke	Ferkessédougou	*S. bovis*	*S. bovis*	-
SA167	Fluke	Ferkessédougou	*S. bovis*	*S. bovis*	-
SA168	Fluke	Ferkessédougou	*S. bovis*	*S. bovis*	-
SA169	Fluke	Ferkessédougou	*S. bovis*	*S. bovis*	-
SA170	Fluke	Ferkessédougou	*S. bovis*	*S. bovis*	-
SA171	Fluke	Ferkessédougou	n/a	*S. bovis*	-
SA174	Fluke	Ferkessédougou	*S. bovis*	*S. bovis*	-
SA175	Fluke	Ferkessédougou	*S. bovis*	-	-
SA176	Fluke	Ferkessédougou	*S. bovis*	-	-
SA177	Fluke	Ferkessédougou	*S. bovis*	-	-
SA178	Fluke	Ferkessédougou	*S. bovis*	-	-
SA179	Fluke	Ferkessédougou	*S. bovis*	-	-
SA180	Fluke	Ferkessédougou	*S. bovis*	-	-
SA181	Fluke	Ferkessédougou	*S. bovis*	-	-
SA182	Fluke	Ferkessédougou	*S. bovis*	-	-
SA183	Fluke	Ferkessédougou	*S. bovis*	-	-
SA184	Fluke	Ferkessédougou	*S. bovis*	-	-
SA185	Fluke	Ferkessédougou	*S. bovis*	-	-
SA186	Fluke	Ferkessédougou	*S. bovis*	-	-
SA187	Fluke	Ferkessédougou	*S. bovis*	-	-
SA188	Fluke	Ferkessédougou	*S. bovis*	-	-
SA189	Fluke	Ferkessédougou	*S. bovis*	-	-
SA190	Fluke	Ferkessédougou	*S. bovis*	-	-
SA191	Fluke	Ferkessédougou	*S. bovis*	-	-
SA192	Fluke	Ferkessédougou	*S. bovis*	-	-

SA193	Fluke	Ferkessédougou	*S. bovis*	-	-
SA194	Fluke	Ferkessédougou	*S. bovis*	-	-
SA195	Fluke	Ferkessédougou	*S. bovis*	-	-
SA196	Fluke	Ferkessédougou	*S. bovis*	-	-
SA197	Fluke	Ferkessédougou	*S. bovis*	-	-
SA198	Fluke	Ferkessédougou	*S. bovis*	-	-
SA199	Fluke	Ferkessédougou	*S. bovis*	-	-
SA200	Fluke	Ferkessédougou	*S. bovis*	-	-
SA201	Fluke	Ferkessédougou	*S. bovis*	-	-
SA202	Fluke	Ferkessédougou	*S. bovis*	-	-
SA203	Fluke	Ferkessédougou	*S. bovis*	-	-
SA204	Fluke	Ferkessédougou	*S. bovis*	-	-
SA205	Fluke	Ferkessédougou	*S. bovis*	-	-
SA206	Fluke	Ferkessédougou	*S. bovis*	-	-
SA207	Fluke	Ferkessédougou	*S. bovis*	-	-
SA208	Fluke	Ferkessédougou	*S. bovis*	-	-
SA210	Fluke	Ferkessédougou	*S. bovis*	-	-
SA211	Fluke	Ferkessédougou	*S. bovis*	-	-
SA212	Fluke	Ferkessédougou	*S. bovis*	-	-
SA213	Fluke	Ferkessédougou	*S. bovis*	-	-
SA214	Fluke	Ferkessédougou	*S. bovis*	-	-
SA215	Fluke	Ferkessédougou	*S. bovis*	-	-
SA216	Fluke	Ferkessédougou	*S. bovis*	-	-
SA217	Fluke	Ferkessédougou	*S. bovis*	-	-
SA218	Fluke	Ferkessédougou	*S. bovis*	-	-
SA219	Fluke	Ferkessédougou	*S. bovis*	-	-
SA220	Fluke	Ferkessédougou	*S. bovis*	-	-
SA221	Fluke	Ferkessédougou	*S. bovis*	-	-
SA222	Fluke	Ferkessédougou	*S. bovis*	-	-
SA223	Fluke	Ferkessédougou	*S. bovis*	-	-
SA224	Fluke	Ferkessédougou	*S. bovis*	-	-
SA225	Fluke	Ferkessédougou	*S. bovis*	-	-
SA226	Fluke	Ferkessédougou	*S. bovis*	-	-
SA227	Fluke	Ferkessédougou	*S. bovis*	-	-
SA228	Fluke	Ferkessédougou	*S. bovis*	-	-
SA229	Fluke	Ferkessédougou	*S. bovis*	-	-
SA230	Fluke	Ferkessédougou	*S. bovis*	-	-
SA231	Fluke	Ferkessédougou	*S. bovis*	-	-
SA233	Fluke	Ferkessédougou	*S. bovis*	-	-
SA234	Fluke	Ferkessédougou	*S. bovis*	-	-
SA235	Fluke	Ferkessédougou	n/a	-	-
SA236	Fluke	Ferkessédougou	n/a	-	-
SA237	Fluke	Ferkessédougou	*S. bovis*	-	-
SA238	Fluke	Ferkessédougou	*S. bovis*	-	-
SA239	Fluke	Ferkessédougou	n/a	-	-
SA240	Fluke	Ferkessédougou	*S. bovis*	-	-
SA241	Fluke	Ferkessédougou	n/a	-	-
SA242	Fluke	Ferkessédougou	*S. bovis*	-	-

SA243	Fluke	Ferkessédougou	*S. bovis*	-	-
SA244	Fluke	Ferkessédougou	*S. bovis*	-	-
SA245	Fluke	Ferkessédougou	*S. bovis*	-	-
SA246	Fluke	Ferkessédougou	*S. bovis*	-	-
SA247	Fluke	Ferkessédougou	*S. bovis*	-	-
SA248	Fluke	Ferkessédougou	*S. bovis*	-	-
SA249	Fluke	Ferkessédougou	*S. bovis*	-	-
SA250	Fluke	Ferkessédougou	*S. bovis*	-	-
SA251	Fluke	Ferkessédougou	*S. bovis*	-	-
SA252	Fluke	Ferkessédougou	*S. bovis*	-	-
SA253	Fluke	Ferkessédougou	n/a	-	-
SA254	Fluke	Ferkessédougou	*S. bovis*	-	-
SA255	Fluke	Ferkessédougou	*S. bovis*	-	-
SA256	Fluke	Ferkessédougou	n/a	-	-
SA257	Fluke	Ferkessédougou	n/a	-	-
SA258	Fluke	Ferkessédougou	*S. bovis*	-	-
SA259	Fluke	Ferkessédougou	*S. bovis*	-	-
SA260	Fluke	Ferkessédougou	*S. bovis*	-	-
SA261	Fluke	Ferkessédougou	n/a	-	-
SA262	Fluke	Ferkessédougou	*S. bovis*	-	-
SA263	Fluke	Ferkessédougou	n/a	-	-
SA264	Fluke	Ferkessédougou	n/a	-	-
SA265	Fluke	Ferkessédougou	*S. bovis*	-	-
SA266	Fluke	Ferkessédougou	*S. bovis*	-	-
SA267	Fluke	Ferkessédougou	n/a	-	-
SA268	Fluke	Ferkessédougou	*S. bovis*	-	-
SA269	Fluke	Ferkessédougou	*S. bovis*	-	-
SA270	Fluke	Ferkessédougou	*S. bovis*	-	-
SA271	Fluke	Ferkessédougou	*S. bovis*	-	-
SA272	Fluke	Ferkessédougou	n/a	-	-
SA273	Fluke	Ferkessédougou	*S. bovis*	-	-
SA274	Fluke	Ferkessédougou	*S. bovis*	-	-
SA275	Fluke	Ferkessédougou	*S. bovis*	-	-
SA276	Fluke	Ferkessédougou	*S. bovis*	-	-
SA277	Fluke	Ferkessédougou	*S. bovis*	-	-
SA278	Fluke	Ferkessédougou	*S. bovis*	-	-
SA295	Fluke	Ferkessédougou	*S. bovis*	-	-
SA296	Fluke	Ferkessédougou	*S. bovis*	-	-
SA297	Fluke	Ferkessédougou	*S. bovis*	-	-
SA298	Fluke	Ferkessédougou	*S. bovis*	-	-
SA299	Fluke	Ferkessédougou	*S. bovis*	-	-
SA300	Fluke	Ferkessédougou	*S. bovis*	-	-
SA301	Fluke	Ferkessédougou	*S. bovis*	-	-
SA302	Fluke	Ferkessédougou	*S. bovis*	-	-
SA303	Fluke	Ferkessédougou	*S. bovis*	-	-
SA304	Fluke	Ferkessédougou	*S. bovis*	-	-
SA305	Fluke	Ferkessédougou	*S. bovis*	-	-
SA306	Fluke	Ferkessédougou	*S. bovis*	-	-

SA307	Fluke	Ferkessédougou	*S. bovis*	-	-
SA308	Fluke	Ferkessédougou	*S. bovis*	-	-
SA309	Fluke	Ferkessédougou	*S. bovis*	-	-
SA310	Fluke	Ferkessédougou	*S. bovis*	-	-
SA311	Fluke	Ferkessédougou	*S. bovis*	-	-
SA312	Fluke	Ferkessédougou	*S. bovis*	-	-
SA313	Fluke	Ferkessédougou	*S. bovis*	-	-
SA314	Fluke	Ferkessédougou	*S. bovis*	-	-
SA315	Fluke	Ferkessédougou	*S. bovis*	-	-
SA316	Fluke	Ferkessédougou	*S. bovis*	-	-
SA317	Fluke	Ferkessédougou	*S. bovis*	-	-
SA318	Fluke	Ferkessédougou	*S. bovis*	-	-
SA319	Fluke	Ferkessédougou	*S. bovis*	-	-
SA320	Fluke	Ferkessédougou	*S. bovis*	-	-
SA321	Fluke	Ferkessédougou	*S. bovis*	-	-
SA322	Fluke	Ferkessédougou	*S. bovis*	-	-
SA323	Fluke	Ferkessédougou	*S. bovis*	-	-
SA324	Fluke	Ferkessédougou	*S. bovis*	-	-
SA325	Fluke	Ferkessédougou	*S. bovis*	-	-
SA326	Fluke	Ferkessédougou	*S. bovis*	-	-
SA327	Fluke	Ferkessédougou	*S. bovis*	-	-
SA328	Fluke	Ferkessédougou	*S. bovis*	-	-
SA329	Fluke	Ferkessédougou	*S. bovis*	-	-
SA330	Fluke	Ferkessédougou	*S. bovis*	-	-
SM008	Miracidium	Ferkessédougou	*S. bovis*	*S. bovis*	-
SM009	Miracidium	Ferkessédougou	*S. bovis*	*S. bovis*	-
SM010	Miracidium	Ferkessédougou	*S. bovis*	*S. bovis*	-
SM011	Miracidium	Ferkessédougou	*S. bovis*	*S. bovis*	-
SM061	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM062	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM065	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM066	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM067	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM068	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM069	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM070	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM071	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM072	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM073	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM074	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM075	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM076	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM078	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM079	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM082	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM083	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM084	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM085	Miracidium	Ferkessédougou	*S. bovis*	-	-

SM086	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM087	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM088	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM089	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM090	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM091	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM092	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM105	Miracidium	Ferkessédougou	n/a	-	-
SM106	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM107	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM108	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM109	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM110	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM111	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM113	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM114	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM115	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM116	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM117	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM118	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM119	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM120	Miracidium	Ferkessédougou	n/a	-	-
SM122	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM123	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM124	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM125	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM126	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM127	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM129	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM130	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM131	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM132	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM134	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM135	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM136	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM137	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM138	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM139	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM140	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM141	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM142	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM143	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM144	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM145	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM146	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM147	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM150	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM151	Miracidium	Ferkessédougou	*S. bovis*	-	-

SM152	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM153	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM155	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM156	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM158	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM159	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM160	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM162	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM163	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM164	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM165	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM166	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM167	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM168	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM169	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM170	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM188	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM189	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM190	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM191	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM192	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM193	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM194	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM196	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM199	Miracidium	Ferkessédougou	*S. bovis*	-	-
SM200	Miracidium	Ferkessédougou	*S. bovis*	-	-
SA358	Fluke	Odienné	*S. bovis*	-	-
SA359	Fluke	Odienné	*S. bovis*	-	-
SA386	Fluke	Bondoukou	*S. bovis*	-	-
SA387	Fluke	Bondoukou	*S. bovis*	-	-
SA388	Fluke	Bondoukou	*S. bovis*	-	-
SA389	Fluke	Bondoukou	*S. bovis*	-	-
SA375	Fluke	Abengourou	*S. bovis*	-	-
SA376	Fluke	Abengourou	*S. bovis*	-	-
SA377	Fluke	Abengourou	*S. bovis*	-	-
SA378	Fluke	Abengourou	*S. bovis*	-	-
SA379	Fluke	Abengourou	*S. bovis*	-	-
SA381	Fluke	Abengourou	*S. bovis*	-	-
SA382	Fluke	Abengourou	*S. bovis*	-	-
SA383	Fluke	Abengourou	*S. bovis*	-	-
SA384	Fluke	Abengourou	*S. bovis*	-	-
SA385	Fluke	Abengourou	*S. bovis*	-	-
SA007	Fluke	Duekoué	*S. bovis*	*S. bovis*	-
SA008	Fluke	Duekoué	*S. bovis*	*S. bovis*	-
SA032	Fluke	Duekoué	*S. bovis*	*S. bovis*	-
SA033	Fluke	Duekoué	*S. bovis*	*S. bovis*	-
SA034	Fluke	Duekoué	*S. bovis*	*S. bovis*	-
SA035	Fluke	Duekoué	*S. bovis*	*S. bovis*	-

SA048	Fluke	Duekoué	*S. bovis*	*S. bovis*	-
SA049	Fluke	Duekoué	*S. bovis*	*S. bovis*	-
SA050	Fluke	Duekoué	*S. bovis*	-	-
SA051	Fluke	Duekoué	*S. bovis*	-	-
SA120	Fluke	Duekoué	*S. bovis*	*S. bovis*	-
SA121	Fluke	Duekoué	n/a	*S. bovis*	-
SA122	Fluke	Duekoué	*S. bovis*	*S. bovis*	-
SA123	Fluke	Duekoué	n/a	*S. bovis*	-
SA124	Fluke	Duekoué	*S. bovis*	*S. bovis*	-
SA331	Fluke	Duekoué	*S. bovis*	-	-
SA332	Fluke	Duekoué	*S. bovis*	-	-
SA333	Fluke	Duekoué	*S. bovis*	-	-
SA334	Fluke	Duekoué	*S. bovis*	-	-
SA335	Fluke	Duekoué	*S. bovis*	-	-
SA336	Fluke	Duekoué	*S. bovis*	-	-
SA337	Fluke	Duekoué	*S. bovis*	-	-
SA338	Fluke	Duekoué	*S. bovis*	-	-
SA339	Fluke	Duekoué	*S. bovis*	-	-
SA340	Fluke	Duekoué	*S. bovis*	-	-
SA341	Fluke	Duekoué	*S. bovis*	-	-
SA342	Fluke	Duekoué	*S. bovis*	-	-
SA343	Fluke	Duekoué	*S. bovis*	-	-
SA344	Fluke	Duekoué	*S. bovis*	-	-
SA345	Fluke	Duekoué	*S. bovis*	-	-
SA346	Fluke	Duekoué	*S. bovis*	-	-
SA347	Fluke	Duekoué	*S. bovis*	-	-
SA348	Fluke	Duekoué	*S. bovis*	-	-
SA349	Fluke	Duekoué	*S. bovis*	-	-
SA350	Fluke	Duekoué	*S. bovis*	-	-
SA351	Fluke	Duekoué	*S. bovis*	-	-
SA352	Fluke	Duekoué	*S. bovis*	-	-
SA353	Fluke	Duekoué	*S. bovis*	-	-
SA354	Fluke	Duekoué	*S. bovis*	-	-
SA355	Fluke	Duekoué	*S. bovis*	-	-
SA356	Fluke	Duekoué	*S. bovis*	-	-
SA357	Fluke	Duekoué	*S. bovis*	-	-
SA360	Fluke	Duekoué	*S. bovis*	-	-
SA361	Fluke	Duekoué	*S. bovis*	-	-
SA362	Fluke	Duekoué	*S. bovis*	-	-
SA363	Fluke	Duekoué	*S. bovis*	-	-
SA364	Fluke	Duekoué	*S. bovis*	-	-
SA365	Fluke	Duekoué	*S. bovis*	-	-
SA366	Fluke	Duekoué	*S. bovis*	-	-
SA367	Fluke	Duekoué	*S. bovis*	-	-
SA368	Fluke	Duekoué	*S. bovis*	-	-
SA369	Fluke	Duekoué	*S. bovis*	-	-
SA370	Fluke	Duekoué	*S. bovis*	-	-
SA371	Fluke	Duekoué	*S. bovis*	-	-

SA372	Fluke	Duekoué	*S. bovis*	-	-
SA373	Fluke	Duekoué	*S. bovis*	-	-
SA374	Fluke	Duekoué	*S. bovis*	-	-
SA019	Fluke	Agboville	*S. bovis*	-	-
SA020	Fluke	Agboville	*S. bovis*	-	-
SA024	Fluke	Agboville	*S. bovis*	-	-
SA025	Fluke	Agboville	*S. bovis*	-	-
SA052	Fluke	Agboville	*S. bovis*	*S. bovis*	-
SA053	Fluke	Agboville	*S. bovis*	*S. bovis*	-
SA054	Fluke	Agboville	*S. bovis*	*S. bovis*	-
SA055	Fluke	Agboville	*S. bovis*	*S. bovis*	-
SA056	Fluke	Agboville	*S. bovis*	*S. bovis*	-
SA057	Fluke	Agboville	*S. bovis*	*S. bovis*	-
SA125	Fluke	Agboville	*S. bovis*	-	-
SA126	Fluke	Agboville	n/a	-	-
SA127	Fluke	Agboville	*S. bovis*	*S. bovis*	-
SA128	Fluke	Agboville	*S. bovis*	*S. bovis*	-
SA129	Fluke	Agboville	*S. bovis*	-	-
SA130	Fluke	Agboville	n/a	-	-
SA131	Fluke	Agboville	n/a	*S. bovis*	-
SA132	Fluke	Agboville	*S. bovis*	*S. bovis*	-
SA139	Fluke	Agboville	*S. bovis*	-	-
SA140	Fluke	Agboville	*S. bovis*	-	-
SA143	Fluke	Agboville	*S. bovis*	-	-
SA144	Fluke	Agboville	*S. bovis*	-	-
SA145	Fluke	Agboville	*S. bovis*	-	-
SA146	Fluke	Agboville	*S. bovis*	-	-
SA001	Fluke	Sikensi	*S. bovis*	-	-
SA002	Fluke	Sikensi	*S. bovis*	-	-
SA009	Fluke	Sikensi	*S. bovis*	-	-
SA010	Fluke	Sikensi	*S. bovis*	*S. bovis*	-
SA011	Fluke	Sikensi	*S. bovis*	*S. bovis*	-
SA012	Fluke	Sikensi	*S. bovis*	-	-
SA015	Fluke	Sikensi	*S. bovis*	-	-
SA016	Fluke	Sikensi	*S. bovis*	*S. bovis*	-
SA017	Fluke	Sikensi	*S. bovis*	-	-
SA018	Fluke	Sikensi	*S. bovis*	*S. bovis*	-
SA044	Fluke	Sikensi	*S. bovis*	*S. bovis*	-
SA045	Fluke	Sikensi	*S. bovis*	-	-
SA046	Fluke	Sikensi	*S. bovis*	*S. bovis*	-
SA047	Fluke	Sikensi	*S. bovis*	*S. bovis*	-
SA065	Fluke	Sikensi	*S. bovis*	*S. bovis*	-
SA082	Fluke	Sikensi	*S. bovis*	-	-
SA083	Fluke	Sikensi	*S. bovis*	-	-
SA090	Fluke	Sikensi	*S. bovis*	-	-
SA091	Fluke	Sikensi	*S. bovis*	-	-
SA096	Fluke	Sikensi	*S. bovis*	*S. bovis*	-
SA097	Fluke	Sikensi	*S. bovis*	*S. bovis*	-

SA105	Fluke	Sikensi	*S. bovis*	-	-
SA106	Fluke	Sikensi	*S. bovis*	*S. bovis*	-
SA107	Fluke	Sikensi	*S. bovis*	*S. bovis*	-
SA108	Fluke	Sikensi	*S. bovis*	*S. bovis*	-
SA109	Fluke	Sikensi	*S. bovis*	*S. bovis*	-

Table S2. Statistical comparison by linear model of between versus within host genetic diversity in Ferkessédougou. Test statistics of the overall test are reported as well as the pairwise contrasts between the "between group" (B, samples from across the host population) and the three cattle hosts of the "within group" (W1-3). A_r, mean allelic richness; H_o, mean observed heterozygosity; H_e, mean estimated heterozygosity; F_{IS}, fixation index.

	Overall test			B vs. W1		B vs. W2		B vs. W3	
Measure	*Df*	*F*	*p*	*t*	*p*	*t*	*p*	*t*	*p*
H_e	3	5.03	0.0025	-3.53	0.0006	-1.136	0.2581	-2.291	0.0235
H_o	3	5.45	0.0014	-1.056	0.2928	-1.44	0.1520	3.16	0.0020
A_r	3	10.45	<0.0001	-0.52	0.6044	-2.65	0.0090	-5.29	<0.0001
F_{IS}	3	4.08	0.0083	-1.18	0.2391	1.05	0.2981	-2.9	0.0039

Table S3. F_{ST} (above diagonal) and R_{ST} (below diagonal) of populations of *Schistosoma* from within eight cattle hosts. Miracidia from live cattle from farms, flukes from slaughtered cattle from abattoirs in Ferkessédougou, Côte d'Ivoire. Values between life-stages are in italics.

	Cfr20	**Cfr78**	**Cfr84**	**195**	**232**	**236**	**BV34**	**BV04**
Cfr20 (flukes)		-0.003	0.019	*0.011*	*0.016*	*0.011*	*-0.002*	*0.047*
Cfr78 (flukes)	-0.014		0.017	*0.023*	*0.010*	*0.008*	*-0.002*	*0.030*
Cfr84 (flukes)	0.022	0.041		*0.043*	*0.055*	*0.038*	*0.019*	*0.053*
195 (miracidia)	*0.000*	*0.006*	*0.011*		0.044	0.039	0.017	0.044
232 (miracidia)	*-0.028*	*-0.013*	*-0.005*	0.006		0.028	0.039	0.034
236 (miracidia)	*-0.006*	*0.018*	*0.000*	0.003	-0.023		0.029	0.063
BV34 (miracidia)	*-0.011*	*0.000*	*0.048*	0.026	-0.030	0.003		0.033
BV04 (miracidia)	*-0.002*	*0.037*	*0.017*	0.013	-0.039	-0.016	-0.022	

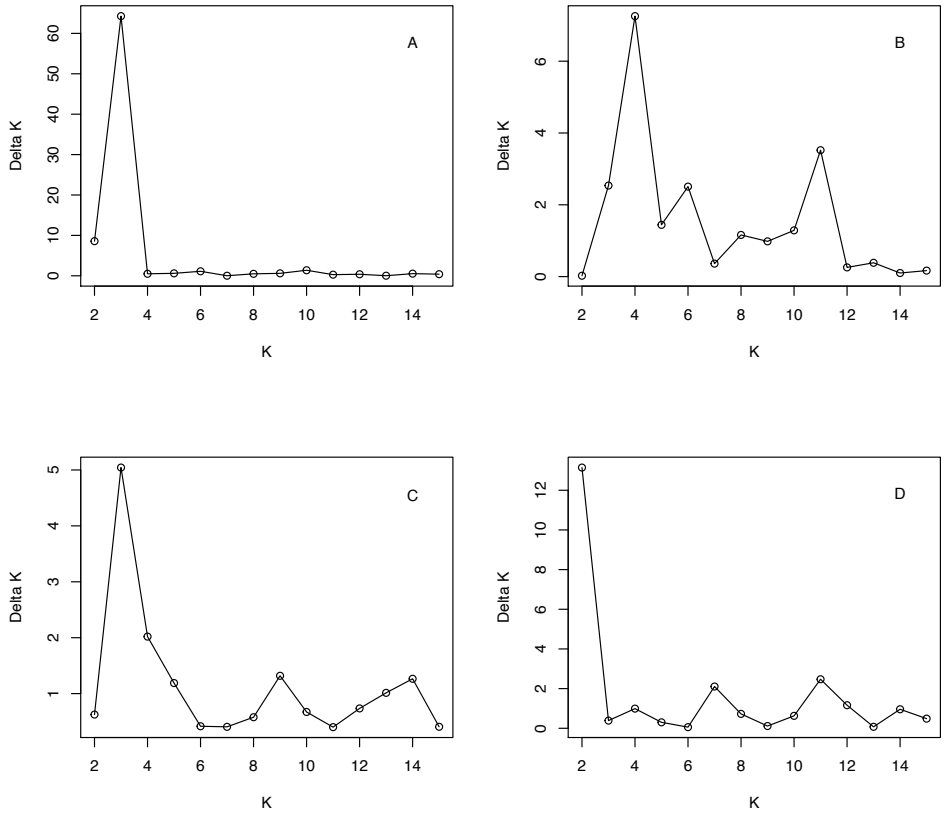

Figure S1. Determination of Best K (largest Delta K) for STRUCTURE analyses depicted in (clockwise from top left) Fig. 2 (A), Fig. 4 (B), Fig. 7 (C) and Fig. 9 (D) using Evanno's method.

Chapter 6c

Genetic Characterization of *Schistosoma* from humans in Côte d'Ivoire

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Working Draft

Abstract

Schistosomiasis is a zoonotic parasitic infection that is a great burden to human health, especially in sub-Saharan Africa. Recently, hybrids between human and livestock schistosomes have been identified, particularly in West Africa. We sampled humans in five locations across Côte d'Ivoire to determine what species of schistosomes were present in humans and to explore genetic diversity and structuring of these populations. Urine from humans was collected and 77 miracidia from Ferkessédougou, 495 from Adzopé, 501 from Agboville, 558 from Duekoué, and 610 from Sikensi were analyzed. In Ferkessédougou 58% of miracidia had mitochondrial DNA from *S. haematobium*, while the other 42% had mitochondrial DNA from *S. bovis*. Only 15% were heterozygous at the *ITS/2* locus, however three of those had traces of *S. curassoni*. Some degree of hybridization was seen in 40% of miracidia from 65% of hosts in Ferkessédougou and 65% of miracidia from the other four sites displayed some degree of hybridization. Overall, high levels of genetic diversity were observed across all five sites, with indications of inbreeding. Structuring and differentiation were present between the Northern and Western sites (Ferkessédougou and Duekoué, respectively) and the Southern sites (Adzopé, Agoville and Sikensi). Hybrids of livestock and human schistosomes are present in humans across Côte d'Ivoire at high prevalences, indicating a stable hybrid zone. Animal reservoirs may pose a risk to control and prevention efforts in Ivorian humans, however, their exact role remains unclear. A better understanding of the role of all hosts, including livestock, wildlife and non-human primates in schistosomiasis transmission is needed in order to effectively plan control and prevention efforts for humans.

Introduction

Schistosomiasis is a parasitic infection of mammals caused by 22 species of the *Schistosoma* genus. Of those, eight are known to infect humans with only five present on the African continent. Yet only two are of significant public health importance in sub-Saharan Africa, *Schistosoma haematobium* and *S. mansoni* (Standley et al. 2012b).

Schistosomiasis is a neglected tropical disease (NTD) that affects 230 million humans in 78 countries with over 90% of infections that require treatment occurring in sub-Saharan Africa (Colley et al. 2014; WHO 2022) and a burden of 1.43 million disability adjusted life years (DALYs) worldwide (GBD 2017 DALYs and HALE Collaborators 2018). The morbidity of schistosomiasis is mainly due to errant eggs, which instead of exiting the body with the urine or feces, become lodged in the organs of the body and calcify over time, causing inflammation and pathology (WHO 2017). This process can lead to hepatitis, splenomegaly, fibrosis of the urinary tract and damage to the kidney and reproductive tracts. The medical sequelae are particularly

concerning in children, who can experience anemia, stunting and a reduced ability to learn (WHO 2017).

Mammalian hosts are infected by *Schistosoma* cercariae, shed from snails in fresh water, which penetrate the skin, therefore, one must only have contact with fresh water to be infected. Domestic activities, such as washing clothes and dishes, fishing and swimming are risk for factors for schistosomiasis infection, which affects mostly poor, rural communities (WHO 2017).

The WHO roadmap for elimination of NTDs is an integrated approach that guides the world response to NTD control and includes schistosomiasis. It combines treatment interventions with other control measures and aims to impact the Sustainable Development Goals (WHO, 2010). The WHO recommends five strategies for the control and prevention of NTDs: a) preventive chemotherapy, b) intensified case-management, c) vector control, d) provision of safe water, sanitation and hygiene and e) veterinary public health (WHO, 2010). Due to these efforts, significant resources are devoted to the control and prevention of schistosomiasis and include improved access to safe water and hygiene (WASH), snail control, health education, behaviour change and most commonly, regular mass drug administration (MDA) of praziquantel, the recommended treatment (Rollinson et al. 2013; WHO 2017).

Côte d'Ivoire is a West African country which is endemic for both *S. mansoni* and *S. haematobium.* A significant effort to map schistosomiasis was undertaken for the first time in Côte d'Ivoire in 2012, in response to the WHO road map, to plan for control measures. Prevalences of schistosomiasis in humans is focal and was more recently estimated to be between 9-35% in Côte d'Ivoire (Chammartin et al. 2014).

Schistosomiasis also affects livestock and in sub-Saharan Africa there are three *Schistosoma* species of veterinary concern: *S. bovis*, *S. curassoni* and *S. mattheei*. *S. bovis* is ubiquitous across Africa, while *S. mattheei* exists mostly south of the 10th parallel and *S. curassoni* is found primarily in West Africa (Standley et al. 2012a; Leger and Webster 2017). Worldwide, it is thought that 165 million cattle are infected. Schistosomiasis in livestock has been reported through-out Côte d'Ivoire and prevalences ranges from 0 to 35% (Achi et al. 2003; Kouadio et al. 2020).

While hybrids between human and animal schistosomes has been suspected by researchers since the early 1940's, the development of molecular methods has verified these observations and recently hybrids of human and livestock *Schistosoma* species (confirmed by molecular methods) have been reported in the West African countries of Benin, Cameroon, Côte d'Ivoire, Mali, Niger, Senegal (Léger et al., 2017; Boon et al., 2019; Tian Bi et al., 2019; Angora et al., 2019; Savassi at al., 2020).

Hybridization between livestock and human schistosomes is concerning for a number of reasons. Firstly, animal reservoirs for human-infective parasites could potentially disrupt current

prevention and control efforts. Secondly it is thought that hybrids may acquire advantageous traits, such as higher fecundity, faster maturation time or wider host range which can affect pathogenicity, prevalence or treatment, also known as hybrid vigour (Huyse et al. 2009). However, at this time it is unclear in which hosts hybridization occur and how often, the host specificity of hybrids and if hybrids are transmitted between different definitive host species. This investigation aimed to identify the species of schistosomes and genetically characterize schistosomes found in human populations at five sites in the north, south and west parts of Côte d'Ivoire.

Materials and Methods

Study sites and sampling

This study was conducted in the West African country of Côte d'Ivoire. Côte d'Ivoire has a population of 25 million people and 1,341,000 cattle and is located on the coast of the Gulf of Guinea (Food and Agricutlure Organization 2005; World Bank 2019). This investigation took place in the Northern Department of Ferkessédougou, where cattle ranching is a significant industry (Food and Agricutlure Organization 2005). In addition to the sedentary Ivorian pastoralists in this area, mobile pastoralists from Mali and Burkina Faso migrate to this region in the dry season to graze their livestock (Bassett 2009).

Five sites were sampled across Côte d'Ivoire, four sites in the south, Adzopé, Agboville, Duekoué and Sikensi, as well as one in the north, Ferkessédougou (Fig. 1).

Feces and urine were collected from humans and cattle in two villages, Village B and Village C, and the villages' farms. The farms are 0.1-5 km from the villages, and they share one water source. Villagers are involved in the care of the herd with some staying on the farms for short periods of time when attending to livestock. The livestock are bred and reared on the same farm for their lifetime.

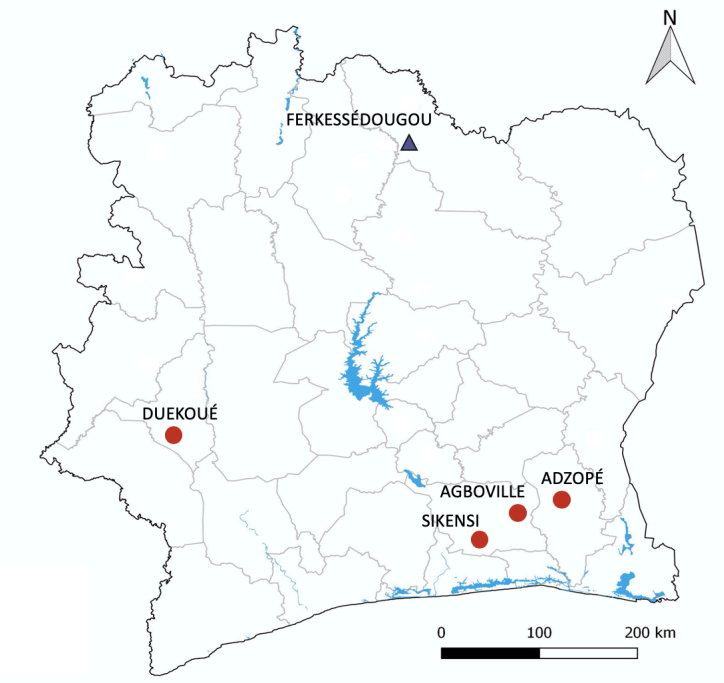

Figure 1. Map of Côte d'Ivoire, West Africa indicating sampling sites for *Schistosoma* specimens. Red circles indicate sampling sites for school age children, purple triangle indicates sampling site for people of all ages.

Specimen collection and storage

In the four southern sites, sampling occurred between January and April 2018. Mid-day urine samples were collected from 1,187 schoolchildren aged 5-14 years (Agboville 402, Adzopé 208, Duekoué 372 and Sikensi 205). Urine samples were screened using the filtration method (Mott et al. 1982), briefly 10 ml of urine was vigorously shaken and filtered through a 40 µm Nytrel filter and examined at 100x under a compound microscope for *Schistosoma* eggs.

Nineteen children with positive urines were randomly selected for the genetic analysis and their urine was subjected to for miracidia hatching as previously described (Adzopé 3, Agboville 5, Duekoué 5, Sikensi 6) (Webster et al. 2012; Boissier et al. 2016; Angora et al. 2020). Briefly, using a stereomicroscope, eggs were transferred from the filter to a Petri dish with tap water, miracidia were collected individually in 3 µl of water and deposited onto a Whatman FTA card (GE Healthcare Life Sciences; Amersham, UK) and were transferred to the University of Perpignan in France for analysis. In the North, the first urine of the day was collected from 146 participants (no age limitations) and 10 ml of urine was screened using the filtration method (Plouvier et al. 1975). The remaining urine from positive samples were filtered through a 30 µm mesh filter into a petri dish and put in semi-shady area for one hour. Samples were examined for miracidia after one hour and every hour thereafter for four hours. Miracidia were collected and stored as described above and were transferred to the Swiss Tropical and Public Health Institute for analysis.

DNA extraction

DNA extraction of miracidia was done using a Chelex protocol (Merck KGaA. Darmstadt. Germany). A 3 mm Harris Uni-core micro punch was used to cut out the spot where the miracidia was deposited on the FTA indicating card. The punch was placed in a 1.5 ml Eppendorf tube with 100 µl of ultra-pure water and left to sit at room temperature for 10 minutes. The water was removed with a pipette and then rinsed once more as just described. Afterwards, 80 µl of 5% Chelex solution was added (Chelex solution was mixed on a magnetic agitator to allow distribution of Chelex beads) and incubated at 65 degrees Celsius with agitation for at least 30 minutes. The temperature was increased to 99 degrees Celsius for a final eight minutes and then the solution was centrifuged at 13,000 rotations per minute (rpm) for 2 minutes. Finally, 60 µl of DNA was pipetted into a new Eppendorf with 5 µl of Low Tris-ethylenediaminetetraacetic acid (EDTA) and stored at -20 degrees Celsius.

PCR species identification

A multiplex *cox1* polymerase chain reaction (PCR) was used to determine species by band length (*S. haematobium* 120 base pairs (bp)*; S. manonsi* 215 bp; *S. bovis* and *S. curassoni* 260 bp). The *cox1* region was amplified in a 10 µl PCR containing 0.5 µl DNA, 5.0 µl of Qiagen Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 3.5 µl water, 1µl of primer mix (4 µl of each primer (100 µM) in 84 µl double distilled water; three forward primers: Sh.F 5'-GGTCTCGTGTATGAGATCCTATAGTTTG -3'; Sm.F 5'-CTTTGATTCGTTAACTGGAGTG -3'; Sb.F 5'- GTTTAGGTAGTGTAGTTTGGGCTCAC -3' and one reverse primer: Shmb.R R5'- CAAGTATCATGAAAYARTATRTCTAA -3'). The PCR protocol was initiated by 3 min at 95 degrees Celsius; followed by 45 cycles of 95 degree Celsius for 10 secs, 52 degree Celsius for 30 secs and 72 degree Celsius for 10 mins and a final extension of 72 degree Celsius for 2 mins.

The PCR product was loaded onto a 2% agarose gel (Promega, Madison, Wisconsin) with 0.5X Tris-borate-EDTA (TBE) (Thermo Scientific, Waltham, Massachusetts) and run for 25 mins at 75 V. Agarose gels were soaked in GelRed (Merck KGaA, Darmstadt, Germany) for at least 30 minutes before being visualized.

A random selection of specimens from the North were further selected for PCR and sequencing of the *ITS1/2* and *cox1* loci. Sequences from the *ITS1/2* marker were used to confirm species by using five variable sites known to distinguish *S. haematobium* from *S. bovis*, of which, one site also distinguishes *S. bovis* from *S. curassoni* (Huyse et al. 2009; Webster et al. 2013). Sequencing of the *cox1* locus was used for phylogeny. The *ITS2* regions of samples from the south and west were characterized using RFLP.

The *ITS1/2* region was amplified in a total volume of 10 µl consisting of 1 µl BD 10X buffer (Solis BioDyne, Tartu, Estonia), 1.3 µl of 25mM $MgCl_2$, 0.8 µl of 10mM dNTP mix (2.5 mM of each dNTP), 0.1 µl of 5U/ml FirePol® Taq polymerase (Solis BioDyne, Tartu, Estonia), 0.5 µl DNA, 5.3 µl water and 0.5 µl of each forward (ITS5: F5'-GGAAGTAAAAGTCGTAACAAG -3') and reverse primers (ITS4 R5'-TCCTCCGCTTATTGATATGC -3'). The PCR protocol was initiated with 4 mins at 94 degrees Celsius; followed by 40 cycles of 30 secs at 94 degrees Celsius, 30 secs at 54 degrees Celsius and 30 secs at 72 degrees Celsius and a final extension of 2 mins at 72 degrees Celsius. PCR reactions were sent to Microsynth (Balgach, Switzerland) for uni-directional Sanger sequencing using reverse primer.

ITS1/2 sequences were compared to other sequences in GenBank using the Basic Local Alignment Search Tool (BLAST) and then aligned and analyzed in CodonCode Aligner version 6.0.2 (CodonCode Corporation, Centerville Massachusetts). *ITS1/2* trace files were inspected to determine heterozygosity at the five variable sites.

Microsatellite analysis

Specimens from all sites were subjected to PCR and sequencing in Perpignan using two panels of microsatellites (Webster et al. 2015), from those, one miracidium from each host was randomly selected to be included in the analysis.

The protocol for these two multiplex PCRs were the same: 5 µl Qiagen Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 1 µl DNA, 3 µl double distilled water and 1 µl 10uM primer mix. The PCR protocol was initiated by 15 mins at 95 degrees Celsius; followed by 45 cycles of 94 degrees Celsius for 30 seconds, 56 degrees Celsius for 1.5 mins and 72 degrees Celsius for 1 min and a final extension of 72 degrees Celsius for 5 minutes. PCR reactions were sent to Microsynth (Balgach, Switzerland) for fragment length analysis using the Applied Biosystems 3730xl DNA Analyzer, where PCR reactions were diluted 1:20 and size standard GS500LIZ was added.

PCR reactions were sent to Genoscreen (Lille, France) for genotyping and size standard GS500LIZ was added.

Microsatellite peaks were called in Genemarker (Southern sites) and Genemapper v. 6.0, ThermoFisher Scientific) (Northern site), size standard GS500LIZ. Automatic bin calling was established based on the samples, and all automatically called peaks were confirmed by visual inspection. As mentioned by Angora et al. (in preparation), loci C131 and Shae08 were removed from the analysis due to poor amplification. Furthermore, loci Shae08, Shae15 and C131 were removed from the analysis as they did not amplify well in northern samples (Tab. 1).

Table 1. *Schistosoma* microsatellite panels, markers used, allele size ranges (base pairs) and fluorescent dye.

Panel	Marker	Allele range	Fluorescent dye
Panel 1	C102	184-196	HEX (green)
	C111	203-221	ATTO565 (red)
	Shae_01	243-279	HEX (green)
	Shae_03	275-305	FAM (blue)
	Shae_06	308-322	ATTO550 (yellow)
	Shae_07	298-307	ATTO565 (red)
	Shae_09	196-223	FAM (blue)
	Shae_14	186-222	ATTO550 (yellow)
Panel 2	Shae_02	167-194	ATTO550 (yellow)
	Shae_04	264-313 OUT	FAM (blue)
	Shae_05	268-304	ATTO550 (yellow)
	Shae_10	183-195	ATTO565 (red)
	Shae_11	189-201	HEX (green)
	Shae_12	244-265	ATTO565 (red)
	Shae_13	163-205	FAM (blue)

Genetic diversity

The allele number (A), rarefied allelic richness (Ar), expected heterozygosity (He), observed heterozygosity (Ho), Hardy Weinberg equilibrium (HWE) and fixation index (F_{IS}) were calculated in RStudio using the package diveRsity (v. 2.9.3.2 ; v.1.9.90) (Keenan et al. 2013; R Core Team 2013). All indices were calculated by loci and by population. Statistical significance was calculated by x^2 in diveRsity and significance was set at <0.05.

A sub-sample of one *Schistosoma* miracidium from each host were randomly selected and the genetic diversity indices were calculated. This random selection of a sub-sample was performed 100 times and the averages of the genetic diversity indices were calculated.

Allele number (A), allelic richness (Ar), fixation index (FIS), expected heterozygosity (He), observed heterozygosity (Ho), Hardy-Weinberg equilibrium (HWE) were calculated in R using the package diveRsity (1.9.90) (Keenan et al. 2013; R Core Team 2013).

Genotypic differentiation

Differentiation was measured by F_{ST} and $R_{ST,}$ which were calculated in RStudio using the package package Genepop (v.1.1.7) (Rousset 2008). Genepop used the following parameters: Minimum distance regression was 0.0001 and number of permutations for Mantel was 1000. Statistical significance was also calculated Genepop with the following Markov chain parameters: dememorization was set at 10,000, 100 batches and 5,000 iterations per batch. Genotypic differentiation for each population was pair was tested with the exact G test. As described above, genotypic differentiation indices were also calculated on 100 random sub-samples, and the average was calculated and reported.

Population structure

The structure analysis was completed using Structure (v. 2.3.4) (Pritchard et al. 2000). All structure analyses were run with a burn-in of 10,000 and 1,000,000 iterations after burn-in. Each K was run 5 times, and then the best K determined by the Evanno method in CLUMPAK (Kopelman et al. 2015) and the best K as determined by Delta was run another 10 times. These results were used to produce the STRUCTURE plot in Clumpak. The DAPC plots were calculated in RStudio using the package adegenet (v. 2.1.3) (Jombart 2008; R Core Team 2013).

Ethical considerations

This study was part of larger project that investigated transmission dynamics of trematodes in humans and livestock Côte d'Ivoire. Ethical approval was obtained from the Comité National d'Ethique de la Recherche (CNER) of Côte d'Ivoire and the Ministère de la Santé et de l'Hygiène Publique de Côte d'Ivoire (reference no. 035/MSH /CNER-kp, reference no. 003–18/MSHP/CNER-kp) and Switzerland (reference no. UBE-2016-00707).
Community leaders, school authorities, teachers, parents/guardians, and participants were informed about the objectives, procedures, and potential risks and benefits of the study after which written informed consent was obtained by the participants, and in the case of children, their oral consent as well as written consent of their legal guardians Following sampling, a praziquantel treatment (40 mg/kg) was offered to those who tested positive for schistosomiasis.

Results

Schistosoma *species identification*

In Ferkessédougou 77/86 (90%) human miracidia produced bands in the multiplex *cox1* PCR (Tab. 2).

Table 2. Locations, number of human hosts and number of *Schistosoma* miracidia included in analysis.

Location	# of hosts	# successful in *cox1*	# successful in *ITS* sequenced	# successful in microsatellites
Ferké	23	77	27	65
Adzopé	?	495	495	440
Agboville	?	501	501	465
Duekoué	?	558	558	527
Sikensi	?	610	610	535

Of those that produced bands, 58% (45/77) of miracidia displayed a band of 120bp in length, indicating that they have mitochondrial DNA from *S. haematobium*, while 42% (32/77) displayed a band of 260bp length, indicating that they have mitochondrial DNA of *S. bovis or S. curassoni*.

Of the 47 samples sequenced for *ITS1/2*, 26 produced good quality sequences, 22 (84.6%) conformed to the pattern of homozygous *S. haematobium*, including 13 that produced bands for *S. bovis* on the *cox1* multiplex PCR. Some degree of heterozygosity at the 5 variable sites was observed in 15% (4/26) of samples, including two samples that had produced bands indicating that they have *S. haematobium* mitochondrial DNA.

Of note, 3/4 of the samples that were heterozygous at *ITS1/2* loci had an "A" at the variable site in the *ITS1* marker (variable site 1). This marker also distinguishes between *S. Bovis* (G) and *S. curassoni* (A), however at this site *S. curassoni* and *S. haematobium* display the same nucleotide (A) (Tab. 3).

Table 3. *Schistosoma ITS1/2* variable sites for species determination. Y, heterozygous T and C; R, heterozygous A and G. * Sample SM102 was a short sequence that only covered 2 variable sites (3 and 4), Site 2 wasn't called but sequence looked fine in trace file and was heterozygous. -, no sequence available; na not applicable.

Specimen	*ITS1/2* Variable Sites					*Cox1* Multiplex PCR result
	1 (52 bp)	2 (709 bp)	3 (764 bp)	4 (814 bp)	5 (884 bp)	
SM017	A	R	Y	G	C	*S. bovis*
SM019	A	R	Y	R	Y	*S. bovis*
SM029	A	R	Y	R	Y	*S. haematobium*
SM102	-	R	Y	R*	-	*S. haematobium*
S. Haematobium	A	G	C	G	C	na
S. Bovis	G	A	T	A	T	na
S. Curassoni	A	A	T	A	T	na

This indicates that the hybrids are either introgressed *S. haematobium* x *S. bovis* hybrids or *S. haematobium* x *S. curassoni* hybrids. At least one indication of hybridization was seen in 40% (34/86) samples from 65% (15) of hosts. No pure *S. bovis* were found (both *cox1* and homozygous *ITS2* were *S. bovis*).

Species identification for samples collected in Agboville, Adzopé, Duekoué and Sikensi are reported in Angora et al. (2020). In short, 84% (2164/2561) of miracidia from 91 children were successfully profiled using the multiplex *cox1 and ITS2* RFLP profiling (Tab. 4). Overall, 36.6% were pure *S. haematobium* (*S. haematobium* at *cox1* and *ITS*), 0.7% were pure *S. bovis* (*S. bovis* at *cox1* and *ITS*), and 63% had at least one indication of hybridization.

Table 4. Proportion of *Schistosoma* miracidia (by site) that conform to six possible genetic profiles as determined by *cox1*(haploid mitochondrial DNA) and *ITS1/2* (diploid nuclear DNA) loci. Sb x SbSb = *S. bovis cox1*, homozygous *S. bovis* at *ITS1/2*; Sh x ShSh = *S. haematobium cox1*, homozygous *S. haematobium* at *ITS1/2*; Sb x ShSb = *S. bovis cox1*, heterozygous *S. haematobium* and *S. bovis* at *ITS1/2*; Sb x ShSh = *S. bovis* cox1, homozygous *S. heamatboium* at *ITS1/2*; Sh x SbSb = *S. haematobium cox1*, homozygous *S. bovis* at *ITS1/2*; Sh x ShSb = *S. haematobium cox1*, heterozygous *S. haematobium* and *S. bovis* at *ITS1/2*.

	Sb x SbSb n (%)	**Sh x ShSh n (%)**	**Sb x ShSb n (%)**	**Sb x ShSh n (%)**	**Sh x SbSb n (%)**	**Sh x ShSb n (%)**	**Total hybrids**	**Total**
Ferkessédougou	0	9 (34.6)	2 (7.7)	13 (50)	0	2 (7.7)	17 (65.4)	26
Duekoué	0	209 (37.5)	19 (3.4)	314 (56.3)	3 (0.54)	13 (2.33)	349 (62.5)	558
Adzopé	3 (0.6)	101 (20.4)	47 (9.5)	320 (64.7)	4 (0.8)	20 (4.0)	391 (79.0)	495
Agboville	9 (1.8)	170 (33.9)	59 (11.8)	90 (18.0)	34 (6.8)	139 (27.7)	322 (64.3)	501
Sikensi	3 (0.5)	311 (51.0)	49 (8.0)	184 (30.2)	2 (0.33)	61 (10.0)	296 (48.5)	610
Total	15 (0.6)	800 (36.5)	176 (8.0)	921(42.1)	43 (2.0)	235 (10.7)	1375 (62.8)	2190

When considering all five sites together, hybrids accounted for 62.8% of the samples and the most common profile was *S. bovis* at *cox1* and *S. haematobium* at *ITS* (42.1%), which was also the most common hybrid profile at all sites individually, except Agboville. Sikensi has the highest proportion of miracidia that were pure *S. haematobium* (51.0%), and also the lowest proportion of hybrids (any indication of hybridization) (48.5%) followed by Duékoué (37.5% and 62.5%), Ferkessédougou (34.6% and 65.4%), Agboville (33.9% and 64.3%) and Adzopé (20.4% and 79.0%) (Tab. 4). There doesn't appear to be any geographical trend, as the two sites at the extremes (Sikensi and Adzopé) are located close to one another. Pure *S. bovis* was found at low proportions in Adzope (0.6%), Agboville (1.8%) and Sikensi (0.49%), but not in Duékoué and Ferkessédougou.

The highest proportion of miracidia that exhibited a *S. bovis* or *S. curassoni* profile at the *cox1* maker were found in Adzopé (67%) (south), followed by Duékoué (51%) (west), Sikensi (45%) (south), Ferkessédougou (42%) (north), and Agboville (34%) (south) (Tab. 5).

Table 5. Proportion of *Schistosoma* miracidia from the five sampling sites displaying signs of hybridization at the *cox1* and *ITS* loci. NYD not yet determined.

Location	**Proportion *cox1 S. bovis* (%, 95% CI)**	**Proportion *ITS S. bovis***	**Proportion *ITS* heterozygous**
Adzopé	54/81 (66.7, 55.3-76.8)	NYD	NYD
Agboville	38/111 (34.2, 25.5-43.8)	NYD	NYD
Duekoué	65/128 (50.8,41.8- 59.7)	NYD	NYD
Sikensi	63/139 (45.3, 36.9-54.0)	NYD	NYD
Ferkessédougou	32/77 (41.6, 30.4-53.4)	0	4/26 (14.4%)

At least one hybrid parasite was found in 97.8% of school children in the southern and western sites, whereas in the north 65.2% (15/23) humans were found to harbour at least one hybrid.

Genetic diversity

There is a deficiency of heterozygotes and high levels of inbreeding across all populations (Tab. 6). Sikensi has a significantly lower level of inbreeding than the other sites.

Table 6. Genetic diversity indices for *Schistosoma* from humans from six locations across Côte d'Ivoire. N, number of samples; A, average number of alleles; A_r, mean allelic richness; H_o, mean observed heterozygosity; H_e, mean estimated heterozygosity; F_{IS}, fixation index; * statistically significant deviation from Hardy-Weinberg Equilibrium using Fisher' exact test ($p< 0.05$) and non-overlapping 95%confidence intervals for F_{IS}.

Population	N	A	A_r	H_o	H_e	HW	F_{IS}
Adzopé	436	8.93 ± 3.83	6.70 ± 2.88	0.49 ± 0.19	0.61 ± 0.20	*	0.19
Agboville	462	9.21 ± 3.79	6.68 ± 2.99	0.51 ± 0.15	0.60 ± 0.16	*	0.15
Duekoué	526	10.79 ± 3.66	8.07 ± 3.19	0.53 ± 0.21	0.63 ± 0.23	*	0.14
Sikensi	528	9.36 ± 3.41	6.81 ± 3.02	0.57 ± 0.19	0.62 ± 0.18	*	0.07*
Ferkessédougou	64	8.00 ± 3.06	7.13 ± 2.78	0.51 ± 0.22	0.61 ± 0.23	*	0.17

Genetic Differentiation

There is little differentiation in the southern sites Adzopé, Agboville, Duekoué and Sikensi, however, there are high levels of differentiation between the northern site (Ferkessédougou) and Adzopé, Agboville and Sikensi, but less with Duekoué (Tab. 7).

Table 7. F_{ST} (above diagonal) and R_{ST} (below diagonal) *Schistosoma* from humans across Côte d'Ivoire.

	Adzopé	Agboville	Duekoué	Sikensi	Ferkessédougou
Adzopé		0.053	0.064	0.048	0.201
Agboville	0.042		0.072	0.043	0.221
Duekoué	0.068	0.045		0.061	0.181
Sikensi	0.034	0.028	0.042		0.210
Ferkessédougou	0.094	0.069	0.028	0.096	

There are 2 cluster and structuring seen between Ferkessédougou and Duekoué, and Adzopé, Sikensi and Agboville (Fig. 6).

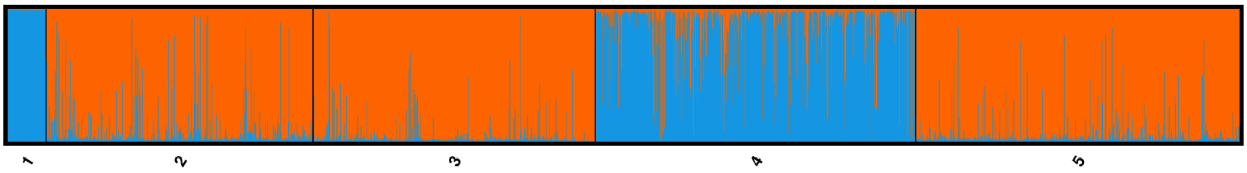

Figure 2. STRUCTURE analysis of schistosomes from humans across Côte d'Ivoire. (K=) (Best K see Supplemental Material Fig. S1), 2 runs, burn-in: 10,000; repeats after burn-in: 200,000. 1 = Ferké, 2= Adzopé, 3= Agboville, 4= Duekoué, 5= Sikensi

The DAPC shows slight differentiation between groups, with Ferkessédougou differentiated from Agboville and Sikensi.

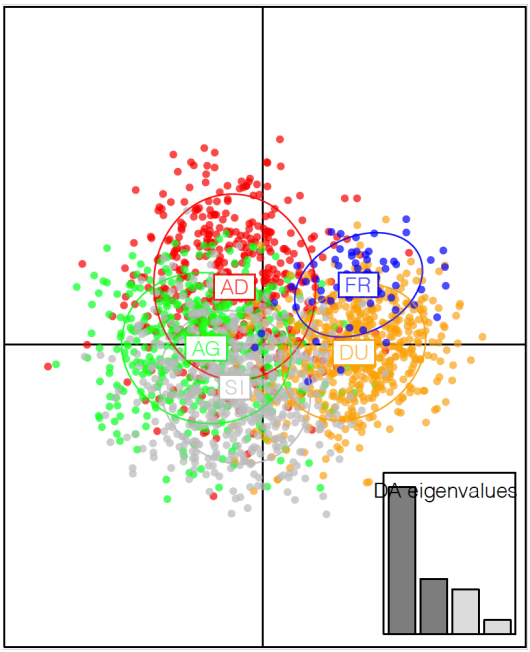

Figure 3. Discriminative analysis of principal components (DAPC) of schistosomes from humans at five sampling sites across Côte d'Ivoire.

Discussion

This analysis is an expansion of the investigation conducted by Angora et al. (2022), which explored the species and genetic diversity of schistosomes from humans at three Southern sites and one Western site in Côte d'Ivoire. Here, we have reanalyzed the data, to include one additional Northern site.

Côte d'Ivoire is endemic for *S. mansoni* and *S. haematobium*, although prevalences vary greatly and are focal (Chammartin et al. 2014). Furthermore, Ivorian cattle are known to be infected with *S. bovis* with prevalences ranging from 0 to 35% (Kouadio et al. 2020). Schistosome hybrids between humans and livestock species have been documented across Africa and Asia. Specifically, *S. haematobium* x *S. bovis* in humans have been reported in Benin (Savassi et al. 2020) and Senegal (Léger et al., 2020). Therefore, it was not unexpected to find *S. haematobium* x *S. bovis* hybrids across all five sites in Côte d'Ivoire. The most commonly observed hybrid profile was *S. bovis* at *cox1* and homozygous *S. haematobium* at *ITS* (42.1%). This is an introgressed form of hybrid, which indicates that hybridization events may occur occasionally, with lots of back-crossing in a what appears to be a stable hybrid zone.

For individual sites, this hybrid was the most common profile in general in Adzopé, Duekoué,and Ferkessédougou. In Agboville and Sikensi it was pure *S. haematobium*. While in Sikensi the most common hybrid profile was *S. bovis* at *cox1* and homozygous *S. haematobium* at *ITS*, in Agboville, the most common hybrid profile was *S. haematobium* at *cox1* and heterozygous *ITS,* which could be first generation hybrids. Agboville also had the highest

proportion of pure *S. bovis* (1.8%). This may indicate that hybridization events are occurring at certain sites (perhaps Agboville) and being transmitted to sites around the country.
Indications of hybridization were much less commonly seen at the *ITS1/2* loci. Interesting, not all miracidia that showed *S. bovis* at the *cox1* loci were heterozygous at the *ITS1/2* loci, while some miracidia that presented *S. haematobium* at the *cox1* loci were heterozygous at *ITS1/2*. This indicates that mitochondrial DNA of hybrids in humans can be either *S. haematobium* or *S. bovis* and therefore, testing the *cox1* loci alone is not enough to determine hybrid status. Furthermore, this result in combination with the pure *S. bovis* that was found suggests that when the male schistosome is *S. bovis*, the schistosome may still dwell in the urogenital system.
Traces of *S. curassoni* were found in humans in Ferkessédougou, however, we are not able to compare this with samples from the South as the variable site used to distinguish the two species is found in *ITS1*, which was not sequenced in the samples from the South. Pure *S. curassoni* was not seen in humans in Ferkessédougou either.
In the Ferkessédougou, 34/77 (44.2%) samples from 15 (65.2%) hosts had at least one indication of hybridization (either *cox1* or *ITS1/2*)**.** While in the south 48% of miracidia in 97.8% of participants had a signature of *S. bovis* or *S. curassoni* in the mitochondrial genome. However, this result must be interpreted with care as the sample size in the north was limited and there were 77 miracidia from 23 hosts analyzed.
Contrary to other investigations conducted in, pure *S. bovis* was found in Côte d'Ivoire at all sites except Ferkessédougou and Duekoué, although in Ferkessédougou this could be due to the small sample size.
Interestingly, the results from cattle revealed no hybrid schistosomes or pure *S. haematobium* in cattle in Agboville, Duekoué, Ferkessédougou or Sikensi. All schistosomes that were subjected to *cox1* amplification and *ITS1/2* sequencing were pure *S. bovis.* Furthermore, the discovery of possible *S. curassoni* in humans in the hybrids in the north was unexpected as schistosomes from cattle at this site, and all sites across Côte d'Ivoire appeared to be pure *S. bovis*. This could be due to several reasons: may likely be imported from other regions where *S. curassoni* is found, although this should be further tested with *cox1* phylogeny and 18S sequencing. Although we are not certain that there are no *S. curassoni* hybrids in the South, there are indications that hybridization may be occurring in humans, as there are pure *S. bovis* and *S. haematobium* in humans.
Schistosome hybrids are concerning as they indicate that animals may be reservoirs for infections that can be transmitted to humans, which would compromise the great efforts to reach elimination by 2030, as outlined by the WHO roadmap. Furthermore, Catalano et al. (2018) found that livestock x humans schistosome hybrids have been found in rats, begging the question if wildlife are a potential reservoir for human disease, and if so, what exactly is their role.

In Angora et al. (2020) the four sites in the South appear distinct, yet when we add the northern site, we see that Ferkessédougou is quite distinct from Adzopé, Agboville and Sikensi, while Duekoué is slightly distinct from the other southern sites, but less distinct than the northern site. There is inbreeding and an excess of homozygotes across all five sites. Glenn et al. (2013) compared a small sampling from Senegal, Zanzibar, Malawi, Nigeria, Mauritius and South Africa, and found low F_{IS} (none significantly different than zero) and no significant deviation from HWE either, but this may have been due to the small sample size. Interestingly, Sikensi has the highest proportion of pure *S. haematobium* miracidia, yet a significantly lower level of inbreeding than all other sites.

Giovanoli et al. (in preparation) analyzed schistosomes from cattle at four of the five sites and found that the humans schistosomes are more structured and distinct than the *S. bovis* found in cattle.

Our investigation has several limitations. First, the sample size in the north was limited, making it difficult to generalize and possibly leading to us having missed pure *S. bovis* in humans. Secondly, our sampling site cover the south, west and north, but the central, northwestern and northeastern parts of the country were not included, and the situation may be very different there. Lastly, our sampling in the south only included school-aged children, whereas the sampling in the north included people of all ages. Furthermore, it is likely that the presence of hybrids may have affected the analyses that used microsatellites as species other than *S. haematobium* don't necessarily amplify well with these primers.

Conclusion

Hybrids between humans and cattle schistosome species exist in humans in both Northern and Southern Côte d'Ivoire, however, pure *S. bovis* did not appear to occur in the north or in the west**.** The WHO recommends veterinary public health as a strategy to combat NTDs, yet this is seldom put into practice. Many resources are devoted to the control and prevention of human schistosomiasis, however, it is important that all potential reservoir hosts are studied and targeted for control programmes in order to not risk current effort in humans.

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Chapter 6d

Host specificity and genetic exchange of *Schistosoma* species found in humans and cattle in Northern Côte d'Ivoire

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Abstract

Schistosomiasis is a zoonotic parasitic infection that infects humans and livestock and has been declared a neglected tropical disease by WHO. Hybrids between livestock and human schistosome species present a threat to current prevention and control efforts in humans. We aimed to determine the species of schistosomes found in humans and cattle who share the same water source, as well as quantify their genetic diversity and population structure using mitochondrial (*cox1*) and nuclear DNA (*ITS1/2*) as well as microsatellites. We collected 86 miracidia from 23 humans and 114 miracidia from 39 cattle. 58% of miracidia from humans indicated that they had mitochondrial DNA of *S. haematobium* in the *cox1* PCR, while the other 42% produced results indicating that they had mitochondrial DNA from *S. bovis or S. curassoni*. *ITS1/2* sequencing of 27 human samples revealed that 85% of specimens conformed to the pattern of *S. haematobium*, and four samples were heterozygous for *S. bovis* and *S. haematobium* at the 5 variable sites. Three of the four heterozygous samples had traces of *S. curassoni*. All miracidia from cattle had mitochondrial DNA of *S. bovis* or *S. curassoni* and all four samples that were subjected to *ITS1/2* PCR were consistent with the pattern for *S. bovis* at all the variable sites. Populations from cattle and humans both showed signs of inbreeding and a distinct structure between the two populations. There appears to be no transmission of schistosomes between cattle and humans in the North of Côte d'Ivoire, as it appears that livestock x human schistosome hybrids are circulating in the human population only. However, this should be monitored to ensure that cattle do not become reservoirs for human schistosomiasis.

Introduction

Schistosomiasis is a zoonotic, parasitic infection found in humans, livestock and wildlife in sub-Saharan Africa. Of the eight *Schistosoma* species known to infect humans, five are present on the African continent, yet only two are of significant public health importance, *Schistosoma haematobium* and *S. mansoni* (Standley et al., 2012).

There are eight species of veterinary concern, with five of particular importance to livestock and three of those are present in Africa (*S. bovis*, *S. curassoni* and *S. mattheei*). *S. bovis* is ubiquitous across Africa, while *S. mattheei* exists mostly south of the 10th parallel and *S. curassoni* is found primarily in West Africa (Leger and Webster, 2017; Standley et al., 2012).

Schistosomiasis affects 230 million humans and 165 million cattle worldwide, with the bulk of human infections occurring in Africa (Colley et al., 2014; De Bont and Vercruysse, 1997). Schistosomiasis is a neglected tropical disease with a burden of 1.43 million disability adjusted life years (DALYs) worldwide. Over 90% of cases are known to occur in sub-Saharan Africa (GBD 2017 DALYs and HALE Collaborators, 2018; WHO, 2017). Control strategies include improved access to safe water and hygiene (WASH), snail control, health education, behaviour change and most commonly, regular

mass drug administration (MDA) of praziquantel, the recommended treatment (Rollinson et al., 2013; WHO, 2017).

Côte d'Ivoire is a West African country where human schistosomiasis is endemic and currently regular MDAs are being carried out**.** Prevalences are focal and range between 0 to 40% (Assaré et al., 2016, 2015).

There is a lack of data regarding schistosomiasis in livestock in Côte d'Ivoire. Achi et al. (2003) found the prevalence of schistosomiasis in slaughtered cattle in the North to be 35%. More recently, Kouadio et al. (2020) found the prevalences in the south and north to range from 5.9 - 53.3% in slaughtered cattle, and 0 -2.4% in the north in live cattle.

Recently hybrids of human and livestock *Schistosoma* species have been found in West Africa in Benin, Cameroon, Côte d'Ivoire, Mali, Niger, Senegal (Léger et al., 2017; Boon et al., 2019; Tian Bi et al., 2019; Angora et al., 2019; Savassi at al., 2020). Hybridization between livestock and human schistosomes is concerning for a number of reasons. Firstly, animal reservoirs for human-infective parasites could potentially disrupt current prevention and control efforts. Secondly it is thought that hybrids may acquire advantageous traits, such as higher fecundity, faster maturation time or wider host range which can affect pathogenicity, prevalence or treatment, also known as hybrid vigour (Huyse et al., 2009).

At this time it is unclear in which hosts hybridization occurs, host specificity of hybrids, how often it occurs and if hybrids are transmitted between different definitive host species.

This investigation aimed to determine to what extent humans and cattle are involved in the same transmission cycle of schistosomiasis. This was achieved by genetically characterizing schistosomes found in humans and cattle who share the same water source in the north of Côte d'Ivoire to answer the following questions: what species of schistosomes are found in humans and cattle and how genetically diverse and distinct are schistosome populations from cattle and humans using the same water source.

Materials & Methods

Study sites and sampling

This study was conducted in the West African country of Côte d'Ivoire. Côte d'Ivoire has a population of 25 million people and 1,341,000 cattle and is located on the coast of the Gulf of Guinea (Food and Agricutlure Organization, 2005; World Bank, 2019). This investigation took place in the Northern Department of Ferkessédougou, where cattle ranching is a significant industry (Food and Agricutlure Organization, 2005). In addition to the sedentary Ivorian pastoralists in this area, mobile pastoralists from Mali and Burkina Faso migrate to this region in the dry season to graze their livestock (Bassett, 2009).

In this investigation feces and urine were collected from humans and cattle in two villages, Village B and Village C, and the villages' farms. The farms are 0.1-5 km from the villages, and they share one water source. Villagers are involved in the care of the herd with some staying on the farms for short periods of time when attending to livestock. The livestock are bred and reared on the same farm for their lifetime.

Fig. 1. Map of sampling sites of humans and cattle in Côte d'Ivoire. Red circle indicates sampling site.

Specimen collection and storage

Sampling occurred at the farms and villages between August 2018 and July 2019.

The first urine of the day was collected from 146 human participants (50 in Village B and 96 in Village C) and 10 ml of urine was screened using the filtration method (Plouvier et al., 1975). The remaining urine from positive samples was filtered through a 30 µm mesh filter into a petri-dish and put in semi-shady area for one hour. Laboratory activities took place at the Hôpital Général de Ferkessédougou. Feces were extracted from the rectums of cattle and transported to the Laboratoire National d'Appui au Développment Agricole (LANADA) laboratories in Korhogo and Ferkessédougou for filtration.

The feces were then filtered through a mesh (400 µm) filter with bottled water, the filtrate was further filtered through the Pitchford filter (inner sieve mesh was 300 µm, outer sieve mesh was 30 µm) (for details see Giovanoli Evack et al., 2020) (Visser and Pitchford, 1972). The filtrate was drained into a Petri dish and was left in a semi-shady area for one hour, to encourage *Schistosoma* eggs to hatch. Petri dishes from humans and cattle were examined for miracidia after one hour and every hour thereafter for four hours. Miracidia were collected individually in 3 µl of water and deposited onto a Whatman indicating FTA card (GE Healthcare Life Sciences; Amersham, UK) and transferred to the Swiss Tropical and Public Health Institute, Switzerland for analysis.

DNA extraction

DNA extraction of miracidia was done using a Chelex® protocol (Bio-Rad Laboratories, Cressier, Switzerland). A 3 mm Harris Uni-core micro punch was used to cut out the spot where the miracidia was deposited on the FTA indicating card. The punch was placed in a 1.5 ml Eppendorf tube with 100 µl of ultra-pure water and left to sit at room temperature for 10 minutes. The water was removed with a pipette and then rinsed once more as just described. Afterwards, 80 µl of 5% Chelex solution was added (Chelex solution was mixed on a magnetic agitator to allow distribution of Chelex beads) and incubated at 65 degrees Celsius with agitation for at least 30 minutes. The temperature was increased to 99 degrees Celsius for a final eight minutes and then the solution was centrifuged at 13,000 rotations per minute (rpm) for 2 minutes. Finally, 60 µl of DNA was pipetted into a new Eppendorf with 5 µl of Low Tris-ethylenediaminetetraacetic acid (EDTA) and stored at -20 degrees Celsius.

PCR Species Identification

A multiplex *cox1* polymerase chain reaction (PCR) was used to determine species by band length (*S. haematobium* 120 base pairs (bp)*, S. manonsi* 215 bp, *S. bovis* and *S. curassoni* 260 bp). The *cox1* region was amplified in a 10 µl PCR containing 0.5 µl DNA, 5.0 µl of Qiagen Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 3.5 µl water, 1µl of primer mix (4 µl of each primer (100 µM) in 84 µl double distilled water; three forward primers: Sh.F 5'-GGTCTCGTGTATGAGATCCTATAGTTTG -3'; Sm.F 5'- CTTTGATTCGTTAACTGGAGTG -3'; Sb.F 5'- GTTTAGGTAGTGTAGTTTGGGCTCAC -3' and one reverse primer: Shmb.R R5'-CAAGTATCATGAAAYARTATRTCTAA -3'). The PCR protocol was initiated by 15 min at 95 degrees Celsius; followed by 45 cycles of 94 degree Celsius for 10 secs, 52 degree Celsius for 30 secs and 72 degree Celsius for 10 secs and a final extension of 72 degree Celsius for 2 mins.

The PCR product was loaded onto a 2% agarose gel (Promega, Madison, Wisconsin) with 0.5X Tris-borate-EDTA (TBE) (Thermo Scientific, Waltham, Massachusetts) and run for 25 mins at 75 V. Agarose gels were soaked in GelRed (Merck KGaA, Darmstadt, Germany) for at least 30 minutes before being visualized.

A random selection of specimens was further selected for PCR and sequencing of the *ITS1/2* and *cox1* loci. Sequences from the *ITS1/2* marker were used to confirm species by using five variable sites known to distinguish *S. haematobium* from *S. bovis*. One of these sites also distinguishes *S. bovis* from *S. curassoni* (Huyse et al., 2009; Webster et al., 2013).

The *ITS1/2* locus was amplified in a total volume of 10 µl consisting of 1 µl BD 10X buffer (Solis BioDyne, Tartu, Estonia), 1.3 µl of 25mM $MgCl_2$, 0.8 µl of 10mM dNTP mix (2.5 mM of each dNTP), 0.1 µl of 5U/ml FirePol® Taq polymerase (Solis BioDyne, Tartu, Estonia), 0.5 µl DNA, 5.3 µl water and 0.5 µl of each forward (ITS5: F5'- GGAAGTAAAAGTCGTAACAAG -3') and reverse primers (ITS4 R5'- TCCTCCGCTTATTGATATGC -3'). The PCR protocol was initiated with 4 mins

at 94 degrees Celsius; followed by 40 cycles of 30 secs at 94 degrees Celsius, 30 secs at 54 degrees Celsius and 30 secs at 72 degrees Celsius and a final extension of 2 mins at 72 degrees Celsius. PCR products were sent to Microsynth (Balgach, Switzerland) for uni-directional Sanger sequencing using the reverse primer.

ITS1/2 sequences were compared to other sequences in GenBank using the Basic Local Alignment Search Tool (BLAST) and then aligned and analyzed in CodonCode Aligner version 6.0.2 (CodonCode Corporation, Centerville Massachusetts). *ITS1/2* trace files were inspected to determine heterozygosity at the five variable sites.

Microsatellite analysis

A sub-selection of specimens (Tab. S1) from all sites were selected to be analyzed using two panels of microsatellites (Webster et al., 2015). These microsatellites were developed for *S. haematobium*, a sister species of *S. bovis*, and therefore some markers produced poor results (Tab. 1). Loci C131 and Shae08 were removed from the reactions in contrast to the original protocol (Webster et al., 2015). During analysis, loci Shae07, Shae10, Shae14, Shae 15 and C102 produced poor results and were dropped from the population genetic analysis.

The protocol for these two multiplex PCRs were the same: 5 µl Qiagen Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 1 µl DNA, 3 µl double distilled water and 1 µl 10uM primer mix. The PCR protocol was initiated by 15 mins at 95 degrees Celsius; followed by 45 cycles of 94 degrees Celsius for 30 seconds, 56 degrees Celsius for 1.5 mins and 72 degrees Celsius for 1 min and a final extension of 72 degrees Celsius for 5 minutes. PCR products were sent to Microsynth (Balgach, Switzerland) for fragment length analysis using the Applied Biosystems 3730xl DNA Analyzer with size standard GS500LIZ.

The trace files were imported into Genemapper (v. 6.0, ThermoFisher Scientific), automatic bin calling was established based on the samples, and all automatically called peaks were confirmed at least twice by visual inspection. Any peaks that were questionable were amplified and sequenced again to confirm the call.

Table 1. *Schistosoma* microsatellite panels, markers used for population genetic analysis, allele size ranges (base pairs) and fluorescent dye.

Panel	Marker	Allele range in humans	Allele range in cattle	Fluorescent dye
Panel 1	C111	203-215	185-209	ATTO565 (red)
	Shae_01	243-279	240-282	HEX (green)
	Shae_03	275-314	291-375	FAM (blue)
	Shae_06	310-320	305-331	ATTO550 (yellow)
	Shae_09	196-223	190-241	FAM (blue)
Panel 2	Shae_02	170-209	152-224	ATTO550 (yellow)
	Shae_04	276-297	264-313	FAM (blue)
	Shae_05	270-306	261-291	ATTO550 (yellow)
	Shae_11	192-201	179-209	HEX (green)
	Shae_12	244-265	241-262	ATTO565 (red)
	Shae_13	169-205	163-229	FAM (blue)

Genetic diversity

The number of alleles (A), rarified allelic richness (Ar), expected heterozygosity (He), observed heterozygosity (Ho), probability of deviation from Hardy Weinberg equilibrium (P_{HWE}) and inbreeding coefficient (F_{IS}) were calculated in RStudio using the package diveRsity (v. 2.9.3.2; v.1.9.90) (Keenan et al., 2013; R Core Team, 2013). All indices were calculated by loci and by population. Statistical significance of P_{HWE} was calculated by χ^2 in diveRsity and significance was set at <0.05. A sub-sample of one *Schistosoma* couple or one miracidium from each host were randomly selected and the genetic diversity indices were calculated. This random selection of a sub-sample was performed 100 times and the distributions of the genetic diversity index values were compared between study groups.

Genotypic differentiation

Differentiation was measured by F_{ST} and $R_{ST,}$ which were calculated in RStudio using the package Genepop (v.1.1.7) (Rousset, 2008). Genepop used the following parameters: Minimum distance regression was 0.0001 and number of permutations for Mantel was 1000. Statistical significance was also calculated in Genepop with the following Markov chain parameters: dememorization was set at 10,000, 100 batches and 5,000 iterations per batch. Genotypic differentiation for each population was pair tested with the exact G test. As described above, genotypic differentiation indices were also calculated on 100 random sub-samples, and the average was calculated and reported.

Population structure

The structure analysis was completed using STRUCTURE (v. 2.3.4,) (Pritchard et al., 2000). All structure analyses were run with a burn-in of 250,000 and 1,000,000 iterations after burn-in. Each K was run 5 times, and then the best K determined by the Evanno method in Clumpak (Kopelman et al., 2015) and the best K as determined by Delta was run another 10 times. These results were used to produce the STRUCTURE plot in Clumpak. PCA was calculated using Genetix (v. 4.05.2) and plotted using Stata (v. 15.1, StataCorp LLC, Texas, USA).

Ethical considerations

This study was part of larger project that investigated transmission dynamics of trematodes in humans and livestock Côte d'Ivoire. Ethical approval was obtained from the National Ethics and Research Committees of Côte d'Ivoire (reference no. 035/MSH /CNER-kp) and Switzerland (reference no. UBE-2016-00707). The "Direction des Services Vétérinaires" of the "Ministère des Ressources Animales et Halieutiques en Côte d'Ivoire" gave authorization to conduct the research. Community leaders and participants were informed about the objectives, procedures, and potential risks and benefits of the study after which, written informed consent was obtained by the participants and

farmers. Following sampling, a praziquantel treatment (40 mg/kg) was offered to humans who tested positive for schistosomiasis.

Results

PCR species identification

DNA from 86 miracidia from 23 humans and 114 miracidia from 39 cattle were extracted. Of the 86 miracidia from humans, 77 produced bands in the multiplex *cox1* PCR. A band of 120 bp in length was produced by 58% (45/77) of the miracidia in the *cox1* PCR, indicating that they had mitochondrial DNA from *S. haematobium* (Tab. 2). The other 42% (32/77) of miracidia from humans displayed a band of 260bp in length indicating that they had mitochondrial DNA from *S. bovis or S. curassoni* (Tab. 2).

ITS1/2 sequencing was performed on 47 of the 86 samples from humans, 27 of these produced good quality sequences. Eighty-five percent (23) conformed to the pattern of *S. haematobium*, including 13 that had produced bands for *S. bovis* on the *cox1* multiplex PCR (Tab. 2). Another four samples produced some degree of heterozygosity (Tab. 3) at the 5 variable sites, two of these samples had produced *S. haematobium* bands in the *cox1* PCR and the other two produced *S. bovis* bands (Tab. 4). Of note, three of the four hybrids had an "A" at the variable site in the *ITS1* locus. This site distinguishes *S. haematobium* (A) from *S. Bovis* (G), but also *S. Bovis* (G) from *S. curassoni* (A). This confuses matters in the case of hybrids, as *S. haematobium* and *S. curassoni* both display "A", indicating that the hybrids are either introgressed *S. haematobium* x *S. bovis* or *S. haematobium* x *S. curassoni*. At least one indication of hybridization was seen in 40% (34/86) samples from 65% (15) hosts.

Table 2. *Schistosoma* species identification by *cox1* PCR and *ITS1/2* sequencing. The *cox1* species identifying PCR produces bands of different length depending on species: 260bp indicates *S. bovis* or *S. curassoni*, 120 bp indicates *S. haematobium*. Five variable sites in the *ITS1/2* marker distinguish *S. bovis* from *S. haematobium*, the first variable site also distinguishes *S. curassoni* from *S. bovis*. *Cox1* PCR was run on all samples, a sub selection of these were *ITS1/2* sequenced.

Host species	*Cox1* species identification		*ITS1/2* species identification		
	S. bovis/S. curassoni	*S. haematobium*	*S. bovis*	*S. haematobium*	Heterozygous
Human	42% (32/77)	58% (45/77)	0	85% (23/27)	15% (4/27)
Bovine	100% (101/101)	0	100% (4/4)	0	0

Eighty-nine percent (101/114) of miracidia from cattle produced bands in the multiplex *cox1* PCR, all of them displayed bands of 260bp in length, indicating that they have mitochondrial DNA of *S. bovis* or *S. curassoni* (Tab. 3). Only 12% (4/34) of the miracidia from cattle that were subjected to the *ITS1/2* PCR produced sequences, all were consistent with the pattern for *S. bovis* at all the variable sites (Tab. 3).

Genetic diversity

A total of 63 miracidia from 23 humans and 99 miracidia from 39 cattle were analyzed using microsatellites. Diversity was high in both populations, although there is a notable heterozygote deficiency, a significant deviation from Hardy-Weinberg equilibrium and significant inbreeding in both cattle and humans. Inbreeding was significantly higher in cattle.

Table 3. Genetic diversity indices for *Schistosoma* from humans and cattle in Ferkessédougou, Côte d'Ivoire. N, number of samples; A, average number of alleles; A_r, mean allelic richness; H_o, mean observed heterozygosity; H_e, mean estimated heterozygosity; F_{IS}, fixation index; * statistically significant from zero.

Host species	N	A ± SD	A_r ± SD	H_o ± SD	H_e ± SD	HWE	F_{IS}
Human	63	12.25 ± 1.86	8.00 ± 3.11	0.54 ± 0.24	0.64 ± 0.26	*	0.15*
Bovine	99	12.83 ± 1.84	10.47 ± 4.99	0.48 ± 0.28	0.74 ± 0.17	*	0.40*

F_{ST} and R_{ST} both showed a high and statistically significant level of differentiation (0.268 and 0.700, respectively) between the two populations.

Structuring

Structure analysis indicated that the microsatellite data are best explained by K = two clusters, with one cluster occurring exclusively in samples from humans and the other occurring almost exclusively in samples from cattle (Fig. 1).

Fig. 2. Structure analysis of *Schistosoma* miracidia from human and cattle in Ferkessédougou, (K=2). 1. Human schistosomes 2. Cattle schistosomes. 5 runs, burn-in: 100,000; repeats after burn-in: 200,000.

PCA also revealed a clear distinction between schistosomes from humans and animals (Fig. 2).

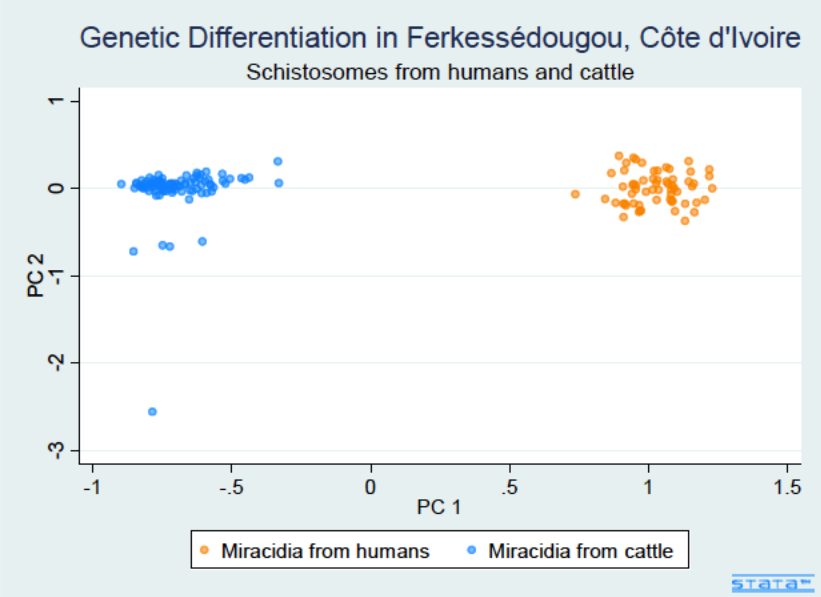

Fig. 3. Principal component analysis of populations of miracidia from cattle and humans in Ferkessédougou

Discussion

This is the first molecular investigation into *Schistosoma* species in humans and bovines sharing the same water source in Côte d'Ivoire**.** Schistosomes infecting humans and cattle appear to be separate and distinct populations, in line with standard expectations that they are two different schistosome species - *S. bovis* in cattle and *S. haematobium* in humans**.** However, our analysis shows that this species distinction is less clear-cut than traditionally thought.

While hybrids between livestock (*S. bovis* and *S. curassoni)* and human species of schistosomes (*S. haematobium*) were identified in this human population, no hybrids were found in cattle**.** Furthermore, the human hybrids appear to have traces of *S. curassoni* and our investigation shows no evidence of *S. curassoni* occurring in cattle in Côte d'Ivoire**.** This may indicate that the hybrids in humans have been imported from other regions, perhaps from the south of Côte d'Ivoire or from Senegal - which is known to have *S. curassoni* in cattle, sheep and goats - and are transmitted amongst humans only (Angora et al., 2020; Léger et al., 2020). In contrast to recent demonstrations of pure *S. bovis* in humans in Southern Côte d'Ivoire and Corsica (Angora et al., 2020; Boissier et al., 2016), we found no evidence of pure livestock schistosomes occurring in people in Ferkessédougou. However, this may be an effect of too limited sampling.

The situation appears mostly the same across West Africa. Humans have been found to be infected with *S. bovis* x *S. haematobium* hybrids in several studies in Benin, Corsica, and Senegal (Boissier et al., 2016; Boon et al., 2018; Léger et al., 2020). Livestock, on the other hand, have repeatedly been found to harbour only pure "livestock-species", e.g. pure *S. bovis* in cattle in Cameroon (Djuikwo-Teukeng et al. 2019), and pure *S. bovis* or *S. curassoni,* or hybrids of the two in cattle, sheep and goats in Senegal (Boon et al., 2019, Léger et al., 2020). There is only one recent publication from Benin

describing *S. bovis* x *S. haematobium* hybrids in cattle (Savassi et al., 2020). However, they it is not clear if they investigated the presence of *S. curassoni*.

It appears that *S. curassoni* is often overlooked. This is likely due to the fact that it cannot be easily distinguished from other *Schistosoma* species in a commonly used multiplex *cox1* PCR and by *ITS1/2* sequencing. In the multiplex *cox1* PCR, *S. curassoni* displays the same band length as *S. bovis* or double bands the lengths of *S. bovis* and *S. haematobium* (Webster, 2013). *S. curassoni* (nucleotide A) is distinguished from *S. bovis* (nucleotide G) at one variable site in the *ITS1* marker. This variable site also distinguishes *S. bovis* (nucleotide G) from *S. haematobium* (nucleotide A), however *S. haematobium* and *S. curassoni* share the same mutation at this site (nucleotide A), making it difficult to determine if the specimen is *S. curassoni* or introgressed *S. haematobium* x *S. bovis.* More sensitive molecular methods are needed to easily distinguish *S. curassoni* from *S. bovis* and *S. haematobium.*

It appears that hybrids are quite common in humans, yet occur only very rarely in cattle in West Africa. Hybridization seems to be one way only and the reason for this is not known. There are numerous possibilities. This study did not investigate where people and cattle are exposed to water. It could be that humans in this location don't wash or play in water in the same location where the cattle drink (and defecate). Perhaps in Ferkessédougou, the hybrids in humans have been imported and are transmitted between humans only and there is no transmission between cattle and humans. Another hypothesis is that the difference in thickness of the epidermal between cattle and humans prevents human schistosomes from penetrating cattle skin.

At the low prevalence (0.7%) of pure *S. bovis* in humans reported in the South of Côte d'Ivoire, it is possible that pure *S. bovis* was simply missed in humans (Angora et al., 2020). It is also possible that with only 39 cattle sampled in two villages, schistosome hybrids could have been missed in the North, although results from Senegal and Cameroon and other locations in Côte d'Ivoire seem to indicate that these hybrids are rare (Boissier et al., 2016; Léger et al., 2020; Savassi et al., 2020). Although a malacological study was undertaken during this investigation, only one positive sail was found and the cercariae were not sequenced (Kouadio, personal communication). Further investigation as to which species of snails are harbouring hybrids and where they are located may shed light on why hybrids have not been found in cattle.

This investigation had a few limitations. The number of hosts for both humans and cattle were limited (23 and 39 respectively) and furthermore, only one site was investigated. This may have affected our ability to detect pure *S. bovis* in humans or hybrids in cattle.

Conclusion

Cattle and humans sharing the same water source on farms in Northern Côte d'Ivoire appear to harbour distinct schistosome populations. Hybrids between humans and cattle schistosome species exist in Ferkessédougou, Côte d'Ivoire in the human population, yet there is no evidence of such

hybrids in the cattle population. This may indicate that hybrids in humans are being imported to the area and transmitted only amongst humans.

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Table S1. List of samples included in analyses. n/a, did not amplify; -, not done

Sample ID	Host Species	Host ID	Life stage	Multiplex *cox1* species ID	*ITS* species ID	*cox1* phylogeny haplotype
SM008	bovine		miracidium			
SM009	bovine		miracidium			
SM010	bovine		miracidium			
SM011	bovine		miracidium			
SM061	bovine		miracidium			
SM062	bovine		miracidium			
SM065	bovine		miracidium			
SM066	bovine		miracidium			
SM067	bovine		miracidium			
SM068	bovine		miracidium			
SM069	bovine		miracidium			
SM070	bovine		miracidium			
SM071	bovine		miracidium			
SM072	bovine		miracidium			
SM073	bovine		miracidium			
SM074	bovine		miracidium			

SM075	bovine		miracidium			
SM076	bovine		miracidium			
SM078	bovine		miracidium			
SM079	bovine		miracidium			
SM082	bovine		miracidium			
SM083	bovine		miracidium			
SM084	bovine		miracidium			
SM085	bovine		miracidium			
SM086	bovine		miracidium			
SM087	bovine		miracidium			
SM088	bovine		miracidium			
SM089	bovine		miracidium			
SM090	bovine		miracidium			
SM091	bovine		miracidium			
SM092	bovine		miracidium			
SM105	bovine		miracidium			
SM106	bovine		miracidium			
SM107	bovine		miracidium			
SM108	bovine		miracidium			
SM109	bovine		miracidium			
SM110	bovine		miracidium			
SM111	bovine		miracidium			
SM113	bovine		miracidium			
SM114	bovine		miracidium			
SM115	bovine		miracidium			
SM116	bovine		miracidium			
SM117	bovine		miracidium			
SM118	bovine		miracidium			
SM119	bovine		miracidium			
SM120	bovine		miracidium			
SM122	bovine		miracidium			
SM123	bovine		miracidium			
SM124	bovine		miracidium			
SM125	bovine		miracidium			
SM126	bovine		miracidium			
SM127	bovine		miracidium			
SM129	bovine		miracidium			
SM130	bovine		miracidium			
SM131	bovine		miracidium			
SM132	bovine		miracidium			
SM134	bovine		miracidium			
SM135	bovine		miracidium			
SM136	bovine		miracidium			
SM137	bovine		miracidium			
SM138	bovine		miracidium			
SM139	bovine		miracidium			
SM140	bovine		miracidium			
SM141	bovine		miracidium			
SM142	bovine		miracidium			
SM143	bovine		miracidium			
SM144	bovine		miracidium			
SM145	bovine		miracidium			
SM146	bovine		miracidium			
SM147	bovine		miracidium			
SM150	bovine		miracidium			
SM151	bovine		miracidium			
SM152	bovine		miracidium			
SM153	bovine		miracidium			
SM155	bovine		miracidium			
SM156	bovine		miracidium			
SM158	bovine		miracidium			
SM159	bovine		miracidium			
SM160	bovine		miracidium			
SM162	bovine		miracidium			
SM163	bovine		miracidium			
SM164	bovine		miracidium			
SM165	bovine		miracidium			
SM166	bovine		miracidium			
SM167	bovine		miracidium			
SM168	bovine		miracidium			
SM169	bovine		miracidium			

SM170	bovine		miracidium			
SM188	bovine		miracidium			
SM189	bovine		miracidium			
SM190	bovine		miracidium			
SM191	bovine		miracidium			
SM192	bovine		miracidium			
SM193	bovine		miracidium			
SM194	bovine		miracidium			
SM196	bovine		miracidium			
SM199	bovine		miracidium			
SM200	bovine		miracidium			
SM001	human	FR115	miracidium			
SM002	human	FR115	miracidium			
SM003	human	FR115	miracidium			
SM004	human	FR056	miracidium			
SM005	human	FR027	miracidium			
SM006	human	FR052	miracidium			
SM007	human	FR067	miracidium			
SM013	human	FR115	miracidium			
SM014	human	FR056	miracidium			
SM015	human	FR056	miracidium			
SM016	human	FR056	miracidium			
SM017	human	FR056	miracidium			
SM018	human	FR027	miracidium			
SM019	human	FR027	miracidium			
SM020	human	FR027	miracidium			
SM021	human	FR027	miracidium			
SM022	human	FR052	miracidium			
SM023	human	FR052	miracidium			
SM024	human	FR052	miracidium			
SM025	human	FR052	miracidium			
SM026	human	FR067	miracidium			
SM027	human	FR067	miracidium			
SM028	human	FR067	miracidium			
SM029	human	FR067	miracidium			
SM030	human	FR029	miracidium			
SM031	human	FR029	miracidium			
SM033	human	FR029	miracidium			
SM034	human	FR127	miracidium			
SM035	human	FR127	miracidium			
SM039	human	FR001	miracidium			
SM042	human	FR001	miracidium			
SM043	human	FR027	miracidium			
SM044	human	FR027	miracidium			
SM050	human	FR027	miracidium			
SM051	human	FR027	miracidium			
SM052	human	FR027	miracidium			
SM055	human	FR014	miracidium			
SM056	human	FR020	miracidium			
SM058	human	FR032	miracidium			
SM093	human	FR053	miracidium			
SM094	human	FR063	miracidium			
SM095	human	FR008	miracidium			
SM096	human	FR011	miracidium			
SM097	human	FR035	miracidium			
SM098	human	FR024	miracidium			
SM099	human	FR018	miracidium			
SM100	human	FR025	miracidium			
SM101	human	FR092	miracidium			
SM102	human	FR046	miracidium			
SM104	human	FR040	miracidium			
SM172	human	FR053	miracidium			
SM173	human	FR063	miracidium			
SM175	human	FR011	miracidium			
SM176	human	FR032	miracidium			
SM179	human	FR046	miracidium			
SM180	human	FR040	miracidium			
SM181	human	FR014	miracidium			
SM182	human	FR032	miracidium			
SM183	human	FR032	miracidium			
SM184	human	FR011	miracidium			

SM185	human	FR092	miracidium			
SM187	human	FR040	miracidium			

Chapter 7a

Molecular confirmation of a *Fasciola gigantic* X *Fasciola hepatica* hybrid in a Chadian bovine

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MOLECULAR CONFIRMATION OF A *FASCIOLA GIGANTICA* × *FASCIOLA HEPATICA* HYBRID IN A CHADIAN BOVINE

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KEY WORDS

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Fasciola gigantica
Fasciola hepatica
Fascioliasis
Goats
Hybrid
ITS1
ITS2
Sheep

ABSTRACT

Fascioliasis is a zoonotic infection of humans and, more commonly, ruminants. It is caused by 2 liver fluke species, *Fasciola hepatica* and *Fasciola gigantica*, which differ in size. The traditional morphological methods used to distinguish the 2 species can be unreliable, particularly in the presence of hybrids between the 2 species. The development of advanced molecular methods has allowed for more definitive identification of *Fasciola* species, including their hybrids. Hybrids are of concern, as it is thought that they could acquire advantageous traits such as increased pathogenicity and host range. In 2013, we collected flukes from *Fasciola*-positive cattle, sheep, and goats slaughtered in 4 Chadian abattoirs. DNA from 27 flukes was extracted, amplified, and analyzed to identify species using the *ITS1+2* locus. Twenty-six of the 27 flukes were identified as *F. gigantica*, while the remaining fluke showed heterozygosity at all variable sites that distinguish *F. hepatica* and *F. gigantica*. Cloning and sequencing of both alleles confirmed the presence of 1 *F. hepatica* and 1 *F. gigantica* allele. To our knowledge, this is the first unambiguous, molecular demonstration of the presence of such a hybrid in a bovine in sub-Saharan Africa.

Fascioliasis is a liver fluke infection affecting ruminants and occasionally humans that, while treatable, is difficult to diagnose. It is acquired by ingesting infective metacercariae on vegetation grown in contaminated water or ingesting the contaminated water itself (Andrews, 1999). Animal fascioliasis leads to economic loss because of reduced herd productivity resulting from diminished fertility, reduced production of wool and milk, and increased mortality (Bernardo et al., 2011). Spillover infections from animals to humans were, until recently, thought to occur only occasionally (Mas-Coma et al., 2009). However, studies suggest that the frequency of human cases is on the rise (WHO, 2015). Human infections, while seldom fatal, cause pain, liver and gallbladder damage, and injury to other organs in the case of ectopic migration (Lotfy and Hillyer, 2003; Fürst et al., 2012).

Fascioliasis is caused by 2 species of the genus *Fasciola*, namely, *Fasciola hepatica* and *Fasciola gigantica* (Mas-Coma et al., 2007). Distinguishing the 2 *Fasciola* species is often accomplished by morphometry or inferred from geography, but this is not reliable as there can be overlaps in the size of the 2 species and in their geographic range (Moazeni et al., 2012; Afshan et al., 2014). Advances in molecular methods now allow species identification and provide a powerful tool to identify potential hybrids (Agatsuma et al., 2000; Huang et al., 2004; Itagaki et al., 2005; Amor et al., 2011b; Shalaby et al., 2013).

Traditionally, it has been thought that *F. hepatica* exists in the temperate regions of the Americas, Europe, and Oceania, while *F. gigantica* is present across Africa and Asia. However, recent reports of *F. hepatica* in Africa and Asia have challenged these long-held beliefs (Agatsuma et al., 2000; Huang et al., 2004; Ali et al., 2008; Walker et al., 2008; Amer et al., 2011; Dar et al., 2012; Mucheka et al., 2015).

In Africa, *F. hepatica* has been confirmed by molecular analysis in Algeria (Farjallah et al., 2009), Ethiopia (Le et al., 2012), and Tunisia (Farjallah et al., 2009). Geographic co-occurrence (but not necessarily co-infection) of the 2 species has been genetically proven in Egypt (Amer et al., 2011; Dar et al., 2012), Niger (Ali et al., 2008), South Africa (Mucheka et al., 2015), Tanzania (Walker et al., 2008), and Zimbabwe (Mucheka et al., 2015). Further reports of *F. hepatica* from Africa based on morphology have been published from Côte d'Ivoire (Utzinger et al., 2010), Ethiopia (Abebe, 2010), Kenya (Ogambo-Ongoma, 1969), and Morocco (Khallaayoune et al., 1991). *Fasciola gigantica* has been documented at Lake Chad (Jean-Richard et al., 2014), but, to date, neither *F. hepatica* nor hybrids have been detected in Chad.

Given that *F. hepatica* is present in a few African countries and that it is difficult to distinguish from *F. gigantica* by clinical, coprological, or immunological methods (Mas-Coma et al., 2005), it is conceivable that *F. hepatica* has merely been overlooked across Africa, including Chad. Furthermore, morphology as a method for species determination has not been rigorously tested in Africa, and hence, *Fasciola* species may have been misidentified. The presence of hybrids complicates matters further (Itagaki et al., 1998; Valero et al., 2005; Inoue et al., 2007; Afshan et al., 2014).

There have been repeated reports of hybrids (or 'intermediate forms') of *F. gigantica* and *F. hepatica* in regions where both species are present (Agatsuma et al., 2000; Huang et al., 2004; Itagaki et al., 2005; Amor et al., 2011b; Shalaby et al., 2013). Hybrids have been reported by molecular means in Egypt (Amer et al., 2011), Islamic Republic of Iran (Amor et al., 2011b), Japan (Itagaki et al., 2005), Korea (Agatsuma et al., 2000), Pakistan (Mufti et al., 2014), People's Republic of China (Huang et al., 2004; Peng et al., 2009), Saudi Arabia (Shalaby et al., 2013), Thailand (Wannasan et al., 2014), and Vietnam (Nguyen et al., 2012). Hybrid forms, as determined by morphology, have been reported in Bangladesh (Ahasan et al., 2016), Egypt (Periago et al., 2008), Islamic Republic of Iran (Ashrafi et al., 2006), and Pakistan (Afshan et al., 2014). These reports are of interest because *F. hepatica* flukes have shown considerable adaptability to new hosts, both intermediate and definitive, environmental conditions, and pharmaceutical treatments (most importantly triclabendazole) raising concerns that hybrid forms may have greater propensity for geographic and host expansion (Seehausen, 2004; Walker et al., 2008; Cwiklinski et al., 2015).

Species-specific single-nucleotide polymorphisms (SNPs) in the nuclear ribosomal DNA are commonly used to differentiate *Fasciola* species. Previous investigations have identified up to 16 polymorphic positions in the internal transcribed spacer 1 (*ITS1*) and *ITS2* (*ITS1+2*) marker; 11 of these are commonly used to distinguish *F. hepatica* from *F. gigantica* (Ali et al., 2008; Itagaki et al., 2009; Amor et al., 2011a, 2011b; Liu et al., 2014; Chaudhry et al., 2016).

In the current investigation, 27 adult *Fasciola* flukes, collected from 19 slaughtered livestock in Chad, were molecularly characterized using the *ITS1+2* nuclear marker to confirm species and any hybrid forms (Itagaki et al., 2009; Liu et al., 2014). This analysis of Chadian *Fasciola* was part of a larger phylogenetic investigation that included Ivorian and Swiss *Fasciola* specimens.

Liver flukes were collected from the carcasses of 24 cattle, 2 goats, and 1 sheep during post-mortem inspection at 4 abattoirs located on the shores of Lake Chad (Bol, Gredaya, and Sidjé) and at 1 abattoir in N'Djamena, the capital of Chad, in 2013. All work was done in accordance with Chadian ethical regulations.

After collection, the flukes were rinsed with sterile water, put in 96% ethanol, and stored at room temperature. In June 2015, the samples were re-preserved for shipping to Switzerland. Twenty-three of the 27 samples were shipped in 96% ethanol, however, 3 (TCDF050, TCDF062.02, and TCDF082) were shipped in neutral buffered 10% formalin (stored for 29 days in formalin). Upon arrival, all flukes were rinsed with double distilled water and fully immersed in 96% ethanol.

Approximately one-quarter of each fluke was cut with a new or bleached scalpel blade and deposited into a lysis solution of 50 mM Tris, 1% SDS and proteinase K, then incubated at 56 C for 12–14 hr with agitation. DNeasy column kits (Qiagen, Hilden, Germany) were used for DNA extraction, adhering to the manufacturer's protocol with the exception of adjusting the amount of lysis solution and proteinase K. Chadian flukes did not necessarily lyse within 24 hr; therefore, more lysis solution was added (up to 380 µl lysis solution and 20 µl proteinase K) and the flukes were left at 56 C until they were fully lysed (up to 25 hr).

The combined *ITS1+2* region of 27 Chadian flukes was amplified in 10 µl polymerase chain reactions (PCRs) containing 1 µl of Buffer BD 10×, 1.6 µl $MgCl_2$ 25 mM, 0.2 µl 10 mM dNTP mix (2.5 mM of each dNTP), 0.4 µl of each 10 mM primer (BD1, forward: 5′-GTCGTAACAAGGTTTCCGTA-3′, and BD2, reverse: 5′-TATGCTTAAATTCAGCGGGT-3′) (Luton et al., 1992), 0.2 µl of FIREPol 5 U/µl (Solis BioDyne, Tartu, Estonia), 1 µl of DNA, and 5.2 µl of sterile water. The PCR protocol was initiated by 3 min at 94 C; followed by 45 cycles of 40 sec at 94 C, 45 sec at 60 C, and 1.5 min at 72 C; followed by a final extension step of 5 min at 72 C.

The PCR products were loaded onto 0.8% agarose (Promega, Madison, Wisconsin) gels with 1× TAE (Thermo Scientific, Waltham, Massachusetts) and run at 50 V for 20 min. Multiple bands were produced, and the band of the correct length, 1,000 base pairs (bp), was excised from the gel for sequencing. The Wizard® SV gel and PCR cleanup system (Promega) was used for gel cleanup. Finally, PCR products were sent to Microsynth (Balgach, Switzerland) for uni-directional Sanger sequencing using the BD1 primer. The sequence was compared to sequences deposited in GenBank using the Basic Local Alignment Search Tool (BLAST) (National Center for Biotechnology Information) and matched *F. gigantica ITS1+2* sequences.

The PCR of the *ITS1+2* loci produced sequences of 970 bp. The sequences from the 27 specimens were aligned and analyzed using CodonCode Aligner version 6.0.2 (CodonCode Corporation, Centerville, Massachusetts). Thirteen SNPs were observed, 11 of those were from known species determining variable sites, plus another 2 sites at positions 107 (3 samples) and 413 (2 samples). These 2 SNPs were heterozygous (R and W, respectively) and did not differ between *Fasciola* species.

Twenty-six of the 27 specimens (96%) conformed to the pattern associated with *F. gigantica* at 10 of the 11 *ITS* species-specific variable sites (GenBank accession nos. MK321604-07, MK321610, MK321614-20, MK321623-26, MK321629-32, MK321634, MK321636, MK321639, and MK321641-43). The exception, specimen TCDF062.02 from a bovine, exhibited heterozygosity at 9 of the 11 sites and a sudden breakdown of the clean sequence after the insertion/deletion (indel) at position 911. This breakdown is the hallmark of a heterozygous variable site, which instead of having a nucleotide mutation has an indel in 1 of the 2 alleles. The Sanger sequence chromatogram for sample TCDF062.02 (Fig. 1) illustrates the sudden breakdown of a clean sequence at a specific bp (position 911, as indicated by the vertical line through the chromatogram). This observation and the heterozygous variable sites indicated that the specimen could be a hybrid. To confirm that there was an *F. gigantica* and an *F. hepatica* allele, the DNA of specimen TCDF062.02 was cloned using a Gibson assembly, and the clones were sequenced (see Suppl. Data for the methods) (Gibson et al., 2009).

Minor SNPs in individual sequences from the clones were due to the Taq polymerase used, and hence, a consensus sequence was built and it revealed heterozygosity at 10 of the 11 variable sites.

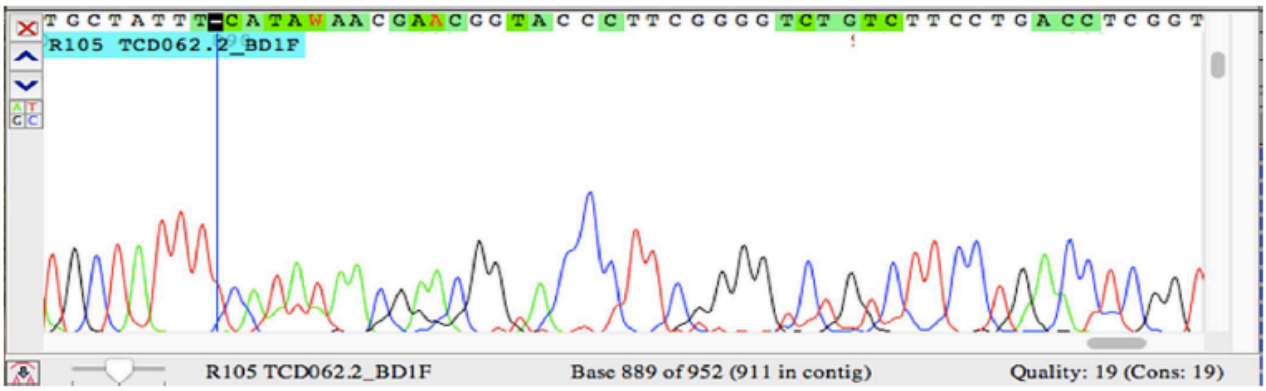

Figure 1. A section of the *ITS1+2* Sanger sequence chromatogram from Chadian *Fasciola hepatica* × *Fasciola gigantica* hybrid sample TCDF062.02. The vertical line indicates the breakdown of the clean sequence at position 911 due to a deletion in 1 of the alleles. Color version available online.

Thirteen clones were sequenced, 1 group (6 clones) matched the expected pattern for *F. hepatica* (GenBank accession no. MK321644) and the other group (6 clones) matched the pattern for *F. gigantica* (GenBank accession no. MK321645), confirming that this specimen is a hybrid between the 2 species.

A second larger analysis of the 11 species determining variable sites was performed using the sequences from all 27 Chadian specimens, in addition to sequences from Ivorian and Swiss specimens and sequences retrieved from GenBank (Table I). It revealed that 1 of the species determining variable sites, position 791, proved problematic for species determination in *F. gigantica*. While this site seems to be "T" consistently in *F. hepatica* specimens, it appears to vary geographically in *F. gigantica* flukes.

Twenty-six of 27 *Fasciola* flukes collected from cattle, goats, and sheep in Chad were found to be *F. gigantica* when analyzed using the 11 species determining variables sites. One specimen, from a bovine, was heterozygous at these sites, and cloning revealed alleles from the 2 *Fasciola* species. It was the only specimen from this bovine included in this analysis. To our knowledge, this is the first unambiguous proof of the occurrence of *Fasciola* hybrids in sub-Saharan Africa and only the second report from Africa. Hybrids have been detected in Egypt (in a buffalo) and in Saudi Arabia (in a goat); however, the goat was imported from Sudan, and it is not known if the hybrid came from Saudi Arabia or Sudan (Amer et al., 2011; Shalaby et al., 2013). There are only a few publications that used advanced molecular methods to determine species of African *Fasciola*, and only 5 sequences are currently available in GenBank. Therefore, the absence of reports of sub-Saharan African hybrids could be due to a paucity of prior investigations.

The hybrid fluke was taken from a cow that belonged to the semi-nomadic ethnic community of Arabs, who, at the end of the dry season, when the pastures around their villages are depleted, bring their livestock to the shores of Lake Chad for grazing (Weibel et al., 2008; Jean-Richard et al., 2014; Greter et al., 2017). At this time, Lake Chad is home to a large population of mobile pastoralists who travel hundreds of kilometers throughout the Sahelian region each year in search of forage. Jean-Richard et al. (2014) and Greter et al. (2017) found the prevalence of fascioliasis in cattle around Lake Chad is high (68% and 31% in slaughtered and live cattle, respectively). Presumably, these mobile livestock are picking-up *Fasciola* parasites and depositing their eggs along the migration route, including at Lake Chad. As the Arab cattle are exposed to Lake Chad, and the mobile livestock there have likely contaminated the area with *Fasciola* from throughout the Sahel, it is difficult to know with any certainty where this hybrid originated.

Site 791 of the *ITS1+2* marker has been included in a panel of 11 variable sites for distinguishing species of *Fasciola* in prior publications. The consensus in the literature is that *F. gigantica* should present a "C" at this site; however, this appears to be true only for *F. gigantica* from Asia and Niger. All *F. gigantica* samples and the hybrid from our investigation, as well as 3 out of 5 sequences from African *F. gigantica* (GenBank accession nos. EF612484, AB010976, and AJ853848) showed a homozygous "T" at this position (although sequence AJ853848 was incorrectly reported as "C" in Liu et al. 2014). The 2 sequences from Niger (GenBank accession nos. AM900371, AM850108) showed "C." *Fasciola gigantica* from the Middle East and Northern Africa also consistently show a "T" at this site, indicating that site 791 is not a reliable marker for distinguishing the 2 species.

There are several limitations to our investigation, which are offered for discussion. While there are a number of publications reporting the species of *Fasciola* found in Africa, many of them do not use the same method to determine species (morphology, molecular methods, or different molecular markers). Consequently, there are only a few *ITS1+2* sequences currently available from African *Fasciola*. Furthermore, among those publications that use *ITS1+2*, there are differences in the approach to the variable sites. Some studies use only one of the *ITS1+2* markers (only *ITS1* or *ITS2*), while others include more variable sites than those mentioned here (Ali et al., 2008; Itagaki et al., 2009; Amer et al., 2011; Choe et al., 2011). The reason for these differences is not clear, as there appear to be only 10 sites that reliably distinguish *F. gigantica* from *F. hepatica*; the 11 reported here minus site 791.

Despite our efforts to obtain a mitochondrial sequence for the hybrid, the sequence was of very poor quality. This may be due to the fact that the specimen was exposed to formalin. Formalin degrades DNA by causing cross-linking between nucleic acids and proteins, which can affect the success of PCR amplification (Vitošević et al., 2018). However, the 3 samples in this investigation that were exposed to formalin amplified in the *ITS1+2* PCR well. Furthermore, only 1 hybrid was found in this relatively small sample. Larger sample size may reveal more

Table I. Comparison of nucleotides at 11 variable positions of the *ITS1/2* marker of *Fasciola* samples collected from Chad, Côte d'Ivoire, and Switzerland and sequences deposited in NCBI GenBank. Abbreviations: n/a, no sequence for this part. Y and W indicate heterozygous positions with nucleotides C/T and A/T, respectively.

			Variable positions in *ITS1/2* sequences											
Species	Origin*	n†	18	108	202	280	300	791	815	854	860	911‡	918	Accession no.
Fasciola hepatica	Switzerland§	1	C	A	C	T	C	T	T	C	C	T	G	MK321597-602
	France	1	C	A	C	T	C	T	T	C	C	T	G	JF708034
	Spain	1	C	A	C	T	C	T	T	C	C	T	G	JF708036
	Niger	5	C	A	C	T	C	T	T	C	C	T	G	AM850107 AM900370
	Tunisia	1	C	A	C	T	C	T	T	C	C	T	G	GQ231546
	Tunisia	1	C	A	C	T	C	T	T	C	C	T	G	GQ231547
	Iran	1	C	A	C	T	C	T	T	C	C	T	G	JF432072
	Egypt	17	n/a	n/a	n/a	n/a	n/a	T	T	C	C	T	G	AB553720-36
	Egypt	67	C	A	C	T	C	n/a	n/a	n/a	n/a	n/a	n/a	LC076128-48 LC076150-52 LC076154-96
	Saudi Arabia	1	C	A	C	T	C	n/a	n/a	n/a	n/a	n/a	n/a	HE972273
	China	1	C	A	C	T	C	T	T	C	C	T	G	JF708026
	Japan	1‖	C	A	C	T	C	n/a	n/a	n/a	n/a	n/a	n/a	AB207145
Fasciola gigantica	Niger	13	T	T	T	A	T	C	C	T	T	—	A	AM900371 AM850108
	Chad§	26	T	T	T	A	T	T	C	T	T	—	A	MK321603-643
	Ivory Coast§	15	T	T	T	A	T	T	C	T	T	—	A	MK321603-643
	Burkina Faso#	1	T	T	T	A	T	T	C	T	T	—	A	AJ853848
	Kenya	1	T	T	T	A	T	n/a	n/a	n/a	n/a	n/a	n/a	EF612472
	Kenya	1	n/a	n/a	n/a	n/a	n/a	T	C	T	T	—	A	EF612484
	Mauritania	2	T	T	T	A	T	T	C	T	T	—	A	HQ197358, HQ197359
	Zambia	6‖	T	T	T	A	T	n/a	n/a	n/a	n/a	n/a	n/a	AB207142
	Zambia	6‖	n/a	n/a	n/a	n/a	n/a	T	C	T	T	—	A	AB010976
	Iran	1	T	T	T	A	T	T	C	T	T	—	A	JN828953
	Egypt	1	T	T	T	A	T	T	C	T	T	—	n/a	KF425321
	Egypt	9	n/a	n/a	n/a	n/a	n/a	T	C	T	T	—	A	AB553694-702
	Saudi Arabia	1	T	T	T	A	T	n/a	n/a	n/a	n/a	n/a	n/a	HE972274
	Indonesia	1‖	T	T	T	A	T	n/a	n/a	n/a	n/a	n/a	n/a	AB207143
	Thailand	1‖	T	T	T	A	T	n/a	n/a	n/a	n/a	n/a	n/a	AB207144
	Indonesia: Thailand	1‖	n/a	n/a	n/a	n/a	n/a	C	C	T	T	—	A	AB207149
	China	3	T	T	T	A	T	C	C	T	T	—	A	JF496709, JF496714 KF543340
	Japan	1‖	T	T	T	A	T	n/a	n/a	n/a	n/a	n/a	n/a	AB207146
	Japan	2‖	n/a	n/a	n/a	n/a	n/a	C	C	T	T	—	A	AB207151, AB207152
	Vietnam	1	n/a	n/a	n/a	n/a	n/a	C	C	T	T	—	A	EU260063
	South Korea	1	n/a	n/a	n/a	n/a	n/a	C	C	T	T	—	A	HQ821455
	India	1	n/a	n/a	n/a	n/a	n/a	C	C	T	T	G	A	KJ720004
Fasciola hybrids	China	1	n/a	n/a	n/a	n/a	n/a	T	T	C	C	T	G	AJ557570 clone I
	China	1	n/a	n/a	n/a	n/a	n/a	T	C	T	T	—	A	AJ557571 clone II
	China	1	Y	W	Y	W	Y	n/a	n/a	n/a	n/a	n/a	n/a	AJ628428
	Egypt	2	C	A	C	T	C	n/a	n/a	n/a	n/a	n/a	n/a	LC076149, LC076153
	Egypt	2	Y	W	Y	W	Y	n/a	n/a	n/a	n/a	n/a	n/a	AB553691, AB553692
	Egypt	1	n/a	n/a	n/a	n/a	n/a	T	Y	Y	Y	T	R	AB553737
	Egypt	1	n/a	n/a	n/a	n/a	n/a	T	Y	Y	Y	—	R	AB553738
	Saudi Arabia	1	C	A	T	A	A	n/a	n/a	n/a	n/a	n/a	n/a	HE972275
	Japan	1‖	Y	W	Y	W	Y	n/a	n/a	n/a	n/a	n/a	n/a	AB207147
	Japan	1‖	n/a	n/a	n/a	n/a	n/a	Y	Y	Y	Y	T	R	AB207153
	South Korea	7	Y	W	Y	W	Y	n/a	n/a	n/a	n/a	n/a	n/a	AB211237
	Chad§	1	Y	W	Y	W	Y	T	Y	Y	Y	—/T	R	MK321644-645

* Origin as reported in GenBank.
† Number of samples investigated.
‡ Dashes (—) = Deletion.
§ Specimens from this study.
‖ One entry in GenBank, but appears to be consensus sequence from numerous specimens from the publication.
Site 791 for this specimen is reported as "C" in Liu et al. (2014), but as "T" in Amor et al. (2011a,b), it is reported as "T" in GenBank.

hybrids and strengthen the case for hybrids in Chad and perhaps elsewhere in sub-Saharan Africa.

Table I lists *ITS1+2* sequences deposited in GenBank, although it is not a comprehensive list of all *Fasciola ITS1+2* sequences available in GenBank. Some samples were not included due to unclear descriptive information. For example, a number of sequences were labeled in GenBank as *Fasciola* spp., although the meaning of this is neither clear nor consistent among samples. In addition, not all studies have used the same method or loci to determine species and, as mentioned previously, some analyses have reported sequences incorrectly. These issues limit our ability to compare specimens from other investigations and underscore the meticulous nature of sequence analysis work. Further investigations from different parts of sub-Saharan Africa with larger sample sizes than reported here are warranted to provide better resolution concerning which species are present in Africa and where exactly they are located.

Fasciola hepatica has been found to adapt quickly to external selection pressures such as new hosts, new environments, and medications leading to a selective advantage (Walker et al., 2008; Cwiklinski et al., 2015). Hybrids of *F. gigantica* and *F. hepatica* are of considerable concern as they may have increased adaptability, allowing them to expand their geographic and host range as well as acquire increased resistance to medications. This poses a threat to animal and human health as well as animal production industries in areas that are endemic for fascioliasis, particularly in resource-constrained settings where prevention and control strategies may be difficult to implement and sustain.

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Chapter 7b

Efficacy of triclabendazole against *Fasciola* infection in cattle in Côte d'Ivoire: a randomised blinded trial

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Efficacy of triclabendazole and albendazole against *Fasciola* spp. infection in cattle in Côte d'Ivoire: a randomised blinded trial

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ABSTRACT

Triclabendazole is the anthelminthic of choice for the treatment of fascioliasis, however, it is only registered in a few countries. We investigated the efficacy of a single-dose of triclabendazole (12 mg/kg) or albendazole (15 mg/kg) against *Fasciola* spp. infection in cattle on farms in the northern part of Côte d'Ivoire in a randomized clinical trial. Faecal samples were obtained from 196 cattle, of which 155 (79.1%) were found positive for *Fasciola* spp. by the sedimentation technique. Cattle infected with *Fasciola* spp. were randomly allocated (3:3:1) to receive triclabendazole (n = 66), albendazole (n = 67) or left untreated to serve as control (n = 22). Follow-up faecal samples were collected on days 21, 28, 90 and 188 post-treatment. No adverse events were observed as reported by farmers in any of the treatment groups. The proportion of non-egg shedding cattle, assessed at day 21 (primary outcome), was significantly higher in cattle treated with triclabendazole (95.4%) compared to those receiving albendazole (70.3%; odds ratio [OR] 8.73, 95% confidence interval [CI] 2.43-31.28, p <0.001). The egg reduction rate (ERR) expressed as number of eggs per gram of faeces, a secondary endpoint assessed at day 21 post-treatment, was significantly higher in the triclabendazole arm (arithmetic mean (AM) ERR = 99.8%) than in the albendazole arm (AM ERR = 92.2%), with a difference of 7.6%-points (95% CI: 0.9-14.5%-points, p=0.026). This is the first report of efficacy of triclabendazole against *Fasciola* spp. in naturally infected cattle in Côte d'Ivoire. Our results confirm that triclabendazole is the most effective treatment of fascioliasis and therefore, should be considered for the control of livestock fascioliasis; if resources allow in combination with intermediate host snail control and raising farmers awareness of pasture and livestock management to avoid reinfection.

1. Introduction

The liver flukes *Fasciola gigantica* and *Fasciola hepatica* are responsible for fascioliasis, a chronic, zoonotic disease of considerable veterinary and public health importance (Bennema et al., 2017; Fürst et al., 2012; Mas-Coma et al., 2018). *Fasciola hepatica* is a cosmopolitan species adapted to temperate regions, while *F. gigantica* is found in tropical and subtropical parts of Africa and Asia (Greter et al., 2017; Malatji et al., 2019; Parkinson et al., 2007). Fascioliasis affects mammals, mainly cattle, goats and sheep, but can also infect humans (Fürst et al., 2012; Gandhi et al., 2019). It causes economic loss in livestock, mainly through reduced fertility and productivity, liver condemnation in abattoirs, stunted growth and premature death (Kaplan, 2001; Suleiman et al., 2015; Yusuf et al., 2016). Fascioliasis is considered one of the most widespread food-borne trematode infections (Fürst et al., 2012).

Since its introduction in the early 1980s in Australia (Boray et al., 1983), triclabendazole – a benzimidazole carbamate – has become one of the most widely used anthelminthic drugs for the treatment and control of fascioliasis in cattle and in humans (Gandhi et al., 2019; Keiser et al., 2005). It has shown over 95% efficacy in reducing the

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excretion of *Fasciola* spp. eggs in cattle and sheep faeces (Keiser and Utzinger, 2004; Rapic et al., 1988; Shokier et al., 2013; Stevenson et al., 2002). Unlike other anthelminthics, which are only efficacious against adult flukes, triclabendazole has a lethal effect against both adult and immature flukes aged 2 weeks and above (Boray et al., 1983; Fairweather, 2005; Smeal and Hall, 1983). The lethal activity against immature flukes is particularly relevant, as the migration of the developing flukes out of the small intestine and into liver is the most damaging stage of the infection (Keiser et al., 2005).

In terms of mechanism of action, triclabendazole and other benzimidazoles (e.g. albendazole and mebendazole) disturb the secretory processes of the fluke. Triclabendazole, in particular, causes the disruption of the tegument of *Fasciola* spp. (Fairweather, 2009) and both triclabendazole and albendazole cause severe damage to the reproductive system of flukes (Fairweather and Boray, 1999). Triclabendazole metabolites reach peak plasma levels at one and three days after administration in cattle (sulfoxide and sulfone, respectively) and are virtually completely eliminated after 10 days (Health Products Regulatory Authority: Ireland, 2018). Triclabendazole-resistance in livestock was first documented in 1995 (Overend and Bowen, 1995), and has since been reported in many countries (Kelley et al., 2016).

Triclabendazole is not registered for use in livestock or humans in many West (Elelu and Eisler, 2018), Central (Greter et al., 2016) and East African countries (Keyyu et al., 2009; Nzalawahe et al., 2018a). In Côte d'Ivoire, despite the high prevalence of fascioliasis (Kouadio et al., 2020), farmers have only two options for treatment: albendazole and nitroxynil, both anthelminthics which only act on adult *Fasciola* spp. Access to efficacious anthelminthic drugs thus remains an issue in this fascioliasis endemic country. Condemnation of *Fasciola*-infected bovine livers from both sedentary and transhumant herds is carried out in slaughterhouses and represent a significant loss of income for livestock producers in Côte d'Ivoire (Achi et al., 2003). Hence, it is in the public interest to control livestock fascioliasis effectively. To our knowledge, there have been no controlled studies of triclabendazole use against fascioliasis in naturally infected cattle in West Africa. We designed a randomised controlled trial to assess the efficacy of triclabendazole against *Fasciola* spp. in naturally infected cattle in Côte d'Ivoire. Triclabendazole was compared to the approved anthelminthic albendazole.

2. Material and methods

2.1. Study design, sampling and safety

This randomised controlled clinical trial was conducted with cattle in Ferkessédougou (9° 35' 37'' N latitude, 5° 11' 50'' W longitude), in the northern part of Côte d'Ivoire (650 km north of Abidjan, the economic capital of the country), from June 2019 to January 2020. The sample size calculation was based on the primary analysis comparing the proportion of non-egg shedding cattle (PNES) of triclabendazole and albendazole. PNES was defined as the proportion of animals who were egg-positive at baseline but became egg-negative at treatment follow-up. Recent randomised trials investigating the efficacy of albendazole and triclabendazole against *F. hepatica* in cattle estimated the proportion of egg-shedding cattle who stop shedding eggs after treatment (apparent cure rate) at 50-70% and 100%, respectively (Nzalawahe et al., 2018b; Shokier et al., 2013). However, the sample size in both trials were low and therefore, we assumed conservatively conversion of egg-shedding cattle to non-egg shedding to be 70% for albendazole and 90% for triclabendazole. To detect a difference with 80% power at a two-sided 5% significance level, a minimum of 60 positive animals per study arm was required. The sample size of the control group was fixed at 20 animals. Assuming a 10% loss to follow-up, we calculated a final sample size of 154 positive cattle (66 per treatment arm and 22 in the untreated control group). The trial statistician (J.H), who was not involved in any field or laboratory work, provided a computer-generated, stratified (by herd), block randomisation code (blocks of size seven) for randomisation of the cattle into treatment arms.

At baseline, faeces were obtained from the rectums of all cattle that could be handled by our team and the cattle were marked with uniquely numbered ear tags. Demographic information (i.e. sex, age, breed and herd location) were recorded. Cattle faeces were transferred to a nearby laboratory and subjected to the sedimentation technique (Sirois, 2017). Cattle were eligible for inclusion if they were 1 year of age or older, were not visibly ill (assessed by a veterinarian) and had *Fasciola* spp. eggs in the faeces. Cattle that met our inclusion criteria were allocated 3:3:1 to the triclabendazole, albendazole and control arms, respectively. Animals were treated over two consecutive days in July 2019. As there is no control programme for fascioliasis in Côte d'Ivoire, the pastures were not and had not been treated. After treatment, faeces were collected from the cattle in the three groups again on days 21, 28, 90 and 188 post-treatment. Farmers and technicians of the veterinary services in Ferkessédougou, assisted the study team catching and treating the animals.

Cattle were assessed by a veterinarian for diarrhoea and other visible signs of serious illness prior to receiving treatment. Farmers were asked to report adverse events, such as diarrhoea, severe illness or death, occurring within 3 weeks after treatment to the researchers. Veterinary technicians were not blinded, however, the microscopists who examined faecal samples for *Fasciola* spp. infection, were blinded as to the treatment group of the cattle. At the end of the study, *Fasciola* spp. positive cattle from the treatment and control arms were given triclabendazole (12 mg/kg) as per the drug marketer's instructions (Agridirect, 2021a).

2.2. Sedimentation method

Three grams of faeces were homogenised with 30 ml of tap water in a conical beaker. The mixture was left to sediment for 3 min, after which the supernatant was decanted. This process was repeated twice, for a total of 6 min of sedimentation. Then, a drop of methylene blue (1%) was added to the sediment, which was placed on a slide for microscopic examination (Giovanoli Evack et al., 2020; Sirois, 2017). Microscopists examined all sediments for *Fasciola* spp. eggs, using a compound microscope at 100X magnification. The number of eggs was quantified per gram of stool (EPG), by dividing the faecal egg count by a factor of 3 to obtain an estimate of EPG. Microscopists did not differentiate *Fasciola* eggs by species (*F. gigantica* or *F. hepatica*) as the size of eggs overlap between the two species and it was not an objective of this investigation to determine infecting *Fasciola* species (Periago et al., 2006; Valero et al., 2009).

2.3. Treatment

Two molecules were included in the trial: triclabendazole (Fasinex, Novartis Animal Health UK Limited; Surrey, UK) dosed at 12 mg/kg body weight and albendazole (Albex, Chanelle Animal Health Limited, Liverpool, UK) dosed at 15 mg/kg body weight, as recommended by the marketer of the anthelminthics (Agridirect, 2021a, Agridirect 2021b). The control group did not receive any molecule or placebo. The weight of each cow was determined by measuring the chest width with a weight tape, and the appropriate dose of medication was calculated and administered orally using a drench gun. The tape was validated by weighing cattle on a scale at the company "Viande de Ferke" in Ferkessédougou, which specializes in livestock breeding and slaughter. No anthelminthic treatment was administered during the follow-ups.

2.4. Endpoints

The primary endpoint of this study was the PNES of *Fasciola* spp. at 21 days post-treatment, using the sedimentation technique. The World Association for the Advancement of Veterinary Parasitology (WAAVP) recommends comparing faecal eggs counts in treated and untreated cattle at baseline and again at least three weeks after treatment by use of

the sedimentation technique (Wood et al., 1995). Therefore, *Fasciola* spp. egg reduction rate (ERR) at day 21 post-treatment was added as a secondary endpoint using faecal egg count reduction test (FECT), in addition to *Fasciola* reinfection rate (RR) at days 90 and 188 post-treatment. Reinfection was assessed in order to determine how quickly cattle become reinfected after treatment. Two time points were chosen, the first 90 days post-treatment was chosen as the time from ingestion of metacercaria to maturity and egg excretion is 7-8 weeks (Andrews, 1999; Mas-Coma et al., 2007). The second at 188 days post-treatment, was chosen to determine the rate of reinfection six months post-treatment.

2.5. Statistical analysis

Data were double entered into a Microsoft Access 2016 database and imported into Microsoft Access 2002-2003 (Redmond, Washington, USA) and cross-checked using the Data Compare tool of EpiInfo version 3.5.4 (Centers for Disease Control and Prevention; Atlanta, Georgia, USA). Statistical analyses were performed using STATA 15.1 (StataCorp; College Station, Texas, USA) and R version 3.6.2 (R Development Core Team; Vienna, Austria).

Descriptive statistics, crude rates (primary analysis) and adjusted logistic regression (adjustment for herd, age, sex and breed) were calculated to determine the efficacy in terms of PNES. The ERR was defined as the percentage of mean reduction at follow-up 1 (21 days post-treatment) compared to baseline and was calculated using both the arithmetic mean (AM) and the geometric mean (GM). Ninety-five percent confidence intervals (CIs) for differences in ERRs were calculated using the bootstrap resampling method with 1,000 replicates and the significance level was set at $p < 0.05$. The PNES and ERR were also calculated on day 28 post-treatment. Reinfection rates were calculated using only animals in the treatment arms that were deemed uninfected on days 21 and 28. Data were analysed using available case population. The PNES, ERR and RR were determined using the following formulas:

$PNES = 100 \times (neg / n)$
neg = *number of negative animals in each group on day 21 post-treatment*
n = *number of animal per group on day 21 post-treatment*
$ERR = 100 \times [1 - (T_x / T_0)]$
T_x = *arithmetic or geometric mean EPG of each group on day 21 post-treatment*
T_0 = *arithmetic or geometric mean EPG of each at baseline*
PNES and ERR were calculated in the same way for day 28 post-treatment
$RR_1 = 100 \times (P_{90} / N_{21\&28})$
RR_1 = *reinfection rate on day 90 post-treatment*
P_{90} = *positive animals on day 90 post-treatment*
$N_{21\&28}$ = *negative animals on days 21 and 28 post-treatment*
$RR_2 = 100 \times (P_{188} / N_{90})$
RR_2 = *reinfection rate on day 188 post-treatment*
P_{188} = *positive animals on day 188 post-treatment*
N_{90} = *negative animals on day 90 post-treatment*

2.6. Ethical considerations

This study was part of a larger project pertaining to the transmission dynamics and hybridization of human and animal trematode infections in Côte d'Ivoire. Ethical approval was obtained from the National Ethics and Research Committees of Côte d'Ivoire (reference no. 035/MSH /CNER-kp) and Switzerland (reference no. UBE-2016-00707). In addition, the "Direction des Services Vétérinaires" of the "Ministère des Ressources Animales et Halieutiques en Côte d'Ivoire" gave authorization to conduct the research. Farmers signed an informed consent form for the sampling and treatment of their cattle and a veterinary technician carried out the treatments.

3. Results

3.1. Baseline characteristics

One hundred and ninety-six (196) cattle were screened for fascioliasis and other livestock parasitic infections. *Fasciola* spp. were found in 79.1% (95% confidence interval (CI): 72.7-84.6%), *Paramphistomum* spp. in 98.5% (95% CI: 95.6-99.7%), *Dicrocoelium hospes* in 2.6% (95% CI: 0.8-5.9%), *Schistosoma bovis* in 2.0% (95% CI: 0.6-5.1%), *Strongilyda* in 55.1% (95% CI: 47.9-62.2%) and *Moniezia* spp. in 1.0% (95% CI: 0.1-3.6%). Cattle positive for *Fasciola* spp. (n= 155) were randomly allocated to the three study arms: triclabendazole (n = 66), albendazole (n = 67) and control (n = 22) (Fig. 1). Faecal specimens could not be collected from all cattle at follow-ups (21, 28, 90 and 188 days post-treatment), either because the animals were no longer available (absent, sold or slaughtered) or because the animals had already defecated. The cattle from whom faeces could be collected at follow-up, were included in the analysis of that follow-up (Fig. 1). At baseline, the treatment groups did not differ considerably in terms of sex, age, breed, chest size, weight and *Fasciola* spp. EPG (Table 1).

3.2. PNES, ERR and safety

The PNES of triclabendazole against *Fasciola* spp. was significantly higher than that of albendazole at day 21 post-treatment (95.4% vs. 70.3%; odds ratio [OR] 8.73, 95% CI 2.43-31.28, $p < 0.001$). Likewise, the arithmetic mean ERR was significantly higher in the triclabendazole group compared to the albendazole group (99.8% vs. 92.2%, respectively; difference: 7.6%-points, 95% CI: 0.9-14.5%-points, p=0.026). In terms of the geometric mean based ERR, triclabendazole was superior to albendazole (99.6% vs. 91.0%; difference: 8.6%-points, 95% CI: 2.4-15.0%-points, p=0.007) (Table 2). A similar pattern was observed at day 28 post-treatment, the PNES of triclabendazole against *Fasciola* spp. was significantly higher compared to albendazole (95.3% vs. 61.2%; OR 12.89, 95% CI 3.66-45.41, $p < 0.001$). The arithmetic mean ERR was significantly higher in the triclabendazole group compared to the albendazole group (99.8% vs. 93.0%, respectively; difference: 6.4%-points, 95% CI: 1.5-11.4%-point, p=0.011) (Table 2). Unexpectedly, the apparent PNES in the control group at days 21 and 28 post-treatment were 50.0% (95% CI 27.2-72.8%) and 38.1% (95% CI 18.1-61.6%), respectively. None of the cattle was visibly ill at baseline and no adverse events were observed by the farmers in either the triclabendazole (12 mg/kg) or albendazole (15 mg/kg) treatment groups during the 3 weeks after treatment.

3.3. Rate of reinfection

Reinfection was assessed at 90 and 188 days post-treatment. Ninety-three cattle who were in the treatment arms and were negative on days 21 and 28 were included in the analysis (Fig. 2). There were fewer infected cattle in the triclabendazole arm compared to the albendazole arm at both 90 and 188 days post-treatment (Fig. 3). Ninety days after treatment, we observed an infection rate of 3/57 (5.3%) in the triclabendazole arm and 8/34 (23.5%) in the albendazole arm. At 188 days, we found 10/54 (18.5%) and 11/33 (33.3%) of cattle were *Fasciola* spp. positive in the triclabendazole and albendazole arms, respectively. *Fasciola* spp. faecal egg counts were lower in the triclabendazole arm compared to albendazole (Fig. 4). Triclabendazole outperformed albendazole in terms of ERR at day 90 (ERRs 99.0% vs 89.9%, difference 9.0%-points, 95% CI: 4.0-16.6%) and day 180 (ERRs 97.4% vs 92.3%, difference 5.1%-points, 95% CI: 1.3-10.0%).

4. Discussion

Triclabendazole has been used in veterinary medicine since the early 1980s and is the recommended treatment for fascioliasis because of its

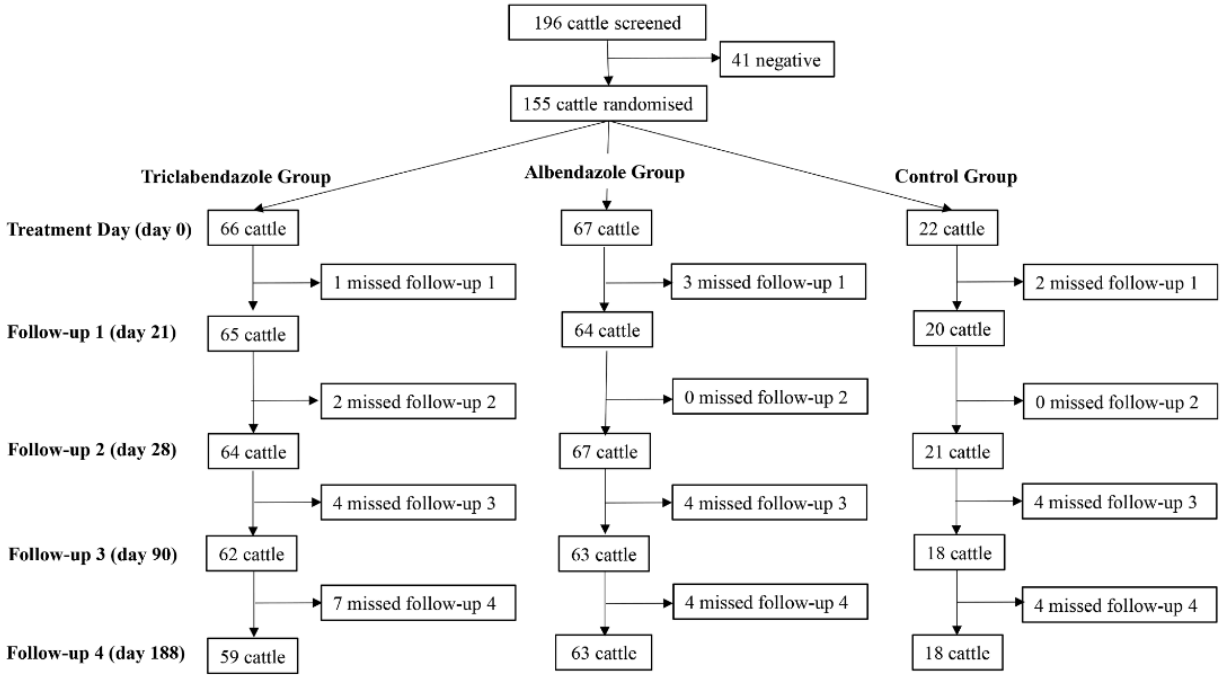

Fig. 1. Trial profile. T1: received triclabendazole; T2: received albendazole; CG: control group (no treatment). Follow-up 1, 2, 3 and 4 correspond to the follow-up faecal sampling and coproscopy on days 21, 28, 90 and 188 post-treatment, respectively.

Table 1
Baseline characteristics of randomised cattle from Côte d'Ivoire in June 2019, stratified by treatment arm. Mean values (standard deviation), EPG = egg per gram of faeces, IQR = interquartile range.

	Triclabendazole	Albendazole	Control
Cattle treated	66	67	22
Sex n (%)			
Female	50 (75.8)	50 (74.6)	14 (63.6)
Male	16 (24.2)	17 (25.4)	8 (36.4)
Breed n (%)			
Zébu n (%)	3 (4.6)	5 (7.5)	3 (13.6)
Taurin x Zébu n (%)	63 (95.4)	62 (92.5)	19 (86.4)
Age [years] mean (SD)	4.8 (2.2)	5.1 (2.3)	5.0 (2.2)
Chest size [cm] mean (SD)	139.0 (15.3)	140.7 (13.5)	146.3 (7.2)
Weight [kg] mean (SD)	232.0 (61.4)	236.0 (58.9)	261.9 (34.7)
Fasciola			
EPG median (IQR)	3.2 (0.7, 6.3)	3.0 (1.0, 7.3)	5.5 (1.0, 11.3)
EPG arithmetic mean	9.2	8.0	8.8
EPG geometric mean	2.9	2.7	3.8

good safety and efficacy profile in animals at all stages and forms of infection (Boray et al., 1983; Gandhi et al., 2019; Kelley et al., 2016). Yet, triclabendazole has not been approved for use in all countries. We present results from the first randomised controlled trial in Côte d'Ivoire and demonstrated that the known efficacy of triclabendazole against fascioliasis in cattle holds true for this West Africa country.

Our findings show that triclabendazole is more efficacious against *Fasciola* spp. infections than albendazole, both in terms of PNES and ERR at 21 and 28 days post-treatment. Our findings corroborate previous studies, which demonstrated high efficacy of triclabendazole (Craig and Huey, 1984; Lecuyer et al., 1985; Rapic et al., 1988; Richards et al., 1990; Stansfield et al., 1987; Suhardono et al., 1991).

The activity of albendazole is restricted to adult *Fasciola* spp. flukes (Johns and Dickeson, 1979; Knight and Colglazier, 1977); hence, immature flukes survive treatment and continue to develop to adulthood. This might explain why the ERR was considerably lower in the albendazole arm compared to the triclabendazole arm, as immature flukes that survived albendazole treatment likely developed into egg-laying, adult flukes. The lower ERR in the albendazole arm might also be explained by drug-resistance developed in flukes as albendazole has been used for many years in Ivorian cattle as an anthelminthic drug. However, this seems unlikely in our study area, as albendazole was still efficacious with an ERR of >90%.

Fifty percent (50%) of the cattle in the control group were found negative at day 21 post-treatment and 38% were negative at day 28. This finding was unexpected. A decrease in PNES and ERR in the control arm is commonly observed in veterinary and human drug trials (Moser et al., 2017). This can be attributed to a regression to the mean phenomenon because only apparent positive animals will be enrolled. However, the effect in the trial reported here was rather high. In theory, the effect should be more pronounced if the day-to-day fluctuation of the parasite is high and the average baseline infection intensity is low. Both are certainly true in this trial, but given the small sample size in the control group, an impact of chance cannot be ruled out. Consequently, the true effect of triclabendazole is likely smaller than estimated but it is important to note that the conclusion of the primary hypothesis "triclabendazole is more efficacious than albendazole" remains valid independently of any potential placebo effect.

Additional factors contributing to the under-estimation of the true prevalence associated with coprology include: variation in the distribution of eggs within a single faecal specimen, daily fluctuations of faecal production and consistency in the host, and daily fluctuations related to oviposition patterns of the parasite (Mas-Coma et al., 2014; Valero et al., 2011, Valero et al., 2002). The Kato-Katz method is

Table 2
Proportion of non-egg shedding cattle (PNES) and egg reduction rate (ERR) against *Fasciola* spp. at days 21 and 28 after the administration of triclabendazole or albendazole in cattle from Côte d'Ivoire. CI = confidence interval, EPG = eggs per gram of faeces.

	Triclabendazole		Albendazole		Control group	
	Day 21	Day 28	Day 21	Day 28	Day 21	Day 28
Resampled cattle	65	64	64	67	20	21
Non-egg shedding cattle after treatment	62	61	45	41	10	8
PNES (95% CI)	95.4% (87.1-99.0%)	95.3% (86.9-99.0%)	70.3% (57.6-81.1%)	61.2% (48.5-72.9%)	50.0% (27.2-72.8%)	38.1% (18.1-61.6%)
EPG arithmetic mean						
Before treatment	9.1	9.5	8.2	8	7.3	7.1
After treatment	0.02	0.03	0.7	0.6	1.7	1.6
ERR (95% CI)	99.8% (99.4-100%)	99.7% (99.1-100%)	92.2% (83.0-96.9%)	93.0% (86.8-96.4%)	76.7% (50.5-92.2%)	78.0% (55.1-89.7%)
EPG geometric mean						
Before treatment	3.6	3.8	3.5	3.5	4.4	4.2
After treatment	0.02	0.02	0.3	0.4	0.9	0.9
ERR (95% CI)	99.6% (98.9-100%)	99.4% (98.5-100%)	91.0% (82.9-95.9%)	89.6% (82.8-94.2%)	80.2% (53.7-93.1%)	77.8% (55.7-89.2%)

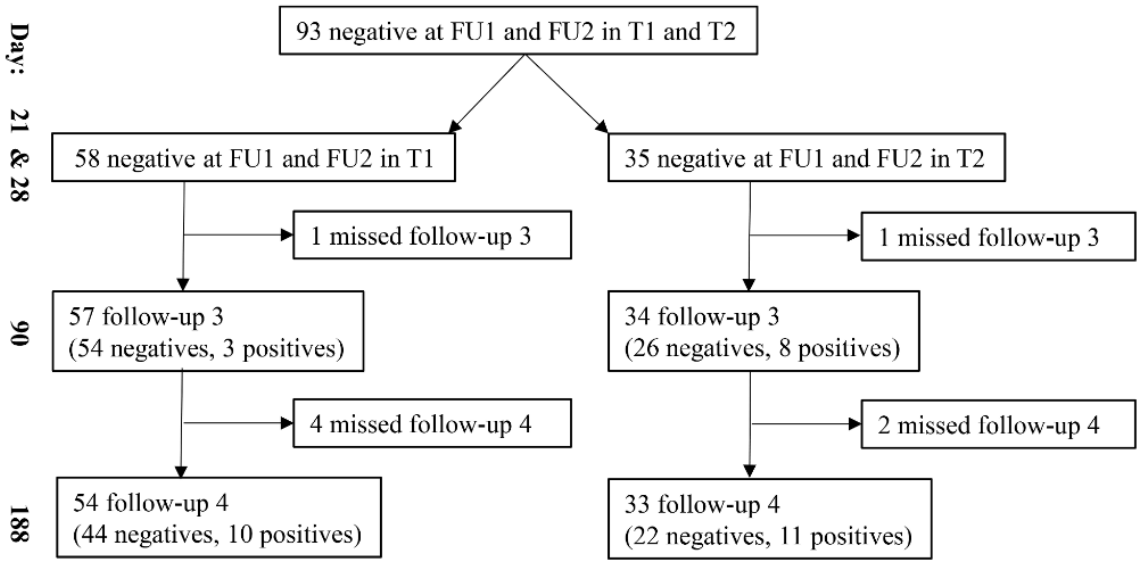

Fig. 2. Flow chart of reinfection. FU1: follow-up 1; FU2: follow-up 2; T1: received triclabendazole; T2: received albendazole. Follow-up 1, 2, 3 and 4 correspond to the follow-up faecal sampling and coproscopy on days 21, 28, 90 and 188 post-treatment, respectively.

commonly used for diagnosis of helminths in human trials (Lamberton et al., 2014), where the standard is to examine multiple slides from multiple samples over 2-3 days. Applying such a strategy to livestock trials would increase sensitivity of diagnosis and improve the accuracy of efficacy estimates.

Our findings reveal that triclabendazole had an effect on post-treatment reinfection that was more pronounced than that of albendazole and was visible for several months, even in a setting of high reinfection. At both 90 and 188 days post-treatment, few cattle in the triclabendazole arm were found to be reinfected with *Fasciola* spp. (5.3% and 18.5%, respectively), compared to those in the albendazole arm (23.5% and 33.3%, respectively). Furthermore, the egg counts were also considerably lower in the triclabendazole arm compared to the albendazole arm at days 90 and 188 post-treatment. The higher reinfection rate in the albendazole group may also be due to the lack of activity of albendazole against immature flukes, as mentioned above (Keyyu et al., 2009).This study was conducted on farms under real life conditions in the northern part of Côte d'Ivoire. Hence, animals pastured and consumed water daily from rivers or dams, where they might become infected with *Fasciola* spp. by ingesting metacercariae. It should be noted that post-treatment reinfection patterns are largely dependent on pasture infestation, and hence, the presence and abundance of intermediate host snails (Brunsdon, 1980). Previous studies from northern Côte d'Ivoire reported the presence of *Lymnaea natalensis* and *Physa acuta* snails, which serve as intermediate hosts for *Fasciola* (Krauth et al., 2017). As we can see from the baseline *Fasciola* spp. prevalence of 79.1%, our study area is an endemic zone for *Fasciola* spp. (Kouadio et al., 2020). Considering that cattle treated with triclabendazole experienced both higher efficacy and fewer reinfections, triclabendazole should be the preferred anthelminthic for the treatment and control of fascioliasis in Côte d'Ivoire.

Despite these promising results, alternating anthelminthics to avoid the development and spread of resistance, as per the manufacturer's recommendations, is highly advisable. Drug resistance in livestock has already been reported in some parts of the world (Fairweather, 2011a, Fairweather, 2011b). In fact, lower efficacy and even resistance to triclabendazole (efficacy <90%) has been documented in Argentina (Olaechea et al., 2011), Australia (Brockwell et al., 2014; Elliott et al., 2015; Kelley et al., 2020), and Northern Ireland (Hanna et al., 2015). Reduction of efficacy or resistance to albendazole has also been

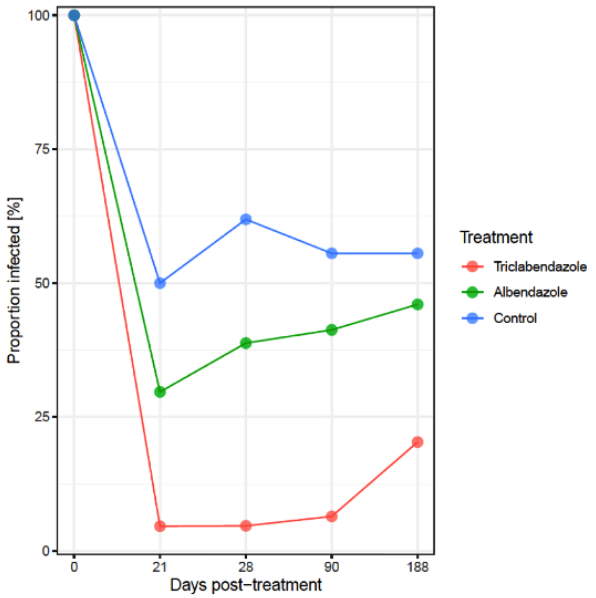

Fig. 3. Variation in the proportion of infected cattle during post-treatment follow-ups.

demonstrated in flukes in Spain (Alvarez-Sanchez et al., 2006), Tanzania (Nzalawahe et al., 2018a) and in a controlled study in Argentina with samples from South America and the UK (Canevari et al., 2014).

Therefore, alternating therapies should be considered as a strategy to avoid or delay the development or spread of resistance (Williams, 1997) and the use of anthelminthics and resistance to them in Ivorian livestock should be further investigated.

Concerning safety, these treatments have been used extensively for many decades and have a well-established safety profile. No adverse events were reported in our study, however under these conditions it is difficult to say if subtler adverse events, such as diarrhoea, occurred. Safety is difficult to assess in the context of real-life field studies with animals such as cattle, particularly in Africa. These animals are large, unpredictable and somewhat dangerous, and with a lack of restraining equipment, it is difficult to control or observe them for more subtle adverse events. Furthermore, it is common in Africa for the herd to spend the day grazing in the pasture, where it is not possible for the handlers to closely monitor 100 or more head of cattle.

5. Conclusion

This study has shown that triclabendazole is safe and highly efficacious against liver fluke infections in cattle in a setting characterised by high fascioliasis prevalence in Côte d'Ivoire. Albendazole, although less efficacious, still plays an important role in the treatment and control of livestock fascioliasis in this country, as it is the registered treatment. To our knowledge, this is the first rigorously designed and executed trial to demonstrate the efficacy of triclabendazole in livestock in Côte d'Ivoire and it is reasonable to assume that these findings are generalisable to other parts of West Africa. Triclabendazole and albendazole should be used for the control of livestock fascioliasis in Côte d'Ivoire as part of a strategic seasonal approach, including intermediate host snail control, as well as raising farmers' awareness of pasture and livestock management. If the seasonal risk of infection is better understood, the treatment could be timed to prolong the effect to a maximum.

Conflict of interest

None

International and national guidelines for the care and use of animals were followed.

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Credit author statement

Jules N. Kouadio, Jennifer Giovanoli Evack, Louise Y. Achi, Oliver Balmer, Jürg Utzinger, Bassirou Bonfoh and Jakob Zinsstag:

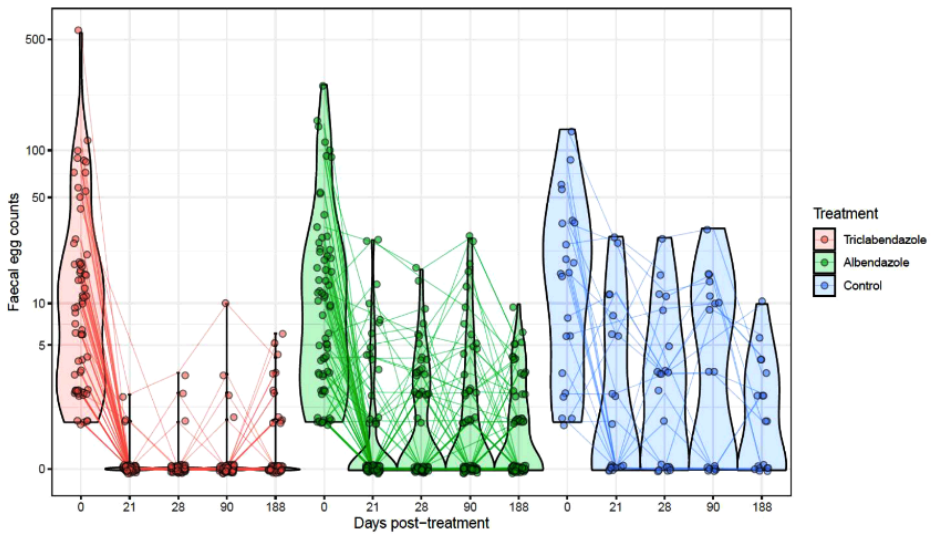

Fig. 4. Density of the distribution of faecal egg (ep3g) counts in the treatment groups at baseline (day 0) and follow-ups (days 21, 28, 90 and 188 post-treatment). ep3g: number of eggs per 3 grams of faeces. Small random noise was added to avoid over plotting.

Conceptualization, Methodology. Jules N. Kouadio, Jennifer Giovanoli Evack and Louise Y. Achi: **Investigation**. Jules N. Kouadio and Jan Hattendorf: **Formal analysis**. Jules N. Kouadio and Jennifer Giovanoli Evack: **Writing- Original draft**. Louise Y. Achi, Jürg Utzinger, Bassirou Bonfoh and Jakob Zinsstag: **Supervision**. Oliver Balmer: **Project administration**. Jules N. Kouadio, Jennifer Giovanoli Evack, Louise Y. Achi, Oliver Balmer, Jürg Utzinger, Eliézer K. N'Goran, Bassirou Bonfoh, Jan Hattendorf and Jakob Zinsstag: **Writing- Reviewing and Editing.** Jürg Utzinger and Bassirou Bonfoh: **Funding acquisition**.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Chapter 8

Discussion

8. Discussion

8.1 Significance of research

Schistosomiasis and fascioliasis present significant health and economic problems in low-resource settings by causing ill health in humans and livestock, and thus reducing productivity of both. Consequently, many resources are directed to the control and prevention of human schistosomiasis, yet to date there is little attention paid to fascioliasis in both humans and livestock, and schistosomiasis in livestock.

The results from this research provide valuable information for Ivorian policy makers concerning control and prevention of these two neglected tropical diseases (NTDs), schistosomiasis and fascioliasis by:

- Confirming the presence of livestock x human schistosome hybrids in humans and the possible role of livestock reservoirs in human schistosomiasis transmission in Côte d'Ivoire,
- Revealing that schistosomiasis and fascioliasis are a significant burden to Ivorian livestock and their holders,
- Uncovering the lack of sensitivity of the current, standard schistosomiasis diagnostic tool in livestock and the degree of underdiagnosis, as well as recommendations for protocol adjustments to improve sensitivity,
- Establishing epidemiological baselines for both infections in livestock across Côte d'Ivoire
- Confirming the effectiveness of triclabendazole for *Fasciola* infected cattle in an Ivorian setting,
- Underscoring the need for:
    - surveillance, monitoring, treatment and control programs for schistosomiasis and fascioliasis in livestock,
    - molecular surveillance of schistosomes and *Fasciola* in all host species to monitor zoonotic activity and hybridization, especially between livestock and human schistosomes.

Prevention and control of NTDs, in general, is a high priority of the World Health Organization (WHO) as witnessed by their focused efforts on NTDs since the early 2000s (Molyneux et al., 2017). Furthermore, both the WHO and the United Nations (UN) have prioritized diseases and issues of poverty, through the WHO's Roadmap and the UN's Sustainable Development Goals (SDGs). The name of the WHO's Roadmap, *Ending the neglect to attain the sustainable development goals: A road map for neglected tropical diseases 2021-2030*, emphasizes the importance and interdependence of these issues. The findings from this research shed light on numerous gaps in control and prevention of these two NTDs, schistosomiasis and fascioliasis, particularly the lack of resources directed to disease in animals. Not only can animals act as reservoirs for human disease, the loss of production and consequent economic losses to farmers in low resource settings from these diseases can perpetuate cycles of poverty. More research is needed to determine the exact role of livestock, wildlife and

transhumance practices on schistosomiasis and fasciolosisis transmission dynamics in Côte d'Ivoire, however the findings from this thesis serve as starting point to inform public and health policy aimed at attaining the targets for both the WHOs NTD Roadmap and the SDGs.

8.2 Innovation, validation and application

This project investigates transmission dynamics of trematodes in humans and livestock in Côte d'Ivoire. The Swiss Tropical and Public Health Institute (Swiss TPH) works across a value chain, from innovation (such as discovering new drugs and diagnostics) and validation (generating evidence in real world settings) to application (integrating new interventions and approaches into everyday policy and practice) in order to achieve their vision, to make the world a healthier place (SwissTPH, 2022). Each chapter in this thesis contributes to the three major pillars of the research mission at the STPH, they are summarized below (Table 1).

Briefly, chapter 4 describes the prevalence of schistosomiasis and fascioliasis in livestock across Côte d'Ivoire, which, as these infections are zoonotic, is relevant to public health. The following comparison of the sedimentation and filtration methods for the diagnosis of schistosomiasis in livestock revealed that the sedimentation technique can be insensitive and the protocol can be adjusted to increase sensitivity. The review of *Schistosoma* population genetics manuscripts summarizes the molecular methods used for revealing genetic diversity and structuring of *Schistosoma haematobium* within and between African countries. The subsequent three chapters describe genetic characteristics of schistosome populations in cattle and humans in the north and south of Côte d'Ivoire. They found that only *S. bovis* is present in cattle in the North and South of Côte d'Ivoire and that these schistosome populations appear to be panmictic, with little structuring and diversity. Furthermore, *S. haematobium*, hybrids of *S. haematobium* x with livestock *Schistosoma* species and pure *S. bovis* are present in human populations in Côte d'Ivoire. The populations of schistosomes from human populations are structured geographically, whereas there is very little structuring in schistosomes from cattle. Finally, schistosome populations in humans and animals that share a water source in the north are distinct and there is limited transmission between the two definitive hosts in that region. The next two chapters discuss *Fasciola* in Chad and Côte d'Ivoire. The first *Fasciola gigantica* x *F. hepatica* hybrid found in sub-Saharan Africa is described (in a bovine), yet in Côte d'Ivoire only *F. gigantica* were found in livestock. The clinical drug trial asserts that triclabendazole is an effective treatment for fascioliasis in cattle in Côte d'Ivoire, in an effort to register triclabendazole for use in cattle in Côte d'Ivoire.

Table 8.1. Overview of the main findings from each chapter and their contribution to the three main research pillars of the Swiss Tropical and Public Health Institute.

	Title	Innovation	Validation	Application
4	Prevalence and distribution of livestock schistosomiasis and fasciolosis in Côte d'Ivoire: results from a cross-sectional survey	√	√	
		First investigation into the prevalence of *Schistosoma* and *Fasciola* across Côte d'Ivoire and in farm livestock. Updates previous prevalence data from abattoir study in Northern Côte d'Ivoire.		
5	Accuracy of the sedimentation and filtration methods for the diagnosis of schistosomiasis in cattle		√	√
		First comparison of sedimentation versus filtration for schistosomiasis diagnosis in livestock. Clarifies protocol optimization for schistosomiasis diagnosis in livestock.		
6a	A review of molecular methods for genetic structure and diversity of *Schistosoma*	√		
		First review of genetic methods used to describe genetic diversity and structure of *S. haematobium* populations.		
6b	Genetic characterization of *Schistosoma* species from cattle across Côte d'Ivoire	√	√	
		First investigation of genetic diversity and structure of *Schistosoma* in cattle in Côte d'Ivoire, second investigation globally. Validates use of microsatellites used for *S. haematobium* on *S. bovis*.		
6c	Genetic Characterization of *Schistosoma* from humans in Côte d'Ivoire		√	
		Describes genetic diversity and populations structure of schistosomes in humans from the north and south of Côte d'Ivoire.		
6d	Host specificity and genetic exchange of *Schistosoma* species found in humans and cattle in Northern Côte d'Ivoire		√	
		First comparison globally of genetic diversity and population structure of schistosome species found in humans and cattle that share the same water source.		
7a	Molecular confirmation of a *Fasciola gigantic* X *Fasciola hepatica* hybrid in a Chadian bovine	√		
		First description of *Fasciola gigantica* x *Fasciola hepatica* hybrid in sub-Sharan Africa		
7b	Efficacy of triclabendazole against *Fasciola* infection in cattle in Côte d'Ivoire: a randomised blinded trial	√	√	
		First randomized controlled trial for the use of triclabendazole for *Fasciola* infection in cattle in West Africa. Validates efficacy of triclabendazole for *Fasciola* infection of livestock in Côte d'Ivoire.		

8.3 Research outputs vs objectives

This PhD project was part of a larger project entitled: Transmission dynamics and hybridization of human and animal trematodes in sub-Saharan Africa and addressed the following objectives: i) to determine what species of *Schistosoma* and *Fasciola* are present in Côte d'Ivoire ii) to quantify genetic diversity and infer transmission dynamics of *Schistosoma* populations within one host and between host species in Côte d'Ivoire; and iii) to infer transmission dynamics of *Schistosoma* populations between geographical locations across Côte d'Ivoire. Over 2,300 specimens were collected: miracidia and flukes of both the *Schistosoma* and *Fasciola* genera from livestock and schistosome miracidia from humans. While all flukes were identified by species molecularly, resource limitations restricted the number of miracidia identified by species and prevented population genetics analysis of *Fasciola* specimens. Furthermore, not all samples could be collected from all host species. Only one snail was found to be infected and not enough sheep and goats were infected with schistosomiasis to allow analysis of schistosome populations from those host species. To date, not all manuscripts for the data collected and analyzed have been written, in particular the manuscripts reporting the prevalences of *Schistosoma* and *Fasciola* in humans and species of *Fasciola* found in Côte d'Ivoire have yet to be written. In this section the outputs for each objective will be discussed.

8.3.1 Objective 1: Trematode species in our sample

Fasciola

Our results revealed no *Fasciola* in humans and only *F. gigantica* in livestock. This is generally congruent with what has been found previously in Côte d'Ivoire on both accounts, although there is not much published data concerning human fascioliasis. A recent systematic review of human fascioliasis in Africa found that fascioliasis has been reported in humans in 12 African countries, but no publications were found on this topic from Côte d'Ivoire (Dermauw et al., 2021). Fascioliasis in Ivorian humans is mentioned in one PhD thesis (Krauth, 2015), where no human fascioliasis was found in Northern Côte d'Ivoire, and in one publication, where one case of human *F. hepatica* was reported (Utzinger et al., 2010).

The systematic review discussed common risk factors for fascioliasis from seven population surveys: female gender, consumption of raw vegetables/seeds, age, owning livestock and use of unsafe drinking sources (Dermauw et al., 2021). Krauth et al. (2015) surveyed the human population in Northern Côte d'Ivoire concerning water access habits, the same area sampled for this thesis, and found 38% of people reported using unimproved and improved water, while 80.0% of people reported consumption of salad and 69.5% reported consumption of raw vegetables. Considering the high prevalence of fascioliasis in cattle in this area, it is somewhat surprising that no fascioliasis was found in humans, as consumption of unsafe water is not necessarily uncommon. A likely explanation could be that water from safe sources is reserved for consumption, while water from unimproved sources is used for domestic activities, not consumption. Indeed, 68% of those surveyed took water used for drinking or preparing food from safe water sources (Krauth et al., 2015). Although only 6.1% of the population surveyed by Krauth et al. (2015) were familiar with fascioliasis, it seems people may be cleaning their raw vegetables with safe water, perhaps to reduce the risk of other food-borne illnesses, which ultimately also reduces their risk for fascioliasis. The lack of human fascioliasis could also be due to misclassifying cases, as only the Kato Katz method was used for diagnosis, when ideally a serological method should also be employed. Coprology presents problem with sensitivity as it relies on egg shedding, which can be low in low-intensity infections or not present at all in new or old infections (Mas-Coma et al., 2014). As mentioned above, not all manuscripts for this project have been written; the manuscript reporting our findings for human fascioliasis is currently in the preparation.

To our knowledge there has not been any molecular species identification of fascioliasis from livestock in Côte d'Ivoire, however previous studies done on fascioliasis prevalences in the north of the country used morphology to determine species and reported only *F. gigantica* (Achi et al., 2003). Our study provides the molecular reassurance that indeed, *F. gigantica* appears to be the only *Fasciola* species infecting livestock in Côte d'Ivoire.

One *F. hepatica* x *F. gigantica* hybrid from a fluke from Chad was identified, the first and only reported *Fasciola* hybrid in Africa. This could be due to the infrequency with which molecular

identification of species is undertaken in Africa. Being that only *F. gigantica* is present in Côte d'Ivoire and from what is known in the countries that feed transhumance in Côte d'Ivoire, it would be unlikely to see hybrids there.

Schistosoma

Our findings reveal that there are high levels of schistosomiasis in cattle, focally, across the country. The schistosomes collected from livestock across Côte d'Ivoire were all *S. bovis*, however in humans, both pure *S. haematobium* and hybrids of livestock (*S. bovis*) and human schistosomes (*S. haematobium*) were found across all five sites in our investigation, while pure *S. bovis* was found in humans in the south only (Angora et al., 2020). The hybrids were found at high prevalences, as has been the case in other West African countries, and many of the hybrids, in both the North and South of Côte d'Ivoire were introgressed, i.e. a result of hybrid backcrosses, suggesting that hybrids are functioning members of the human schistosome population in a stable hybrid zone. Furthermore, the high prevalences indicate that hybridization occurs and hybrids are quite common in humans. Yet, at the four sites where schistosomes were also collected from cattle, Ferkessédougou, Sikensi, Agboville or Duékoué, no hybrids in cattle were observed, suggesting that hybridization is unidirectional and occurs only in humans.

The presence of pure *S. bovis* in humans is concerning, and it is curious that they were only found in the South, although it explains the hybrids. It could be that the pure *S. bovis* was present in humans in the North, but we were unable to detect it due to the small sample size. Furthermore, from our limited view of the genome (2-3 markers, *cox1*, *ITS1*and *ITS2*), it is possible that the pure *S. bovis* found in humans are hybrids too, but display *S. bovis* at the genomic loci we are observing.

It is remarkable that there are forms of *S. bovis* in humans, but no traces of *S. haematobium* in cattle. There are several hypotheses as to why this might be, as the ability of the cercariae to infect the host depends on their ability to locate the host, penetrate the skin and avoid the immune system. It is conceivable that the thinner skin of humans makes it feasible for *S. bovis* to penetrate humans, but not *S. haematobium* to penetrate cattle. Or that, as *S. haematobium* is phylogenetically ancestral to *S. bovis*, perhaps *S. bovis* has retained the ability to infect humans (Huyse et al., 2009).

Strangely, in Ferkessédougou, where schistosomes from humans and cattle who share the same water source were analyzed, the hybrids in humans had traces of *S. curassoni* at the *ITS1* loci, another species of livestock schistosomes. This is odd as the cattle there harboured exclusively *S. bovis,* with no traces of *S. curassoni*. This may indicate that in the North, hybrids in humans are part of a different transmission cycle that include hybrids imported from other regions where *S. curassoni* is present in livestock, perhaps due to the transhumance that occurs in this Northern region. Unfortunately, we do not know if the schistosomes from humans in the South have traces of *S. curassoni*, as they were analyzed as part of another PhD thesis (Dr. Etienne Angora) and were not sequenced at the *ITS1* marker, preventing us from determining if there were traces of *S. curassoni.*

Schistosoma curassoni is not well documented, however it has been reported in livestock and in hybrids from humans in Senegal (Léger et al., 2020; Webster et al., 2013), and since it is difficult to distinguish from *S. bovis*, it may often simply be overlooked. It is also possible that there is so little *S. curassoni* that were we not able to detect it, or that it exists in regions where we did not sample or that it is present in other definitive hosts, such as rodents. The role of *S. curassoni* in human transmission cycles should be further investigated across West Africa.

It is worth noting that livestock are not the only reservoir for numerous schistosome species, including *S. haematobium* x *S. bovis* hybrids. A recent report found prevalences of schistosomiasis of 1.9 – 28.6% in wild rodents in Senegal, the infected species included *S. mansoni*, *S. bovis*, and also *S. haematobium* x *S. bovis* hybrids (Catalano et al., 2018). There is no information on rodent infections in Côte d'Ivoire, but it is possible that rodents may be part of the human transmission cycle. This is of great concern as it suggests that rodents are a natural definitive hosts of *S. haematobium* hybrids.

Hybrids are of great concern in general, as it is thought that they may have a predilection for advantageous traits. Léger et al. (2016) report the first case of a hybrid of livestock species (*S. bovis* x *S. curassoni*) infecting a human, a child in Niger. Léger et al. (2016) argue that this illustrates the possibilities of hybrids having advantageous traits (hybrid vigour) as neither of these schistosome species are known to be able to fully develop in humans or non-human primates (Léger et al., 2016). This kind of adaption could greatly change the schistosomiasis transmission landscape in humans and threaten control and prevention programs.

In summary these results indicate that hybrids are circulating widely amongst humans, but not livestock, and that Côte d'Ivoire is a stable hybrid zone. There appears to be exchange of schistosomes from cattle to humans, but not necessarily the other way round. It is likely that there is occasional spill-over of *S. bovis* from livestock to humans, leading to limited hybridization events and exchange of schistosomes from cattle to humans with the hybrids becoming a permanent feature of the human schistosome population. Yet, important questions remain, such as how long hybridization has been occurring, how frequent hybridization events are and where and in which host species they are occurring.

Furthermore, the ubiquitous nature of hybrids across all sites would indicate that there is gene flow across the sites, which reduces structuring, or that hybridization events are occurring at different locations. As the hybrids in the North had traces of *S. curassoni*, it could be that hybrids in the North are imported from neighbouring countries that harbour *S. curassoni*, as mobile pastoralists frequent this region during their migration during the dry season (Bassett, 2009). The possibility that there is gene flow into Côte d'Ivoire from other countries is concerning as it would further complicate prevention and control efforts in Côte d'Ivoire, however, before a proper investigation into the distribution and prevalence of *S. curassoni* in the region is undertaken, this remains purely speculative.

8.3.2 Objective 2: Genetic diversity and transmission dynamics of parasites within one host and between host species

Genetic diversity and transmission dynamics within and between hosts were analyzed for schistosomes from cattle only, as limited resources and a lack of specimens prevented analysis of other host species (i.e. goats, sheep ad snails) and *Fasciola* populations. Our findings from cattle hosts suggests that the genetic diversity of adult schistosome flukes inside a host individual is comparable to that found across the host population, that is to say that individual cows harbored as much diversity as was found in the overall schistosome population from cattle. This finding contrasts with patterns found in *Schistosoma* miracidia in urine of school children in the South of Côte d'Ivoire, where many children harbored parasites that were genetically very similar and much less diverse than across hosts (Angora et al., 2022). The biological reason for this discrepancy may be that different life stages of the parasite were analyzed in each investigation: miracidia in children and flukes in cattle. Flukes arise from infection with cercariae individually entering the host, and although there are hundreds of asexually produced cercariae emitted from the snail that could be in the same area, their infection of the definitive host is independent at the time of infection and certainly more independent than miracidia. The miracidia are hatched from eggs that are produced inside the host by fluke couples, therefore, miracidia from the same host may be siblings. Our data thus suggest that - in cattle at least - the behavior of the definitive host does not reduce the genetic diversity of schistosomes present in the environment at the time of infection. They also suggest that many flukes from few individual cattle can represent the local parasite population in population genetic analyses, since they do not appear to be more closely related than those from many hosts. However, we would like to caution that theoretical considerations and the findings by other researchers nonetheless advocate for using as broad a host sampling as possible. And it must be kept in mind that the same is not necessarily true for miracidia, i.e. for studies using live hosts.

8.3.3 Objective 3: Genetic diversity and transmission dynamics of parasites by geography

Genetic diversity and transmission dynamics were analyzed only for schistosomes from humans and cattle, as there were not enough samples from sheep, goats and snails.

Our results indicate that there is some geographical structuring in the human schistosome population, although not in an expected manner, while there is very little in the cattle schistosome population. When comparing these results to the African context, our results are comparable to others, showing moderate levels of genetic diversity and structuring between regions in humans (Gower et al., 2013) and very little structuring within countries in cattle schistosomes (Boon et al., 2019; Djuikwo-Teukeng et al., 2019).

The analysis on schistosome populations from humans showed some structuring between the Western and Northern sites (Duekoué and Ferkessédougou) and the Southern sites (Adzopé, Agboville and Sikensi), signalling that there may not be a lot of gene flow between the two groups. It is odd that the

Western and Northern sites appear similar, as transhumance practices occur along a North- South corridor, from Mali or Burkina Faso to Ferkessédougou and Niakaramadougou, making the exchange of schistosomes more likely along this corridor (Achi, 2022; Bassett, 2009). These mobile pastoralists graze their cattle in Côte d'Ivoire in the dry season and return to their homes in the rainy season, providing the opportunity for parasite to disperse across great distances. Another opportunity for movement of schistosomes is provided by the shipment of cattle by train or truck across borders for commercial purposes. The cattle are transported to Bouaké, Yamoussoukro or Agboville to be sold, after which they may be slaughtered or taken to abattoirs in other centers by truck or foot (Achi, 2022). For these reasons it is curious that Ferkessédougou is clustered with Duekoué - which is far from these routes - and not with the southern sites, which are on or near the routes. Although experts in Côte d'Ivoire have discovered that mobile pastoralists are now travelling to the West of Côte d'Ivoire, which may explain this result (Achi, 2022). This finding raises questions as to how hybrids, or advantageous traits such as drug resistance, would spread through the humans schistosome population in Côte d'Ivoire.

There appears to be little structuring and restriction to gene flow of schistosomes in cattle. This contrasts with the situation in humans, where geographical structuring was observed and could indicate that humans are more restricted to their geographical range than cattle, or that *S. bovis* populations have recently been introduced in Côte d'Ivoire, which is unlikely.

8.3.4 Sundry results

There were additional investigations outside the initial objectives that were carried out: the efficacy of triclabendazole on *Fasciola* infected cattle in northern Côte d'Ivoire and the comparison of the sedimentation and filtration methods for diagnosing schistosomiasis in cattle. The efficacy trial confirmed that triclabendazole is an effective treatment for *Fasciola* infected cattle in an Ivorian setting. Furthermore, during the trial, the *Fasciola* infection status of some cattle in the control group changed from one follow-up time point to another (i.e. from positive to negative), suggesting an insensitivity of the method. At the same time, the findings from the comparison of filtration and sedimentation demonstrated issues with the sensitivity of the sedimentation method for schistosomiasis diagnosis in cattle. Both studies emphasize the need for improved diagnostics and control programs for livestock schistosomiasis and fascioliasis.

8.4 Public Health Relevance

8.4.1 Implications for public and human health

This work revealed that livestock, especially cattle, are heavily burdened by schistosomiasis and fascioliasis in Côte d'Ivoire and that they are potential reservoirs for human disease (*Schistosoma*

hybrids). This heavy burden of disease perpetuates the cycle of poverty as it causes economic losses and threatens the economic stability of farmers. Furthermore, livestock and wildlife reservoirs may be relevant factors for the epidemiology of human *Schistosoma* in Côte d'Ivoire. They pose a risk to current control and elimination schemes and should be considered in any strategy (Catalano et al., 2018; Gower et al., 2017).

Many resources are dedicated to the control and prevention of schistosomiasis in humans in an effort to reach elimination, however there is very little, if any, resources directed towards the control and prevention of these two zoonoses in animals. In animals, prevalences are often unknown, treatment is not provided and infecting species are often not confirmed molecularly (World Health Organization, 2010). Consequently, the epidemiological status of these zoonotic NTDs in livestock and wildlife is not well understood, which poses a risk for human health in terms of infection, but also economic stability, making it a public health problem in need of intervention.

Public health professionals often classify health interventions by the level of prevention: primordial prevention aims to prevent disease at a systemic level with interventions that target the entire population to inhibit the establishment of factors that are known to increase risk (e.g. enacting a law to outlaw tobacco), primary prevention is risk reduction and aims to prevent the onset of a disease by addressing the personal risk factors at a population level (e.g. a campaign for smoking cessation), secondary prevention aims to detect and treat disease in individuals early (regular pap smears to detect cervical cancer in women), while tertiary prevention focuses on early rehabilitation and reducing the impact of the disease (Donovan et al., 2018). The current toolbox for human schistosomiasis prevention and control by level of prevention is displayed in Table 2.

Currently, the mainstay of prevention and control interventions for human schistosomiasis lies within secondary prevention: screening and mass drug administration (MDA), as it has proven very successful for controlling morbidity. Nevertheless, there is also some effort put towards primordial and primary prevention through improving health practices by way of education, improved water, sanitation and hygiene (WASH) and vector control.

Table 8.2. Toolbox of schistosomiasis prevention and control by levels of prevention.

Primordial Prevention	Primary Prevention	Secondary Prevention	Tertiary Prevention
• Improved sanitation • Vector control	• Education regarding hygiene and sanitation to encourage behaviour change	• Schistosomiasis screening • MDA	

MDAs for schistosomiasis take place in Côte d'Ivoire currently and reduces prevalences and morbidity, but it is not enough for elimination (Fenwick and Jourdan, 2016). Furthermore, primordial

and primary prevention would reduce the risk of acquiring the infection, along with many other water-borne infections, whereas screening and MDA aim to reduce morbidity of those already infected.
In order to reach elimination of schistosomiasis in humans a more integrated approach which includes prevention and control in animals, is necessary (Campbell et al., 2020; Colley et al., 2020; King et al., 2020). Yet traditionally, this type of integrated approach is not often considered or attempted. One reason for this could be that public health interventions are often guided by the population health framework (Fig. 1), which is human centric, and doesn't address veterinary or environmental influences or reactions. Furthermore, its lowest level of consideration is that of the individual; it does not address what occurs within the individual (e.g. a single cell or molecule).

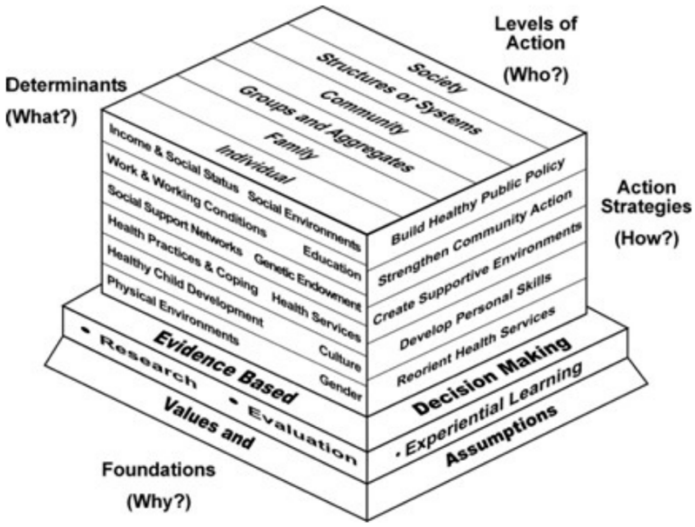

Figure 8.1. The population health promotion model (Vollman Robinson et al., 2004)

A Population Health Model that integrates One Health could be useful in addressing schistosomiasis and other zoonotic neglected tropical diseases by providing public health practitioners with a more holistic perspective. For example, integrating the Generalized Systems Dynamic Framework of Health of Humans and Animals in Social-ecological Systems by Schwabe (1984) and the Generalized systems dynamic framework of health of humans and animals extended from Ostrom (2007), Young et al. (2008) and Rock et al (2009) (see the introduction of this thesis for more details), would add the dimensions of animal health and environmental integrity into model. For example, on the top side of the box, under the individual, a larger unit "population" could be added as well as smaller units of "tissue", "single cell" and "molecule". On the left side of the box, determinants could include three columns: determinants of human health, determinants of animal health (e.g. physical environments, access to safe water and food, exposure to vectors and pathogens etc.) and determinants of ecological

health (sustainability, resilience, vegetation, erosion, climate change, industrial and agricultural production etc.). One side of the box in the back could include ecological factors (Agro-ecology and general ecology of ecosystems and wildlife such as vectors, pathogens, livestock, wildlife, atmosphere, soil and water).
Schistosomiasis and fascioliasis affect humans, livestock and wildlife, like many other pathogens, and therefore require public health professionals to widen their lens and consider more than just human health factors and outcomes of these infections.

8.4.2 One health

Livestock contribute to the livelihood of more than 70% of the world's rural poor, including those south of the Sahel (Ashley et al., 1999). Aside from zoonoses, human health is linked to animal welfare in terms of economics, from both agriculture and the meat industry (Ashley et al., 1999). Fascioliasis and schistosomiasis are an economic burden to farmers as they cause reduced livestock productivity.

This project used a one health approach and was able to find added value by:

- Revealing the transmission dynamics of schistosomes between cattle and humans to determine if cattle are reservoirs for human schistosomiasis,
- Describing *Schistosoma* species across Côte d'Ivoire from a Veterinary perspective – revealing that in the North traces of *S. curassoni* are present in hybrids in humans, while in cattle *S. bovis* appears to be the predominant, and perhaps only, infecting species,
- Allowing veterinary and human health professionals to work together, learn new skills and know more about these zoonoses from another perspective,
- Verifying that fascioliasis is prevalent in livestock in Côte d'Ivoire, especially in the North, but was not found in humans, indicating that there is likely not transmission between livestock and humans.

8.5 Outlook and Recommendations

The WHO has set goals for the elimination and control of NTDs over the last 20 years, however, attainment of these goals has proven exceedingly difficult and complex due to the complicated biology of the pathogens (intermediate hosts, vectors and animal reservoirs) as well as the profound influence of social determinants of health. Ultimately, many NTDs cannot be eliminated with single-pronged interventions. Integrated approaches that effectively interrupt transmission at the interface of the human and animal hosts and the environment are required, through treatment, environmental sanitation and hygiene, vector control and behaviour change (Campbell et al., 2020; Colley et al., 2020; King et al., 2020).In the light of the COVID-19 pandemic, there is an understanding and

readiness for integrated and systems thinking to develop solutions that address the complexity of NTDs and the SDG; for this a One Health approach is very fitting.

This project revealed that fascioliasis prevalence in cattle is high in northern Côte d'Ivoire, yet appropriate treatment is neither registered, nor provided. As mentioned above, fascioliasis is also an NTD, and despite the high economic burden of livestock fascioliasis and the fact that veterinary public health and treatment are listed as strategies to combat NTDS, surveillance and treatment of livestock fascioliasis receives very little attention. Livestock producers loose income when animals are ill, further contributing to poverty and making people more at risk for poverty associated diseases. The triclabendazole trial verified that triclabendazole is an effective treatment for fascioliasis in cattle in Côte d'Ivoire and should be registered and made available to farmers.

Hybrids of human and livestock schistosomes are concerning as they threaten decades of investment and effort into the control and prevention of schistosomiasis. Current strategies are not sufficient to reach goals of elimination and that a more integrated approach is necessary (Campbell et al., 2020; Colley et al., 2020; King et al., 2020).

The evidence generated by this research should be used to inform public policy to address transmission between regions and host species. The policy changes may include:

a. Normalize and incentivize One Health interventions by encouraging Ministries to work together (e.g. MDA of schistosomiasis for humans and schistosomiasis and fascioliasis for cattle),
b. Create awareness of the limitations of diagnostics methods for livestock and encourage adjustment of institutional protocols to increase sensitivity of these methods,
c. Encourage and fund research for better schistosomiasis and fascioliasis diagnostics for livestock,
d. Fund surveillance of schistosomiasis and fascioliasis in both humans and livestock, including molecular species identification,
e. Registering Triclabendazole for use against fascioliasis in livestock.

Questions remain and further research is needed regarding the role of other animal hosts in the transmission of human schistosomiasis in Côte d'Ivoire, such as rats, as well as effect of human and animal migration on transmission cycles.

8.6 Conclusion

In conclusion, we have found that schistosomes in humans are structured geographically across Côte d'Ivoire and hybrids between human and livestock schistosomes are present in human populations. This is not the case in cattle, where schistosome populations are panmictic with little structuring, and they seem to have some degree of resistance to *S. haematobium* and hybrids. Structuring helps to

prevent or slow down dispersal of advantageous traits for parasites, however, in cattle the spread of advantageous traits, such as drug resistance, could occur quickly. Further research concerning the prevalence of humans and livestock hybrids, *S. curassoni* prevalence and *S. bovis* in humans is needed. This emphasizes the necessity of a 'One Health' approach to schistosomiasis control and prevention, in order to prevent further hybridization events and possible livestock reservoirs, treatment for livestock schistosomiasis should be considered across West Africa. Further research into the prevalence of *S. curassoni* in humans and cattle as well as its role in human and livestock hybrids is warranted.

There is a desperate need for better diagnostics for both *Fasciola* and *Schistosoma* in general, but particularly for livestock in resource constrained settings where these infections are of grave concern and a great burden to farmers.

The current approach to disease control in livestock poses a risk for human health in terms of animal reservoirs of disease and perpetuating cycles of poverty due to the high disease burden in livestock and associated economic loses. We hope that these results will assist local governments to plan control and prevention interventions, support the registration of triclabendazole and encourage diagnostic protocol change to improve sensitivity.

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