Human and animal schistosomiasis and fascioliasis in a mobile pastoralist setting at Lake Chad: A One Health approach

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Dekan

For the people of Lake Chad

Table of contents

List of Figures

List of Tables

List of Abbreviations

ABZ	Albendazole
API	Active pharmaceutical ingredient
CCA	Circulating cathodic antigen
CSSI	Centre de Support en Santé International, N'Djamena, Chad
DALYs	Disability Adjusted Life Years
DNA	Deoxyribonucleic Acid
DRE	Délégations Régionals d'Elevage
DSS	Demographic Surveillance Study
EPI	Expanded Immunization Programme
FAO	Food and Agriculture Organization
FBZ	Flubendazole
FGDs	Focus group discussions
HSES	Health in social-ecological systems
IRED	Institut de Recherche en Elevage pour le Développement, N'Djamena, Chad
MBZ	Mebendazole
MDA	Mass drug administration
MERA	Ministère de l'Elevage et des Resources Animales du Tchad
NTDs	Neglected Tropical Diseases
PCR	Polymerase chain reaction
PCT	Preventive chemotherapy
POC	Point-of-care
RDT	Rapid diagnostic test
REPIMAT	Réseau d'épidémiosurveillance des maladies animales au Tchad
SSA	Sub-Saharan Africa
Swiss TPH	Swiss Tropical and Public Health Institute, Basel, Switzerland
THZ	Thiabendazole
TCZ	Triclabendazole
WHO	World Health Organization

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Summary

Background: Throughout the Sahelian belt of Africa, mobile pastoralism is a highly adapted life style and livestock breeding by mobile pastoralists contributes significantly to the economies of Sahelian countries. The Lake Chad basin is a unique ecosystem and provides livelihoods to millions of people, including fishermen, farmers and specifically mobile pastoralists. But mobile people are disadvantaged with regard to access to social services such as health care and education. Chad ranks among the poorest countries in the world and the national human and veterinary health services cover only very basic services which do not meet the needs of the population, especially in rural areas. For more than 20 years, the Swiss Tropical and Public Health Institute, in close partnership with Chadian institutions, have pursued collaborative health research and development. The collaboration aims to sustainably improve human and animal health of mobile pastoralists through a transdisciplinary One Health approach. In this way public and animal health workers engage closely with communities and authorities to develop locally adapted health care solutions for humans and animals. This project was initiated to address the pastoralists' concern about considerable economic losses due to fascioliasis caused by liver flukes. In Chad, livestock fascioliasis is caused by *Fasciola gigantica*, a water transmitted trematode that uses a snail vector as an intermediate host in its life cycle, similar to the blood flukes of the genus *Schistosoma*. *Schistosoma* blood flukes affect humans and animals. Livestock schistosomiasis is caused by *Schistosoma bovis*, while *S. haematobium* and *S. mansoni* cause human disease. Human schistosomiasis is listed among the neglected tropical diseases and the World Health Organization (WHO) aims for elimination by 2025.

Objectives: This PhD project aims to deepen the systemic understanding of the epidemiology of schistosomiasis and fascioliasis in humans and cattle in the social-ecological system of a mobile pastoralist setting at Lake Chad. Specific objectives include: (i) to determine the prevalence and distribution of human and animal trematodes in humans, cattle and snails, in view of potential development of joint human, veterinary and malacological prevention and control measures; (ii) to elucidate mobile pastoralist disease perception, health seeking behavior and access to and use of treatment for human worm disease and livestock fascioliasis, including the test of available drugs for their active ingredients; and (iii) to engage in strengthening parasitological laboratory capacities by validating point-of-care diagnostic tools and to test triclabendazole treatment of livestock fascioliasis in a mobile pastoralist husbandry system.

Methods: On the eastern shores of Lake Chad, 19 groups of pastoralists of four different ethnicities participated in the study. From September 2013 to December 2014, randomly selected individuals voluntarily submitted urine and stool samples for parasitological screening. Urine filtration, reagent strip testing for microhematuria and point-of-care circulating cathodic antigen urine cassette test as well as microscopy of Kato-Katz thick smears from stool samples were performed in a mobile field

laboratory in proximity to the pastoralist's camp sites. Additionally, stool samples were conserved in sodium acetate-acetic acid-formaline (SAF) solution for transport and subsequent ether-concentration preparation in a laboratory in Basel. Cattle were randomly selected and individually ear tagged. A fecal sample was collected from each animal and conserved in SAF solution for subsequent analysis with the sedimentation technique in laboratories in N'Djamena and Zurich. During questionnaire interviews with schistosomiasis patients, disease perception, treatment strategies and health seeking behavior were assessed. Livestock health priorities, treatment strategies and outcome satisfaction were addressed during focus group discussions. Locally available anthelminthic drugs were tested for their active ingredient and quantity using the high pressure liquid chromatography-UV method combined with tandem mass spectrometry. A single-dose triclabendazole treatment against fascioliasis was administered to 375 individually ear tagged naturally infected cattle. At the same time, a fecal sample was obtained from each animal for parasitological analyses. After six months, 198 cattle were re-sampled and re-infection was assessed. A malacological study on snail species composition, distribution and abundance in two seasons was conducted in N'Djamena.

Results: The ethnic groups participating in the study were the Arabs, the Gorane, the Fulani and the Buduma. A total of 413 people and 534 cattle were randomly selected for participation. Overall, *S. haematobium* was the most prevalent helminth in humans with a prevalence of 8.1% (95% CI: 5.0-12.8). Stool sample analyses revealed a *S. mansoni* prevalence of 0.4% (95% CI: 0.05-2.4), whereas point-of-care circulating cathodic antigen (POC-CCA) urine cassette tests revealed a *S. mansoni* prevalence 9.2% (95% CI: 5.1-16.2). In cattle, *F. gigantica* prevalence was 31.3% (95% CI: 23.3-40.6) and *S. bovis* prevalence was 20.3% (95% CI: 13-30.2). Trematode prevalence varied significantly between ethnic groups and showed comparable patterns in humans and cattle of the same ethnic groups. Among 57 schistosomiasis cases, more than 60% sought treatment in health centers and from the informal market. Fascioliasis awareness among pastoralists was high and self-mediated therapy for animals is the common practice. Mebendazole and albendazole are the drugs locally available which are used for human worm diseases and for livestock fascioliasis treatment. Tests revealed that the majority of the products containing an over-concentration of the active compound (up to 150% of the labeled amount).

The field trial of triclabendazole showed rapid re-infection with fascioliasis. A total of 46% of treated cattle were shedding *F. gigantica* eggs six month after treatment, which has implications for the future planning of locally adapted interventions. In N'Djamena, several intermediate host snail species of *Schistosoma* spp and *Fasciola* spp were found in both seasons sampled. Generally, cercarial shedding experiments revealed low snail infection. Snail species composition varied seasonally.

Conclusions: Human urogenital schistosomiasis and bovine fascioliasis and schistosomiasis are endemic in the mobile pastoralist populations and their cattle at Lake Chad. When stratified by ethnic groups, prevalence shows similar patterns for human and cattle trematode infections. This illustrates the strong linkage between the culturally defined husbandry system and human and animal exposure to trematode infection risks. For both, human and animal trematode infection, the most effective drugs, praziquantel and triclabendazole, are currently not available in the study zone. Introducing efficacious treatment against human schistosomiasis and livestock fascioliasis will not only positively impact human and animal health, but also result in economic benefits by improving livestock productivity and reducing treatment costs. The One Health approach applied in this research project has the potential to translate into economic savings through joint intervention planning for the sustainable improvement of human and animal health.

Résumé

Contexte : Tout au long de la bande sahélienne de l'Afrique, le pastoralisme mobile qui est un mode de vie et d'élevage très adapté par les pasteurs mobiles, contribue de manière significative à l'économie des pays situés au sud du Sahara. Le bassin du lac Tchad est un écosystème unique de son genre qui fournit des moyens de subsistance à des millions de personnes, tels que des pêcheurs, des agriculteurs et surtout les éleveurs mobiles. Cependant, la mobilité de ce groupe social est un facteur défavorable à l'accès aux services sociaux de base tels que la santé et l'éducation. Le Tchad est classé parmi les pays les plus pauvres du monde avec des services de santé humaine et vétérinaire nationaux ne couvrant que le minimum de services, répondant ainsi difficilement aux besoins de la population rurales.

Depuis plus de 20 ans, l'Institut Tropical et de Santé Publique Suisse, en étroit partenariat avec des institutions tchadiennes poursuivent des activités de recherche-développement en santé. Cette collaboration vise à améliorer durablement la santé humaine et animale des pasteurs mobiles grâce à une approche transdisciplinaire « Santé Unie ». Ainsi, les professionnels de la santé publique et de la santé animale s'engagent avec les communautés et les autorités à rechercher des solutions de santé humaine et animale tout en s'adaptant aux conditions locales. Ce projet a été initié suite aux soucis soulevés par les éleveurs victimes des pertes économiques considérables liées à la fasciolose animale.

Au Tchad, la fasciolose animale est causée par *Fasciola gigantica*, un trématode transmis de l'eau à l'aide d'escargot comme hôte intermédiaire dans son cycle de vie semblable aux douves du sang du genre Schistosomiase qui affectent les êtres humains et aussi les animaux. La schistosomiase animale est causée par *Schistosoma bovis*. Cependant, la schistosomiase humaine, causée par *S. haematobium* et *S. mansoni*, figure parmi les maladies tropicales négligées listés par l'Organisation Mondiale de la Santé (OMS) à éliminer d'ici 2025.

Objectifs : Ce projet de thèse vise à approfondir la compréhension systémique de l'épidémiologie de la schistosomiase et de la fasciolose chez l'homme et l'animal dans un système socio-écologique du Lac Tchad fréquenté par des pasteurs mobiles. Les objectifs spécifiques sont: (i) déterminer la prévalence et la distribution des trématodes d'origines humaine et animale chez les hôtes respectives (homme, bétail et escargots), dans l'objective de développer des mesures de contrôle et de prévention conjoints humaine-animale-mollusques, (ii) élucider la perception des helminthes humaines et animales par les pasteurs mobile, leurs comportements sanitaires et leurs accès aux médicaments vétérinaires et humains ainsi que leurs modes d'utilisation afin de tester des médicaments usuels; (iii) s'engager dans le renforcement de capacité de laboratoire de parasitologie

en validant des méthodes de diagnostic de point et tester le triclabendazole pour le traitement de la fasciolose de bétail dans un élevage mobile.

Méthodes : Au total, 19 groupes de pasteurs de quatre différentes ethnies ont participé à l'étude dans la rive est du lac Tchad. De Septembre 2013 à Décembre 2014, des échantillons d'urine et de selles ont été collectés sur des individus choisis au hasard pour le dépistage parasitologique. Des différents types de tests de diagnostic ont été effectués sur ces échantillons notamment la filtration de l'urine, des bandelettes pour microhématurie, *point-of-care circulating cathodic antigen urine cassette test* ainsi que la microscopie de Kato-Katz sur des épais frottis à partir d'échantillons de selles ont été effectués dans un laboratoire mobile sur le terrain à proximité des campements de pasteurs. En outre, des échantillons de selles ont été conservés dans une solution d'acétate-acétique formole (SAF) pour le transport et plus tard la préparation d'éther-concentration dans un laboratoire à Bâle.

Des bovins ont été choisis au hasard et individuellement marqués à l'oreille. Des échantillons de selles ont été collectés et conservées dans une solution SAF pour le transport puis soumis à la technique de la sédimentation dans des laboratoires à N'Djamena et à Zurich. Au cours des entretiens basés sur des questionnaires avec des patients de la schistosomiase, la perception de la maladie, les stratégies de traitement et les comportements sanitaires ont été évalués. Des discussions de groups FGD ont permis d'identifié les priorités en santé du bétail, les stratégies de traitement et la satisfaction des résultats ont été aussi abordées au cours de ces discussions de groupes. Les médicaments anthelminthiques disponibles ont été testés pour leur ingrédient actif et aussi la quantité administrée en utilisant la méthode de liquide à haute pression chromatographie-UV couplée à la spectrométrie. Un traitement à dose unique de triclabendazole contre la fasciolose animale infectés naturellement a été administré à des bovins marqués à l'oreille. Des échantillons de selles ont été prélevés à partir de chaque animal pour des analyses de parasitologie. Après six mois, les bovins ont été ré-échantillonnés et la réinfection a été évalué. Enfin, une étude malacologique sur la composition des espèces d'escargot, la distribution et l'abondance en deux saisons a été menée à N'Djamena.

Résultats : Les groupes ethniques participant à l'étude étaient les Arabes, le Gorane, les Peuls et le Boudoumas. Un total de 415 personnes et 534 bovins ont été sélectionnés au hasard pour participer à cette étude. Dans l'ensemble, *S. haematobium* était l'helminthe le plus répandu chez l'homme avec une prévalence de 8,1% (IC 95%: 5,0 à 12,8). Les analyses d'échantillons de selles ont révélé une prévalence de *S. mansoni* de 0,4% (IC à 95%: 0,05 à 2,4), lors ce que les tests POC-CCA ont révélé une prévalence de *S. mansoni* de 9,2% (IC 95%: 5,1 à 16,2). Chez les bovins, la prévalence de *F. gigantica* était de 31,3% (IC à 95%: 23,3 à 40,6) et la prévalence de a été de 20,3% (IC à 95%: de 13 à 30,2). La

prévalence de tous les trématodes variait significativement entre les groupes ethniques et a montré des modèles comparables chez les humains et le bétail des mêmes groupes ethniques. Parmi 57 cas de schistosomiase, plus de 60% ont cherché un traitement dans les centres de santé et du marché informel. La sensibilisation et connaissance de la fasciolose était élevé parmi les éleveurs et la thérapie d'auto-médiation pour les animaux est une pratique courante. Les Mebendazole et l'albendazole sont des médicaments disponibles localement et utilisés pour les maladies du ver de l'homme et pour le traitement du bétail de la fasciolose. Des tests ont révélé que la majorité des produits étaient de qualité inférieure aux normes, principalement due à une trop forte concentration de la substance active (jusqu'à 150% de la quantité marquée).

L'essai sur le terrain de triclabendazole a montré une réinfection rapide de la fasciolose. Un total de 46% des bovins traités ont excrétés des œufs de *F. gigantica* six mois après le traitement. Cette découverte a des implications pour une future planification des interventions sanitaires adaptées aux conditions locales. À N'Djamena, plusieurs espèces intermédiaires d'escargot hôte de *Schistosoma* spp et *Fasciola* spp ont été trouvés dans les deux saisons de l'échantillonnage. D'une manière générale, les excrétions cercaires révélaient une faible infection des mollusques. La composition des espèces varie selon les saisons.

Conclusions : La schistosomiase urogénitale humaine, la fasciolose bovine et la schistosomiase sont endémiques dans la population pastorale mobile et leur bétail au Lac Tchad. Après stratification par groupes ethniques, les prévalences montrent des tendances similaires pour les infections humaines et animales des trématodes. Cela illustre le lien étroit entre le système d'élevage défini culturellement et l'exposition humaine et animale à des risques d'infection par des trématodes. Pour les deux, l'infection des trématodes humaine et animale, les médicaments les plus efficaces, à savoir le praziquantel et le triclabendazole, ne sont pas disponibles dans la zone d'étude en ce moment. L'introduction d'un traitement efficace contre la schistosomiase humaine et animale et la fasciolose aura un impact non seulement positivement sur la santé humaine et animale, mais aussi des avantages économiques par l'amélioration de la productivité de l'élevage et de la réduction des coûts de traitement. L'approche « Santé Unie » dans ce projet de recherche à un potentiel de se traduire en gains économies à travers la planification des interventions conjointes pour une amélioration durable de la santé humaine et animale.

1. Introduction

1.1. Neglected parasite infections in humans and animals

A diverse group of disabling infectious diseases predominantly endemic to tropical and subtropical regions of Africa, America and Asia are categorized by the World Health Organization (WHO) as neglected tropical diseases (NTDs). Worldwide, over 85% of the NTDs' disease burden is caused by helminth infections (Hotez, P. J. and Kamath, A., 2009). The WHO initiates and coordinates NTD specific control and prevention programs. Several NTDs are on the agenda for elimination and eradication, among these schistosomiasis (WHO, 2012a). Many of those most affected by NTDs live in remote rural areas, informal urban settlements or regions that are affected by conflicts. Poverty facilitates the acquistion and persistence of NTDs and is also a consequence of these diseases (WHO, 2015a). People with NTDs are often impaired to work, attend school or contribute to community social and economic life. Treatments exist for the majority of NTDs but are simply not available for those in need due to economic challenges or lack of access to control programs and health services (Obrist, B.*et al.*, 2007, Hotez, P. J.*et al.*, 2009, Tambo, E.*et al.*, 2015). Voluntary and forced population movements are prone to the aforementioned factors and have an impact on the epidemiology of NTDs (Aagaard-Hansen, J.*et al.*, 2010). The control of NTDs is further challenged by the lack of appropriate, accurate diagnostic tools which can be easily performed within the capacity of the health centers in the endemic areas (Utzinger, J. and de Savigny, D., 2006, Bergquist, R.*et al.*, 2009, Bergquist, R.*et al.*, 2015).

Rural populations in Africa largely generate their livelihoods through subsistence farming, agriculture and livestock breeding, and the Sahelian belt is one of the most important livestock production areas on the continent (Robinson, T. P.*et al.*, 2014). In these settings, parasitic infection and multiparasitism affecting ruminants are a constant threat causing losses due to expenses for veterinary services and reduced productivity and reproduction. Poor animal health directly affects the economy of livestock breeding societies, specifically the livelihoods of mobile pastoralists who are fully dependent on livestock. Animal productivity losses led to reduced income and lack of financial means leds to food deficits, showing how human and animal health are intrinsically interconnected (Zinsstag, J.*et al.*, 1998). During a recently conducted demographic surveillance study (DSS) on the south-eastern shores of Lake Chad, mobile pastoralists repeatedly emphasized fascioliasis caused by *Fasciola gigantica* as a major veterinary health problem leading to serious economic losses (Jean-Richard, V., 2013).

Because of the similarities of the life cycles of all trematode species, this project addressed human and animal schistosomiasis and fascioliasis concurrently. The aim of this PhD project is to deepen the

understanding of *Schistosoma spp* and *Fasciola* spp transmission in humans and cattle in the social-ecological system of a mobile pastoralist setting at Lake Chad. With a one health approach, the mutual predictive potential of human and animal trematode infections is explored in view of developing joint human and veterinarian prevention and control measures.

1.2. **Trematode biology**

The blood flukes of the genus *Schistosoma* and the liver flukes of the genus *Fasciola* belong to the obligate parasitic class *Trematoda*, subclass *Digenea*. The subclass's name refers to the fact that all of its members complete their lifecycle within two hosts, the intermediate host being a mollusc species and the principal host being a vertebrate species (Mehlhorn, H., 2008).

Schistosoma species: the blood flukes

Schistosoma eggs are excreted either with urine (*S. haematobium* in humans) or feces (in humans: *S. mansoni, S. intercalatum, S. japonicum*; in ruminants: *S. bovis, S. mattheei, S. magrebowiei, S. leiperi*). These eggs contain a ready-to-hatch miracidium. Once in contact with water, the miracidium emerges from the egg and seeks to infect a water snail, predominantly *Biomphalaria* spp. (*S. mansoni*), *Bulinus* spp. (*S. haematobium, S. bovis*) and *Planorbis* spp. (*S. bovis)*.

Figure 1.1. The life cycle of the human blood flukes, illustrated here for *Schistosoma haematobium* and *S. mansoni*, is comparable to the life cycle of *S. bovis,* which infects ruminants.

(Photos by W. Moser, A. A. Batil, H. Greter)

Within the snail over a timespan of 3 to 5 weeks, the miracidium develops into a sporocyst that multiplies into more sporocysts and redia, leading to massive asexual reproduction and the generation of cercariae. A snail infected with a single miracidium can shed thousands of cercariae over a period of several days (Figure 1.1). The cercariae freely swim in the water in search of a susceptible host and then actively penetrate the human or animal skin. In the venous bloodstream, the parasite undergoes several larval stages. It travels through the body until it reaches the intestinal mesenteric venules (*S. mansoni, S. bovis*), or the venous plexus of the urinary bladder (*S. haematobium*), where it attaches to the blood vessel epithelium (Figure 1.2) (Mehlhorn, H., 2008). A specialty of the genus *Schistosoma* in comparison to other trematode genera is that they are dioecious and monogamous (Moné, H. and Boissier, J., 2004, Loker, E. S. and Brant, S. V., 2006, Beltran, S. and Boissier, J., 2008). Once a male and a female worm come together, the female remains in a special gynaecophoric canal of the male worm and the pair can produce fertilized eggs for several years when the infection remains untreated (Southgate, V. R.*et al.*, 1998).

Figure 1.2. Adult *Schistosoma* flukes, here *S. bovis* in slaughtered cattle, live in the mesenteric veins (left). The spindle-shaped egg of *S. bovis* has a characteristic terminal spine (right). (Photos: A. A. Batil, H. Greter)

***Fasciola* species: the liver flukes**

The liver flukes of the *Fasciola* family have a comparable lifecycle to *Schistosoma* spp., with two main differences. First, the miracidia develop within the egg in the environment over several days. And second, after completing the asexual multiplication within the snail host, *Fasciola* cercariae emerge from the snail. The swimming cercariae attach to vegetation and encapsulate. In this stage they are called metacercariae and can persist for several weeks (Morley, N. J., 2015). *Fasciola* flukes reach their definitive host passively when ruminants feed on contaminated vegetation. Once in the

ruminant gastro-intestinal tract, the metacercariae penetrate the wall of the small intestine and migrate through the peritoneal cavity to the liver into the biliary tract, where they develop into adult flukes. *Fasciola* flukes are hermaphrodites. The eggs are shed into the environment with the host's feces (Mehlhorn, H., 2008). Once in contact with water, the lifecycle starts over again with the development of the miracidia and the infection of the intermediate host snails, predominantly a *Galba* species (Figure 1.3).

Figure 1.3. Life cycle of *Fasciola gigantica*. (Photos: W. Moser, fileshare.org, H. Greter)

1.3. Human and animal trematode infections: burden, diagnosis and treatment

Human schistosomiasis in Africa

Schistosomiasis is a major public health problem with around 240 million people worldwide suffering from the chronic intestinal or urogenital forms of the disease (WHO, 2013), which represents a burden of 3.3 million disability-adjusted life-years (DALYs) (Murray, C. J.*et al.*, 2012). Over 90% of these human schistosomiasis cases occur in sub-Saharan Africa (SSA). In view of their plan for schistosomiasis elimination by 2025, WHO estimated 210 million people in need of preventive chemotherapy in Africa in 2013 (WHO, 2015b). Multifaceted integrated control programs including targeted treatment through mass drug administration (MDA), strengthening of health and social

systems, improved sanitation infrastructure, vector control and health education and promotion have been established in many endemic countries (Gray, D. J.*et al.*, 2010, Rollinson, D.*et al.*, 2013).

The three species infecting humans in Africa are *S. mansoni, S. intercalatum* and *S. haematobium* (Figure 1.4). Infections may show mild, unspecific symptoms or occur seemingly asymptomatic, but chronic schistosomiasis can cause severe morbidity (King, C. H. and Dangerfield-Cha, M., 2008, King, C. H., 2015). It is not the presence of the adult worms which induces pathological reaction, but rather stray eggs that circulate through the blood vessels and become trapped in organ tissue of the bladder, liver, intestines, or various other tissues (Gryseels, B.*et al.*, 2006). Besides environmental and climatic factors determining and influencing snail host distribution, human activities involving water contact and sanitation and hygiene related behavioral aspects have a strong impact on the endemicity of the disease and its presence, persistence and transmission within a community (Utzinger, J.*et al.*, 2011, Colley, D. G.*et al.*, 2014).

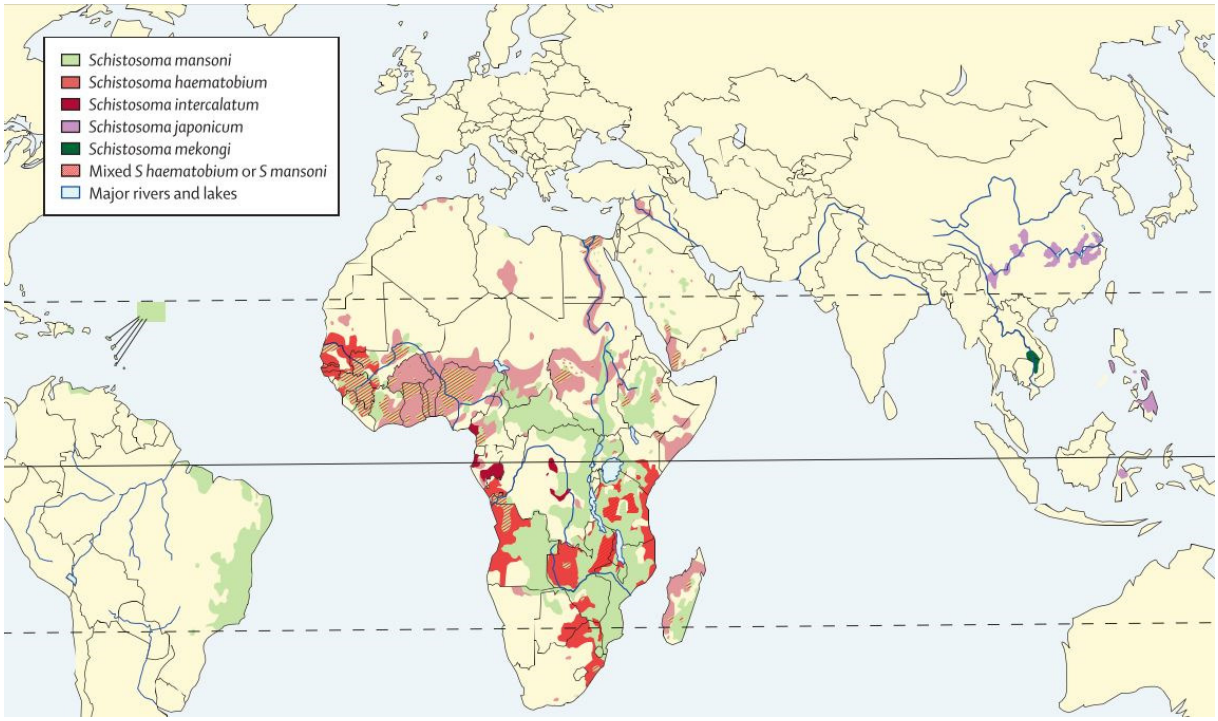

Figure 1.4. Global distribution of *Schistosoma* species causing human schistosomiasis. (Colley, D. G. et al. (2014). The Lancet)

Standard diagnostic procedures for schistosomiasis involve microscopy for parasite eggs in stool or urine samples. For urogenital schistosomiasis a filtration method is applied to urine samples, followed by microscopy. An active schistosomiasis infection is often accompanied by micro-hematuria, so the detection of blood in urine using reagent strips is widely used as an indicator for urogenital schistosomiasis (Mott, K. E.*et al.*, 1983). The standard diagnostic approach for intestinal schistosomiasis is the quantitative Kato-Katz method that uses methylene blue staining of parasite eggs in fecal samples (Katz, N.*et al.*, 1972). With the elimination goal in view, schistosomiasis

diagnosis has received new attention and more easy-to-use, highly sensitive and rapid methods need to be developed (Utzinger, J.*et al.*, 2015). Besides the development of molecular techniques with high sensitivity such as polymerase chain reaction (PCR) or antibody detection methods (ELISA), rapid tests with a high sensitivity that can be easily applied, do not need laboratory infrastructure and are affordable promise the highest impact. One example is a newly available point-of-care (POC) rapid diagnostic test (RDT) for intestinal schistosomiasis that applies a lateral-flow principle to detect the circulating cathodic antigen (CCA) of *S. mansoni* in urine samples (Colley, D. G.*et al.*, 2013).

Praziquantel is the drug of choice to treat both forms of human schistosomiasis. It is a highly effective single-dose treatment that is used in clinical cases as well as in preventive chemotherapy programs worldwide (WHO, 2015b). In Chad currently, no national schistosomiasis control program is in place (Figure 1.5) (Rollinson, D.*et al.*, 2013).

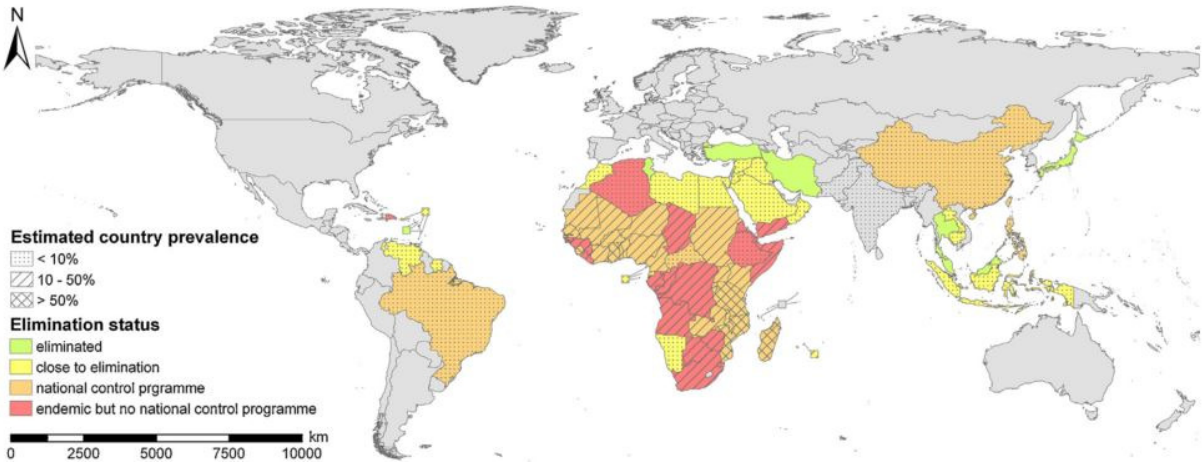

Figure 1.5. World map highlighting countries where schistosomiasis has been eliminated (green color), is close to elimination (yellow color) or where national control programs or some preventive chemotherapy are in place (orange color). Marked in red are countries where schistosomiasis is endemic and national control programs are not yet implemented (Rollinson, 2013).

Animal schistosomiasis in Africa

Globally, 19 *Schistosoma* species have been reported to naturally infect animals, with those affecting ruminants receiving particular attention due to their recognized veterinary importance[1]. In Africa, three species affecting ruminants are of specific interest: *S. mattheei* is endemic to southern and eastern Africa, while *S. curassoni* has been described in western Africa from Senegal to Nigeria. *S. bovis* has the widest range, stretching across the African continent to include northern, western, and eastern Africa, and expanding into the Mediterranean region and the Middle East (Figure 1.6) (Over, H. J.*et al.*, 1992, Calavas, D. and Martin, P. M., 2014). Livestock schistosomiasis can be diagnosed using a sedimentation technique on fecal samples, but sensitivity is low and prevalence is therefore

[1] http://www.merckvetmanual.com/mvm/circulatory_system/blood_parasites/schistosomiasis.html

widely underestimated[1] (Habtamu, A. and Mariam, S. W., 2011). During meat inspection, adult *Schistosoma* worms are visible in the intestinal mesenteric veins of slaughtered animals (Figure 1.2). Infected animals show nonspecific symptoms such as weight loss and reduced productivity (Christensen, N. O.*et al.*, 1983). Pathogenesis does not arise from the presence of adult worms in the veins, but is mainly caused by the migration of millions of eggs through the host intestinal wall and accumulation in the tissue (Mehlhorn, H., 2008). Schistosomiasis in ruminants can be treated with repeated praziquantel administration, but the volume of dead worms following chemotherapy may cause problems since dead worms lodge in small veins or in the liver and provoke inflammatory reactions (Mehlhorn, H., 2008). The risk of these adverse effects, together with the nonspecific symptoms result in the disease remaining untreated in most of the endemic areas of Africa[1] (Mehlhorn, H., 2008).

Little recent epidemiological data on livestock schistosomiasis is available and the epidemiology in Africa and beyond is not well studied currently (Christensen, N. O.*et al.*, 1983, Vercruysse, J.*et al.*, 1994, Moné, H.*et al.*, 1999).

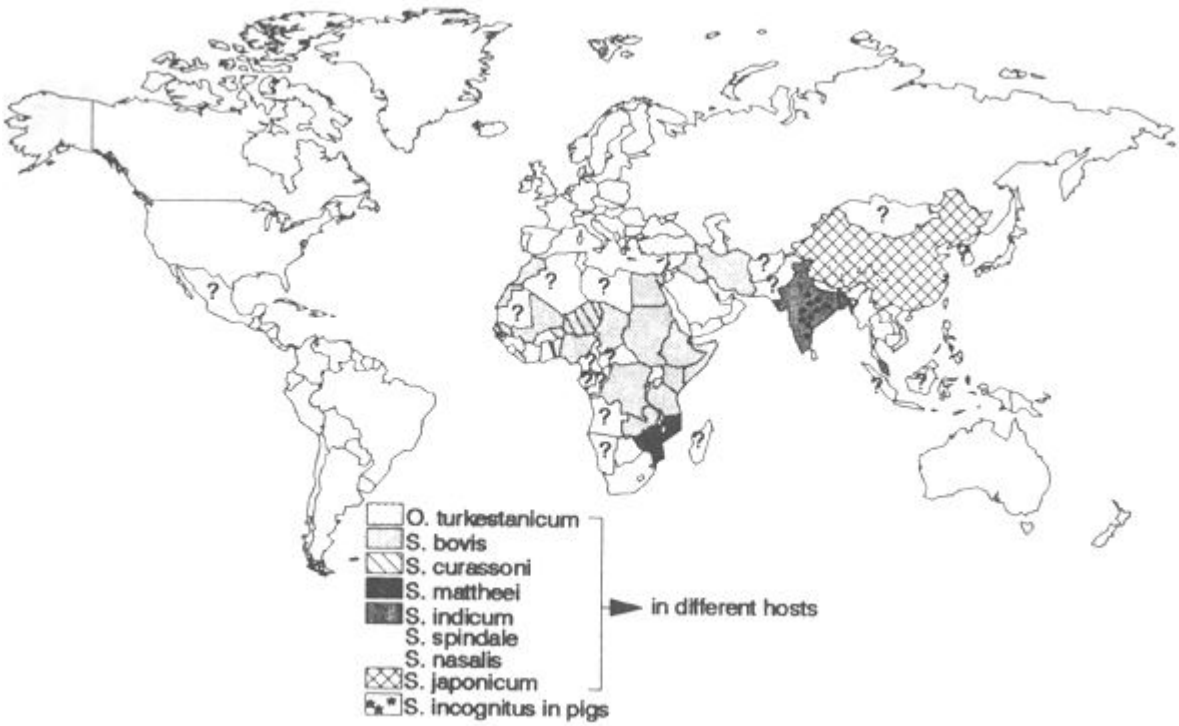

Figure 1.6. Global distribution of *Schistosoma* species which affect livestock (Over et al. 1992).

In Africa, the different ruminant infecting *Schistosoma* species distributions show some overlap (Figure 1.6) and interaction has been reported, resulting in interspecific hybridization, specifically from two species found in cattle, *S. bovis* and *S. curassoni* (Southgate, V. R.*et al.*, 1998). More recently through application of newly developed molecular tools, natural hybridization has also been described from the two most important species causing human schistosomiasis in Africa, *S. mansoni*

and *S. haematobium* (Huyse, T.*et al.*, 2013). Natural hybridization of all three ruminant infecting species with *S. haematobium* was described last year (Moné, H.*et al.*, 2015). This discovery is of biologic interest, since the host switching event must have happened either from the ruminant infecting species into humans or from *S. haematobium* into ruminants (Brant, S. V. and Loker, E. S., 2013). Additionally, from a public health perspective this discovery is of concern since an adaptation of *S. bovis* or *S. bovis / S. haematobium* hybrids to humans as a final host may interfere with the elimination goals for schistosomiasis (Moné, H.*et al.*, 2015).

Human and animal fascioliasis in Africa

In Africa, livestock fascioliasis is caused by *Fasciola gigantica* or *F. hepatica* (Figure 1.7). The disease is of veterinary significance due to the severe economic losses it causes (Charlier, J.*et al.*, 2014). Infected animals show reduced growth, low milk production and weak reproduction rates (Bechir, M.*et al.*, 2015). Treatment costs and condemnation of contaminated meat after slaughter are further factors that have a negative impact on the livelihoods of livestock dependent people (Ogurinade, A. and Ogunrinade, B. I., 1980, Torgerson, P. R. and Macpherson, C. N., 2011).

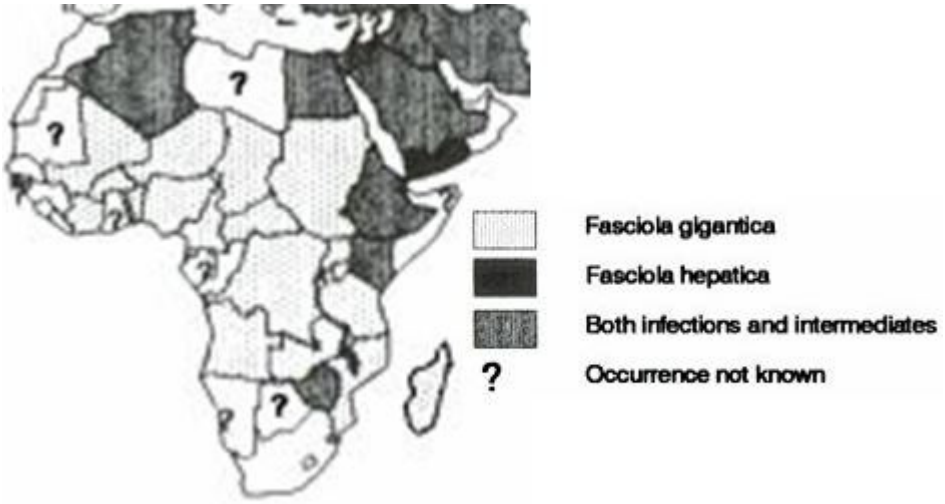

Figure 1.7. Distribution of *Fasciola gigantica* and *F. hepatica* in Africa (adapted, Over et al. 1992).

The standard method for the diagnosis of livestock fascioliasis is sedimentation of fecal samples for egg concentration followed by microscopy. Antibody detection in blood serum by ELISA and coproantigen tests using fecal samples have been developed (Alvarez Rojas, C. A.*et al.*, 2014). *Fasciola* has a zoonotic potential and can accidentally infect humans through consumption of contaminated raw vegetation or drinking water containing cercariae (Mas-Coma, M. S.*et al.*, 1999, Nyindo, M. and Lukambagire, A. H., 2015, WHO, 2015a). Until recently, the number of human infection has been largely underestimated: in the late 1990s about 2000 cases were reported worldwide annually. Today, the estimate is about 2.4 – 17 million cases per year worldwide, the most affected group being children age 5 to 15 years (Mas-Coma, S., 2004, Fürst, T.*et al.*, 2012).

Represented by the two species *Fasciola hepatica* and *F. gigantica,* the disease shows a global distribution (Figure 1.8).

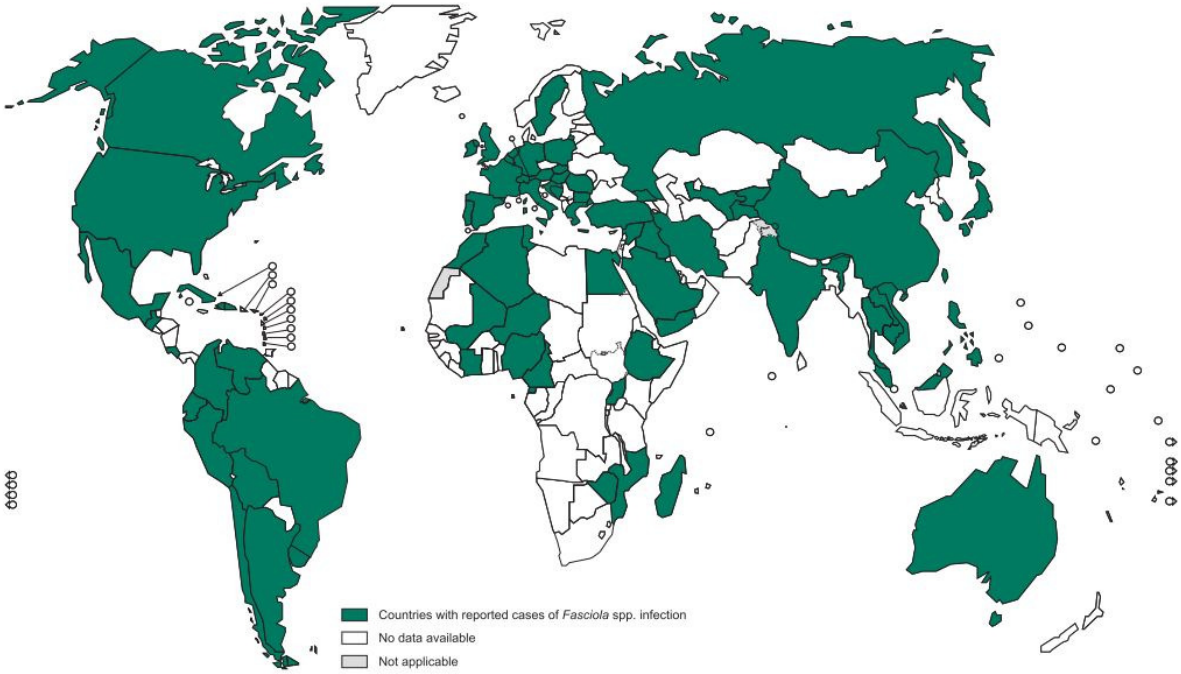

Figure 1.8. Distribution of human fascioliasis worldwide, latest year available (WHO, 2015).

With the recent rise in awareness of human fascioliasis, specific diagnostic tools have been developed (Mas-Coma, S.*et al.*, 2014). Alongside classical parasitological methods using sedimentation techniques for fecal samples and microscopy, antibody ELISA and antigen assays play an important role in the diagnosis of pre-patent infection or infection with sterile flukes (Mas-Coma, S.*et al.*, 2014). Today, triclabendazole is the recommended treatment against human (10mg/kg) and animal (12mg/kg) fascioliasis (Keiser, J.*et al.*, 2005, WHO, 2007).

1.4. **Study area: Chad and Lake Chad**

This project was carried out on the eastern shore of Lake Chad in Chad and in the capital city of N'Djamena. Chad is situated in central Africa with the majority of its territory covering Saharan desert and the Sahelian belt (Figure 1.9).

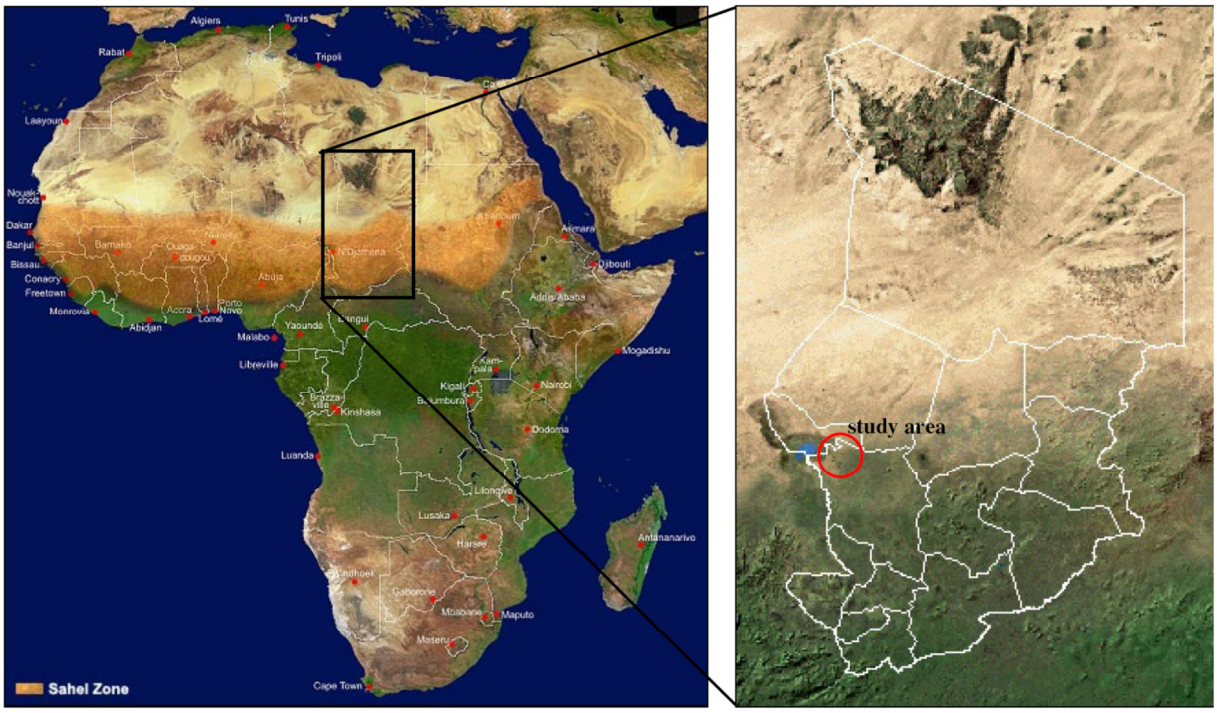

Figure 1.9. Chad is situated in central Africa. The red circle indicates the study area on the eastern shore of Lake Chad.[2]

The climate consists of three distinct seasons. From November to February, a dry and fresh wind, the Harmattan, blows from the North-East. From March to June a dry and worm period follows, characterized by high monthly mean temperatures of around 30° Celsius. Finally, a warm rainy season lasts from mid-June to mid-October (Magrin, G.*et al.*, 2015). These seasons have a strong impact on the two most important water bodies of the country: The Chari River and the Lake Chad. Lake Chad is a very unique ecosystem. The lake bed is very shallow, so an increase in water inflow from the river Chari during the rainy season leads to fluctuations of the lake water level causing oscillating flooding of large areas which become swamps and islands for weeks at the time (Leblanc, M.*et al.*, 2011). When the annual rainfalls end, due to evaporation and water used for irrigation, the lake reduces in surface area slowly during the dry season. Human activities, climate change and changes in rainfall quantities have a huge impact on this fragile ecosystem, and during the last fifty years, Lake Chad expanded to a very large surface area but also contracted to the smallest recorded (Sarch, M. T. and Birkett, C., 2000). During the last decade, the lake seems to have regained size (Figure 1.10). Nevertheless, extreme fluctuations, as seen during the last five decades, are concerning because the livelihood of millions of people depends directly or indirectly on the lake natural resources (Onuoha, F. C., 2008).

[2] (Maps: http://www.eduspace.esa.int/Worksheet/images/Africa_Sahel.jpg)

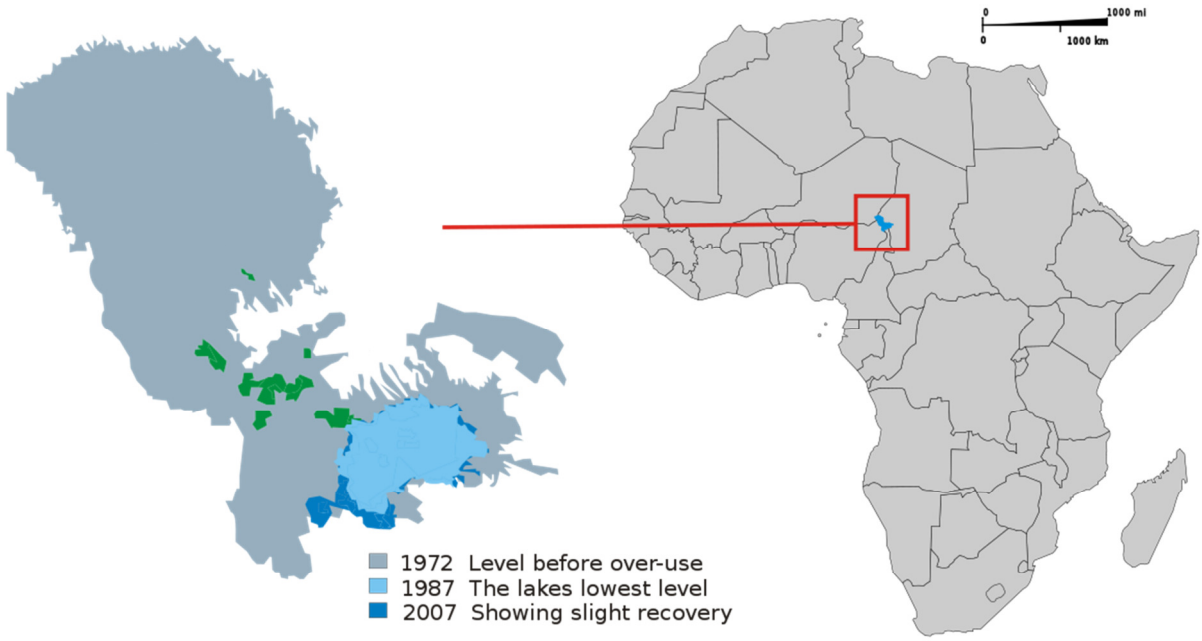

Figure 1.10. Map showing Lake Chad at its largest surface area (grey, 1972) and at its smallest size (light blue, 1987). During the last decades, the lake seems to be slowly increasing in size.[3]

The land where this annual flooding occurs is very fertile and plays an important role in human and animal nutrition and thus also impacts the Chadian economy. The pasture growing in this area after the rainy season is one of the most important feeding grounds for mobile pastoralist livestock as well as for wild ruminants. Besides fishing activities, sedentary human populations also use the fertile land for agricultural activities. The nutrition of the entire human, livestock and wildlife population depends on this short but very fertile vegetation period (Onuoha, F. C., 2008).

Economically, Chad is one of the poorest countries of the world, with an estimated population of 12'825'000 people, ranking among the bottom four on the Global Multidimensional Poverty Index (WHO, 2012b, Alkire, S.*et al.*, 2015). Along with the export of crude oil and cotton, livestock an important export product. According to the "Deuxième Recencement Général de la Population et de l'Habitat" (2009), at least 3.5% of the total population are estimated to be mobile pastoralists. About 75% of Chadian livestock is managed in a nomadic or semi-nomadic way, which accounts for 80% of the entire pastoral production and creates at least 25% of the national export revenues (MERA, 1998).

[3](https://commons.wikimedia.org/wiki/File:Lake_Chad_map_showing_receding_water_area_and_level_1972-2007.svg)

1.5. **Mobile pastoralism at Lake Chad**

Mobile pastoralism is a highly adapted lifestyle in grassland ecosystems (Niamir-Fuller, M., 1998). In Chad, the mobile pastoralist way of life is determined by the seasonally changing conditions of the semi-arid environment ranging from the sub-humid savannah (Sudanese zone) to the Sahelian belt and the Sahara desert (Wiese, M.*et al.*, 2004b, Jean-Richard, V.*et al.*, 2014d). Resource availability, namely access to water and seasonally available pasture, defines the mobility pattern of the people and their livestock (Ellis, J. and Galvin, K., 1994). This mobility allows for maintenance of large ruminant herds in the face of scarce forage availability (Krätli, S. and Schareika, N., 2010). Livestock trading and the trade with livestock products such as dairy, meat and hides generates the mobile pastoralist's livelihood. Large, productive herds define and reflect the social status of the owners. Movement patterns and transhumance routes show constant adaptation to an ever changing environment (Stenning, D. J., 1957, Galvin, K. A., 2009). During the past 40 years, these adaptations were mainly a reaction to recurrent droughts that occurred throughout the Sahelian belt in 1970s and 1980s (Loutan, L., 1989, Wiese, M.*et al.*, 2004b). The droughts led to significant decrease of the Lake Chad water levels (Leblanc, M.*et al.*, 2011). Other factors like upstream irrigation from the Chari and Logone Rivers and climatic variability led to further shrinking of Lake Chad (Ahmed, M. A., 2015). Today, mobile pastoralists graze herds closer to Lake Chad (Wiese, M.*et al.*, 2004b). The fertile grounds of the former lake area led to an increase of agricultural activities and establishment of new villages (Sarch, M. T. and Birkett, C., 2000, Jean-Richard, V.*et al.*, 2015). Towards the end of the dry season, when the mobile pastoralists and their livestock gather there, the area becomes quite crowded (Jean-Richard, V.*et al.*, 2015). The resilience of this lifestyle in view of the impact of climate variability and change on the Sahel illustrates its important potential for the future. Conversely, adaptation to changing conditions also brings risks. The intrusion into new environments may expose humans and livestock to social and health related hazards (Bonfiglioli, A. M. W., C., 1992, Wiese, M.*et al.*, 2004b, Onuoha, F. C., 2008). A mobile lifestyle can also expose humans and animals to health risks or provide opportunity to avoid risky areas (Sheik-Mohamed, A. and Velema, J. P., 1999, Aagaard-Hansen, J.*et al.*, 2010). Crowding around wells at the end of the dry season is an example of a health risk. Equally, mobile communities can also use flexibility to avoid certain disease risks, especially those appearing seasonally, by adapting movement patterns and reacting quickly in the face of outbreaks of epidemics. The recent changes in movement patterns, in adaptation to climate change, lead to concerns about emerging human and livestock diseases, which were previously limited or unknown to the mobile pastoralists. The mobile pastoralists associate these diseases strongly with proximity to Lake Chad (Schelling, E., 2002, Wiese, M.*et al.*, 2004a, Jean-Richard, V.*et al.*, 2014b).

The mobile pastoralist populations at the shore of Lake Chad belong to different ethnic groups and subgroups, and a detailed ethnographic description goes beyond the frame of this PhD thesis. The description of the ethnic groups given here is simplified and based on documentation of the Chadian Ministry of Livestock and Animal Resources (MERA), which distinguishes the groups as follows (MERA, 2008):

- Those living in the driest zones in the north-eastern part of Chad, up to the border of Libya, are camel-keeping **Toubou** groups. Subgroups of the Toubou are present in our study zone. These are called "**Dazagada**" or "**Gorane**" and they also breed cattle and small ruminants.
- **Arab** people living in the central part of Chad are predominantly camel breeders who practice fully mobile pastoralism. The Arab communities in our study zone keep cattle and small ruminants, with most living semi-nomadic lifestyle, where they leave their villages towards the end of the dry season, seeking pasture closer to Lake Chad. The MERA distinguishes between the "Arabs of the North", the camel breeders, and the "Arabs of the south" in our study area.
- The **Fulani**, in French called Peulh or Foulbé, live throughout the Sahelian belt and may seasonally migrate through several countries. Many Fulani groups from the study area move from Chad to Cameroon or Nigeria. The Fulani breed cattle and small ruminants. In the study zone, many sub-groups such as the Wodabé / Ouda, Weila, Maré or Jayejaye are present.
- The islands of Lake Chad are home to the **Buduma** and **Kouri** who are known as agro-pastoralists and fishermen. Their Kouri cattle breed is unique to the Lake Chad area and is highly adapted to the specific husbandry system because the animals reach their pasture by swimming from island to island, following the reed boats of their herdsmen.

The seasonal moving patterns of Fulani, Arab and Gorane pastoralists have been assessed during a two year period from 2011 – 2012 by Vreni Jean-Richard (Figure 1.11) and illustrate clearly how each ethnic group occupies specific geographic areas. Towards the end of the dry season when pastures get scarce, pastoralists gather closer on the shores and islands of Lake Chad (Jean-Richard, V.*et al.*, 2014c). The Buduma and Kouri live on islands within the lake year round and spend only about two to three month at the lakes shore, when the lake water level is at its highest and the islands are submerged.

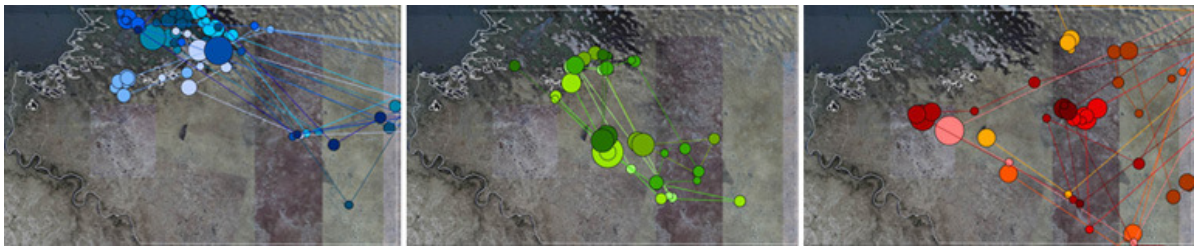

Figure 1.11. Moving patters over one year of Fulani (blue), Arab (green) and Gorane (red) mobile pastoralists at the south-eastern shores of Lake Chad. The size of the dot indicates the duration of the stay at one specific site. By comparing the three movement patterns, clearly distinct land usage patterns can be seen for the three ethnic groups (Jean-Richard et al. 2014f).

1.6. **Human and animal health in Chad**

For the last decade, the Chadian health sector has officially been financially supported by state-generated income from crude oil exports. Despite this investment, the health system still struggles to provide even basic services to the population, especially in rural areas (Djimouko, S. and Mbairo, P., 2014). Although life expectancy at birth for both sexes has increased by 5 years over the period from 2000 – 2012, it is still very low at only 51 years of age (WHO, 2012b). Analyses of the millennium development goal (MDG) indicators reveals that progress towards achievement is slow and lags behind the WHO regional average (WHO, 2012b). Maternal mortality and under-5 mortality are still unacceptably high while childhood vaccination coverage is insufficient[2]. A constant lack of skilled health personnel may partly explain the poor performance of the health system, as currently, there are 3.7 physicians per 100 000 population with 2.1 nurses and midwives per 100 000 population. Specifically, 65% of the physicians and 35% of the nurses and midwives are concentrated in the region of N'Djamena[4]. Besides limited access to health services, the lack of clean drinking water is another crucial factor that jeopardizes human and animal health in rural Chad (Schelling, E., 2002, Omosa, E. K., 2005). In 2013, less than 50% of the Chadian population had access to improved drinking-water sources and less than 20% accessed improved sanitation facilities. Parasitic diseases represent an important burden in human health, especially in children (Beasley, M.*et al.*, 2002, Bechir, M.*et al.*, 2012a).

The veterinary services in Chad were privatized in the 1990s. Activities of private veterinarians and veterinary technicians focus mainly on livestock vaccination. However, after the mandatory rinderpest vaccination regulation was discontinued many actors abandoned their posts (MERA, 2008). Simultaneously, import and distribution of veterinary medications was also privatized.

[4] http://www.who.int/workforcealliance/countries/tcd/en/

Alongside official pharmacies, an unofficial sector evolved in parallel, importing and selling medicines on an unregulated basis (Schelling, E., 2002, MERA, 2008).

Today, the decentralized veterinary system consists of 18 regional delegations for livestock (Délégations Régionales de l'Elevage (DRE)). These are subdivided into 56 sectors and 199 veterinary posts. Since 1995, a disease surveillance system has been in place, encompassing 106 surveillance posts which currently monitor eight livestock diseases (Réseau d'Epidémio- Surveillance des Maladies Animales au Tchad (REPIMAT)) (Hendrikx, P.*et al.*, 1997, Ouagal, M.*et al.*, 2008, Ouagal, M.*et al.*, 2012). Animal movement within the country and across borders is uncontrolled. Animal diseases are pervasive and effective interventions are rare due to financial constrains and the constant shortage of qualified human resources. Nevertheless, the rural development plan aims to fight these problems by strengthening the productivity and revenues from the rural sector by improving market access, also mentioning issues related to good governance (MERA, 2008). Additionally, non-governmental organizations (NGOs) engage to improve both the human health and the veterinary sector.

The human and veterinary health sectors in Chad face similar challenges. About 20 years ago, the Swiss Tropical and Public Health Institute established a research partnership with Chadian institutions, namely the Institut de Recherche en Elevage pour le Développement (IRED) and the Centre de Support en Santé International (CSSI). The partnership provides the framework for pursuing collaborative health research and development, aiming to sustainably improve human and animal health of mobile pastoralists through a transdisciplinary One Health approach (Schelling, E.*et al.*, 2008). As a result of the long-term commitment and the associated research and stakeholder processes, joint human and animal health interventions were developed, tested and evaluated in the study area (Bechir, M.*et al.*, 2004, Schelling, E.*et al.*, 2007a). In this way, public and animal health workers engage closely with communities and authorities, and this process is crucial in building stakeholders' ownership (Schelling, E.*et al.*, 2008). Such intervention programs aim to improve human and animal health simultaneously and add value through financial and human resource savings. Today, these intersectoral interventions have been adopted by the Chadian government at border crossing points to neighboring countries but have not yet been applied on a broader scale as part of the expanded programme of immunisation (EPI) in Chad (Zinsstag, J.*et al.*, 2009, Bechir, M.*et al.*, 2012c, Montavon, A.*et al.*, 2013).

1.7. **Underlying concepts of this research project**

Health today is understood more as an on-going process than a fixed constant since it depends on a wide variety of factors of physiological and biological origin (Bircher, J., 2005). Many other factors such as the environment, climate, social and economic status or cultural background also influence

health and wellbeing (Forget, G. and Sanchez-Bain, W. A., 1999, Forget, G. and Lebel, J., 2001, McMichael, A. J., 2013). Most of these associated factors undergo constant change, similar to health. This principle is true for both human health and animal health. To better understand these changes, the relationships between the most important factors have to be assessed carefully and evaluated in a systems context. Here, an introduction to the underlying concepts of this research project is provided.

One health, ecohealth and health in social-ecological systems

A one health approach promotes integrated human and animal health research and interventions and closer collaboration of the human and animal health sectors with the aim of adding value or generating synergistic effects for the benefit of human and animal health (Zinsstag, J.*et al.*, 2015a). Added value may be of an economic nature through financial savings by sharing resources and infrastructure such as laboratories or cold chains by human and animal health providers (Zinsstag, J.*et al.*, 2012). The one health approach may also lead to synergistic effects of improved human and animal health, or environmental services, for example, clean water for humans and animals resulting from behavior change to reduce open defecation and improved sanitation infrastructure (Zinsstag, J.*et al.*, 2009). The latter example involves sectors beyond the health sector by including environmental aspects and shows the close, inextricable interconnection of humans and animals and their social and ecological context. These interactions are complex with non-linear relationships and feedback loops at different scales (Zinsstag, J.*et al.*, 2011).

Systems thinking and its advantages for epidemiology

The contemporary complex health problems cannot be solved by individual disciplinary approaches. In contrast, they require systems thinking (Atun, R. and Menabde, N., 2008, De Savigny, D. and Adam, T., 2009). To better understand and describe the complexity of the "reality", systems thinking allows for analysis of non-linear relationships in complex networks. Mathematical disease transmission models are an example for such a network analysis (Zinsstag, J.*et al.*, 2015b). Besides the result of better understanding of transmission at the human-animal-environment interface, such models also allow for the testing of potential intervention strategies at all levels. Together with economic cost effectiveness analyses, mathematical transmission models contribute to select and develop interventions with the highest possible leverage (Zinsstag, J.*et al.*, 2015b). This may include unexpected linkages that cannot be detected by applying linear statistics alone. A systems understanding of health relates to the concept of ecohealth and "health in social-ecological systems" (HSES), which includes the one health concept (Zinsstag, J.*et al.*, 2011, Zinsstag, J., 2012).

The project presented here studies snail-vector transmitted parasites which affect humans and animals and have important environmental life stages. In order to gain knowledge on the interactions of these organisms with each other and with their environment, the similarities and differences of the four trematode species in their intermediate and final hosts were studied and described (Figure 1.12). The project includes the training of two MSc students in Epidemiology, one in Switzerland at Swiss TPH and one in Chad at IRED, and the PhD thesis presented here.

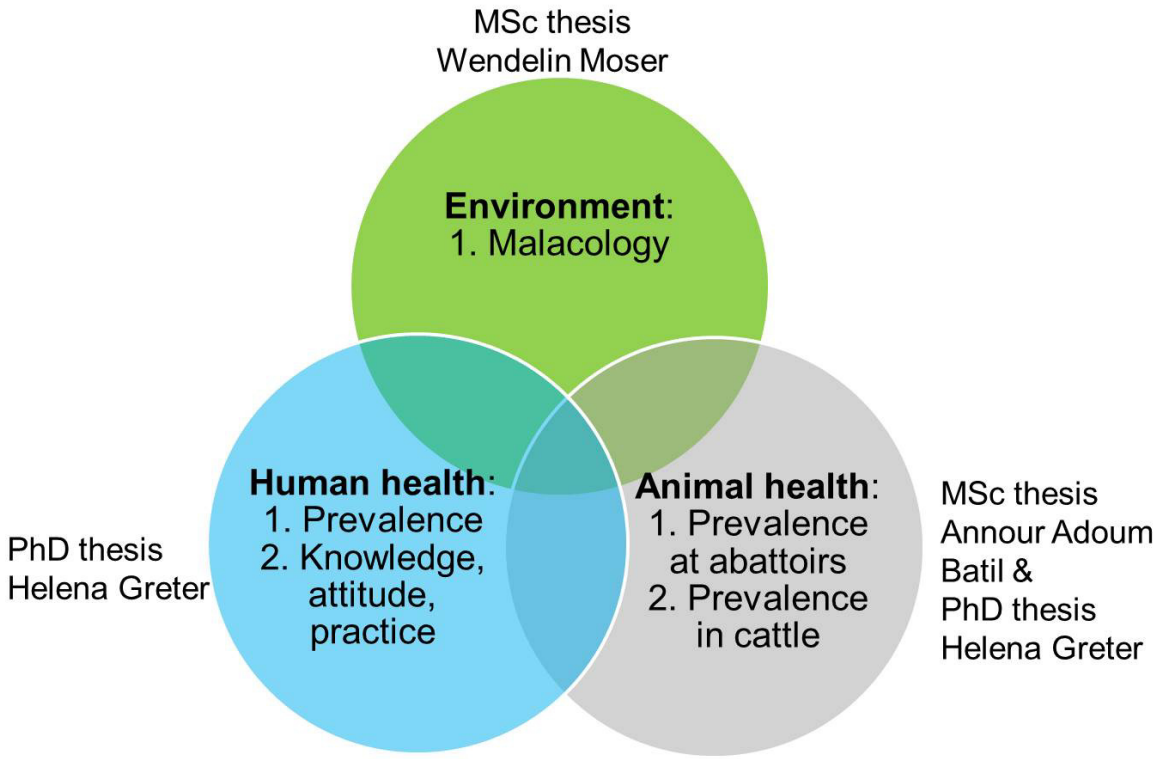

Figure 1.12. Scheme showing the planned research at the human-animal-environment levels within the One Health approach. The project includes a Chadian and a Swiss MSc thesis as well as the PhD thesis presented here.

These data will provide a basis for development of a mathematical transmission model (Figure 1.13). In this figure the hypothetical seasonal dynamics of trematodes are depicted. Empirical data from the companion PhD thesis by Stefanie Krauth and this study will allow for the calibration of transmission dynamic models. Further, the mutual predictive potential of human and animal disease and *vice versa* can be explored. The transmission models will enable testing and development of locally adapted interventions, possibly leading to new joint human and animal intervention strategies.

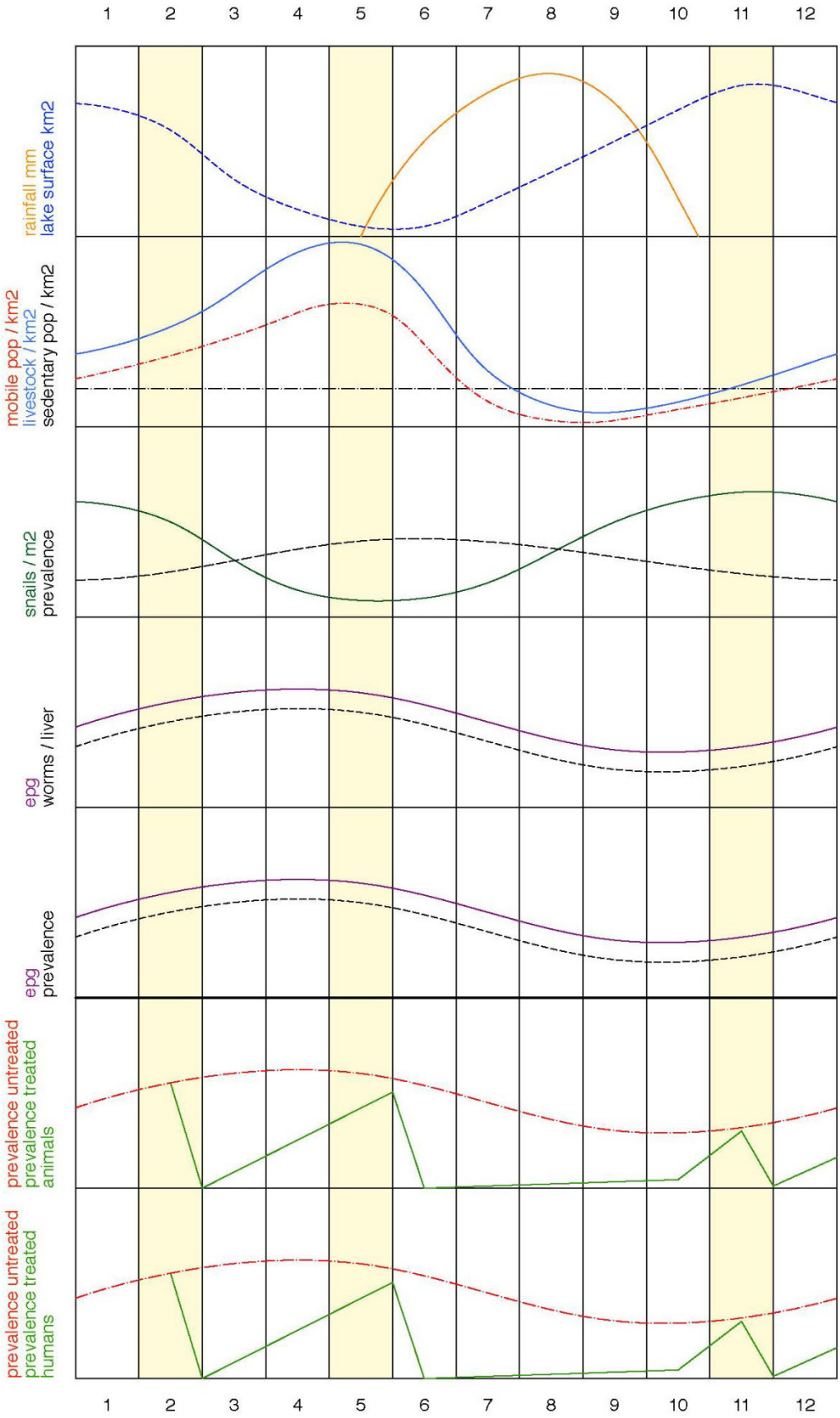

Figure 1.13. Synopsis of the dynamic processes involved in human and animal schistosomiasis and fascioliasis transmission in the social-ecological system in the study area on the eastern shores of Lake Chad. These basic data will allow for development of a mathematical transmission model.

From interdisciplinary to transdisciplinary research

Systems thinking in health research and systems understanding of human and animal health require inter- and transdisciplinary approaches (Min, B.*et al.*, 2013). The positive impact of an interdisciplinary research approach has been summarized and critically discussed in a recent special issue of Nature (Vol 525, September 2015). Analyses of published research reveal a remarkable increase in multi- and interdisciplinary referencing in the past 15 years, equally in natural science and social science (Van Noorden, R., 2015). In fact, health research is the field with the most interdisciplinary referencing (go.nature.com/z9m3gy).

Our planet consists of a large diversity of habitats and human populations have evolved highly adapted lifestyles and societal forms that are defined by natural conditions including climate and topography, but also by man-made perceptions of the universe, including religions, cultures, and languages that define rules and regulations within a society. In our research work in Switzerland and Chad, success only comes through close collaboration of all institutions and the engagement of every person involved. The culturally defined societal system described above brings together people who may have different conceptual understandings of health and disease, healing and death. To better understand the specific disease systems in their context, incorporating all knowledge, whether scientific or non-scientific, increases the chance to elaborate research results that translate into practice (Gehlert, S.*et al.*, 2010, Zinsstag, J.*et al.*, 2011).

Transdisciplinary (TD) methods consider involving academic and non-academic knowledge for solving problems of societal importance (Hirsch Hadorn, G.*et al.*, 2008). Based on principles of TD approaches, stakeholders on the national and local level were involved in the research process from the beginning of this project (Hirsch Hadorn, G.*et al.*, 2008). Discussions on the research planning with partners at Centre de Support en Santé International (CSSI) and Institut de Recherche en Elevage pour le Dévelopement (IRED) were initiated in N'Djamena in September 2012, which served as a basis for the joint development of the research protocol. Following this first meeting, the identification of the Chadian Master student started. Preceding the launch of the field work phase, the first stakeholder meeting took place in N'Djamena from 15. – 16. March 2013 (Figure 1.14). Attending participants were representatives from the Ministry of Public Health, CSSI, IRED, Swiss TPH, University of N'Djamena and the chiefs of mobile pastoralists groups from the Lake Chad area. This stakeholder meeting was of great value for the project as important contacts with pastoralist associations and community chiefs as well as the Ministry of Public Health were initiated. The presence of schistosomiasis in the study population was suspected because pastoralist representatives reported the observation of blood in urine, especially in children. Access to water and availability of clean water was reported as a major problem in daily life, as well as the lack of

accessible veterinary and medical health services. These concerns were integrated and addressed in the frame of this project. Discussions with the representatives of the mobile pastoralists were extremely useful regarding the inclusion of their knowledge about human and animal diseases, traditional treatment methods, local practices, customs and beliefs relating to parasitic disease.

Figure 1.14. Stakeholder meeting at the start of the project, which took place in N'Djamena, 15.-16. March 2013. (Photo: W. Moser)

1.8. Identified research gaps, aims and objectives

Identified Research Gaps

Substantial efforts have been made for spatially explicit risk mapping and prediction for schistosomiasis in Africa (Raso, G.*et al.*, 2006, Schur, N.*et al.*, 2011). In the study area at Lake Chad, two species of *Schistosoma* are present (Beasley, M.*et al.*, 2002). *Schistosoma mansoni*, causing intestinal schistosomiasis, was rarely found in Chad with prevalence among school children of 1%. *Schistosoma haematobium*, infecting the urinary tract including the bladder, was found at prevalence 11.8% to 13.7% among school children, with the highest prevalence found in the Sahelian belt, where our study area is located (Massenet, D.*et al.*, 1995, Beasley, M.*et al.*, 2002). For fascioliasis, however, most of the work on risk prediction from climate dates back to the late 1960s, defining an index using temperature and rainfall data (Ollerenshaw, C. B. and Smith, L. P., 1969). Apart from a very recent abattoir study that reported prevalence of up to 68% in cattle (Jean-Richard, V.*et al.*, 2014a), no

recent data on *Fasciola* infections in livestock from Chad are available. Published work dates from the 1960s and 1970s (Graber, M., 1966, Bouchet, A.*et al.*, 1969, Tager-Kagan, P., 1978, Graber, M. and Thal, J., 1979). For human fascioliasis, no data for Chad could be found. The recent abattoir study showed that *Fasciola* infection is directly related to proximity to the lake and infection rates reflected husbandry practices (Jean-Richard, V.*et al.*, 2014a). The present study includes a larger abattoir study (by the Chadian MSc student) in combination with a cohort study among livestock herds to complete the picture of the fascioliasis prevalence in livestock in the area.

To better understand human and animal schistosomiasis and fascioliasis, disease and transmission dynamics, we aim to describe the presence, seasonality and disease prevalence of schistosomiasis and fascioliasis among humans, cattle and intermediate host snails at Lake Chad.

Hypotheses:

1. A deeper understanding of the social-ecological context of parasite and host dynamics will allow for assessing the mutual predictive potential of fascioliasis and schistosomiasis risk.

2. Current fascioliasis and schistosomiasis control is not effective or absent in the study area. Participatory involvement of communities, authorities and scientists will enable the identification of current knowledge, attitude and practice and reveal the potential for rapid intervention improvement.

3. Determining the social-ecological factors of human and animal fascioliasis and schistosomiasis allow for the development of a mathematical transmission model which enables identifying critical time periods for effective and locally adapted interventions.

Aims and specific objectives

With this project we aim to deepen the understanding of complex interactions of ecological, behavioral and biological factors that influence human and animal schistosomiasis and fascioliasis in a challenging climatic and economic environment such as the eastern Lake Chad area in Chad. The outcome of this study will contribute towards development of truly effective, locally accepted interventions to promote human and animal health in the mobile pastoralist community in Chad.

Specific objectives

1. Establish and characterize the prevalence of schistosomiasis and fascioliasis in humans and cattle in relation to the prevailing social-ecological system of mobile pastoralists

2. Investigate the mobile pastoralist's knowledge, attitudes and practice regarding human schistosomiasis and livestock fascioliasis: disease perception, health seeking behavior and use of and access to treatment

3. Engage in knowledge transfer through strengthening parasitological laboratory capacities by introducing new diagnostic tools and training of laboratory technicians and validating diagnostic methods for human and animal schistosomiasis and fascioliasis in a mobile field laboratory

4. Test the mutual predictive potential for human and animal disease in the context of the mobile pastoralist setting

1.9. **Field activities: planned procedures and unforeseen challenges**

Field work activities for data collection took place between 2012 and 2014 on the eastern shore of Lake Chad in the districts of Hadjer Lamis, Chari Baguirmi and Lac as well as in N'Djamena. The collaboration in the frame of this project was based on a joint contract of all partners involved: in Chad the Centre de Support en Santé International (CSSI) and the Institut de Recherche en Elévage pour le Développement (IRED) and in Switzerland the Swiss Tropical and Public Health Institute (Swiss TPH). The contract defined responsibilities and accountability of each partner. The start of the first field work phase was planned for early 2013. It had to be postponed due to security issues related to expanding terrorist group activities in northern Cameroon and Nigeria (Boko Haram). As a first consequence, the protocol for the malacological research project had to be adapted and the activities translocated to N'Djamena. Likewise, every following field work phase was influenced by temporarily restricted access to the study area due to the fragile security situation in the region. As a result, the research work could not be executed as planned initially and certain adjustments to the protocol were necessary. Nevertheless, thanks to the close collaboration between all partners and the study population involved, a majority of our goals could be achieved in the face of difficult circumstances. This strong partnership builds on the long standing tradition of the Swiss TPH of equal partnership and on the principles set out by the KFPE's Guide for Transboundary Research

Partnerships[5] (Schelling, E.*et al.*, 2008). The success of this project is further proof of the power of close collaboration, as shown before during a crisis period in Côte d'Ivoire (Bonfoh, B.*et al.*, 2011b).

1.10. **Collaborating Partners**

This research project was undertaken within the SNF funded project entitled "*Systems epidemiology of human schistosomiasis and livestock fascioliasis in sub*-Saharan Africa" (grant no. 320030_141246/1).

Collaborating partners were:

- The Swiss Tropical and Public Health Institute (Swiss TPH), Basel, Switzerland
  Department of Epidemiology and Public Health
  Department of Molecular Parasitology and Infection Biology
  Department of Medical Services and Diagnostics
- Institut de Recherche en Elevage pour le Développement (IRED), N'Djamena, Chad
  Division de la Santé Animale
  Service de la Parasitologie
- Centre de Support en Santé International (CSSI), N'Djamena, Chad
- The Institute of Parasitology (IPZ), Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
- Schistosome Collection at the Natural History Museum (SCAN), London, UK

[5]http://www.naturalsciences.ch/organisations/kfpe/11_principles_7_questions?_ga=1.260369357.1362482225.1453647434

2. Outline of Thesis

This PhD thesis is structured in five thematic parts, as grouped in individual chapters. The individual chapters are presented as peer-reviewed scientific articles, followed by a general discussion and conclusions in the final chapter (chapter 12).

One Health research among mobile pastoralists

- The benefits of 'One Health' for pastoralists in Africa (chapter 3)
- Human and animal health surveys among pastoralists (chapter 4)

Epidemiology of human and animal trematode infections at Lake Chad

- Prevalence of *Fasciola gigantica* infection in slaughtered animals in south-eastern Lake Chad area in relation to husbandry practices and seasonal water levels (chapter 5)
- Human and livestock trematode infections in a mobile pastoralist setting at Lake Chad: added value of a One Health approach beyond zoonotic diseases research (chapter 6)

Treatment of human and animal helminth infections at Lake Chad

- Treatment of human and livestock helminth infections in a mobile pastoralist setting at Lake Chad: Attitudes to health and analysis of active pharmaceutical ingredients of locally available drugs (chapter 7)
- Re-infection with *Fasciola gigantica* 6-month post-treatment with triclabendazole in cattle from mobile pastoralist husbandry systems at Lake Chad (chapter 8)

Diagnostic tools and their performance at Lake Chad and beyond

- Validation of a point-of-care circulating cathodic antigen urine cassette test for *Schistosoma mansoni* in the Sahel, and potential cross-reaction in pregnancy (chapter 9)
- All that is blood is not schistosomiasis: experiences with reagent strip testing for urogenital schistosomiasis with special consideration to very-low prevalence settings (chapter 10)

Malacology

- The spatial and seasonal distribution of *Bulinus truncates, Bulinus forskalii* and *Biomphalaria pfeifferi,* the intermediate host snails of schistosomiasis, in N'Djamena, Chad (chapter 11

3. The benefits of 'One Health' for pastoralists in Africa

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3.1. Abstract

'One Health' is particularly suited to serve mobile pastoralists. Dinka pastoralists in Sudan inspired Calvin Schwabe to coin the term 'one medicine', indicating that there is no difference in paradigm between human and veterinary medicine. Our contemporary definition of 'One Health' is any added value in terms of improved health of humans and animals or financial savings or environmental services resulting from a closer cooperation of human and animal health sectors. Here we present a summary of 'One Health' studies with mobile pastoralists in Africa which were done in research partnership, demonstrating such an added value. Initial joint human and animal health studies revealed higher livestock vaccination coverage than in the pastoralist community, leading to joint animal and human vaccination intervention studies which demonstrated a better access to primary health care services for pastoralists in Chad. Further simultaneous animal and human serological studies showed that camel breeding was associated with human Q-fever seropositivity. In Borana communities in Ethiopia, human cases of *Mycobacterium bovis* infection could be related to strains isolated from cattle. A challenge remained with regard to how to assess vaccination coverage in mobile populations. With the advent of mobile phones, health and demographic surveillance could be established for mobile pastoralists and their animals. This presents vast possibilities for surveillance and control of human and animal diseases. Pastoralists prefer a 'One Health' approach and therefore contribute toward the validation of this concept by showing real added value of the cooperation between human and animal health services.

Keywords Africa, mobile pastoralists, One Health

3.2. Introduction

The Human and Animal Health Unit at the Swiss Tropical and Public Health Institute, Basel, Switzerland, is a multidisciplinary research unit working with partner institutions in nine countries in Africa (Ethiopia, Kenya, Chad, Mali, Mauritania, Côte d'Ivoire) and Asia (Kyrgyzstan, Mongolia, Vietnam). We focus on health research for mobile pastoralists and the control of zoonotic diseases in developing countries. Several of these diseases are emerging and many zoonotic diseases are also categorised as neglected diseases. Pastoralist populations in these countries are often marginalised by government healthcare systems because of the remote location of their camps and villages or high mobility (Schelling, E.*et al.*, 2010). Several of our studies have shown that the application of the 'One Health' concept is especially valuable in the settings of mobile pastoralism (Schwabe, C., 1964). This review summarises the importance of 'One Health' for pastoralists in Africa.

The Sahel ecosystem and its inhabitants

The Sahelian belt is a semi-arid zone which borders the southern aspect of the Sahara Desert, spanning Africa east to west from Ethiopia to Mauritania. It forms a corridor through the continent where highly mobile populations have lived for a long time. The ecological zone in the central regions includes a rainy season from June to September and a dry season from October to May. This unique ecosystem led to the development of specific agricultural inventions. One important form is mobile pastoralism which allows for the breeding of large herds of cattle, camels, goats and sheep by following the cycle of pasture growth during the seasons (Wiese, M., 2004). Many different ethnic groups practise this way of life in the Sahelian belt. In Chad, for example, the Fulani, Gorane and Arab groups prevail in the Lake Chad basin (Cerezo, M.*et al.*, 2011, Jean-Richard, V.*et al.*, 2015). Social science and anthropological studies have investigated the requirements for adequate healthcare in different mobile pastoralist ethnic groups. These studies show, amongst other things, that it is crucial to plan health interventions for populations with respect to their cultural definition of the human body and the forces influencing their health (Münch, A. K., 2012).

2.3. The evolution of 'One Health'

In the early 1960s, Calvin W. Schwabe, a veterinarian with a background in biology working in Southern Sudan at the time, observed that Dinka pastoralists maintained an integrated attitude toward humans and animals. Inspired by this observation, Schwabe developed the concept of 'one medicine', stating that 'human and veterinary medicine share a common body of knowledge in anatomy, physiology, pathology and the origins of diseases in all species' (Schwabe, C., 1964) and thereby recognising the mutual benefits available through the connection of veterinary medicine and human health. Today, this concept is expanded to 'One Health': further recognising the inextricable linkage of human, livestock, companion animal and wildlife health and implying an added value to the health and wellbeing of humans and animals (Zinsstag, J.*et al.*, 2011). Closer cooperation of human and animal healthcare provision can also lead to financial savings in different sectors (Zinsstag, J.*et al.*, 2005). The concept of 'One Health' has important potential, especially in developing countries such as many of the Sahelian nations, where our unit works in close collaboration with local partners, communities and ministries.

Past research, results and consequences

A 1999–2000 study on the health of mobile pastoralists in Chad, where approximately 1000 people and animals were examined, found hardly anyone who did not report a health problem (Daugla, D. M.*et al.*, 2004). More cattle had been vaccinated than children and women; and no children had

been vaccinated completely according to the standards of the Expanded Programme on Immunization of the United Nations (EPI). Nevertheless, the people rarely sought care in health centres, coming only at late stages of illness, with the result that many diseases remained untreated (Schelling, E.*et al.*, 2005). Similar studies in Mali showed higher child mortality in mobile pastoralist populations than in the sedentary population (Münch, A. K., 2012). In addition, mobile pastoralists face a higher risk for zoonotic diseases than do sedentary populations because of their deep dependence on and close contact with their livestock (Dean, A. S.*et al.*, 2013). Practices such as consumption of raw milk and meat favour the transmission of brucellosis, bovine tuberculosis and anthrax. It has also been possible to show the direct relationship of camel breeding and human Q-fever seroprevalence (Schelling, E.*et al.*, 2003). On the basis of these findings, a first collaborative intervention project applying 'One Health' was initiated, planning and implementing a joint vaccination campaign for both livestock and children in mobile pastoralists in Chad (Bechir, M.*et al.*, 2004, Zinsstag, J.*et al.*, 2005, Schelling, E.*et al.*, 2007a). This campaign showed not only a health benefit but also the economic benefit in terms of savings made by the Chadian Public Health and Animal Health Ministries from closer cooperation between the public health and veterinary sectors. It laid the foundation for further joint health interventions in the country.

Combined investigations of zoonotic disease surveillance, performed simultaneously in both humans and animals, have also been shown to be highly effective in terms of identifying epidemiological linkages. Studies in Ethiopia on bovine and human tuberculosis demonstrated that bovine tuberculosis can be transmitted to humans and vice versa (Gumi, B.*et al.*, 2012).

Human nutrition includes a wide variety of animal-source foods. For mobile pastoralists, their entire diet depends on their livestock, as meat and milk are consumed directly or sold in order to gain money to buy cereals (Münch, A. K., 2012). This strong dependence of the people on their livestock shows clearly the importance of healthy herds. Herd losses as a result of prolonged dry seasons or disease have a direct effect on the mobile pastoralists, resulting in malnutrition and vitamin deficiencies. Because fruits and vegetables are not part of the mobile pastoralist diet, milk is the primary source of vitamin A. This interrelationship was shown by studies on vitamin A in Chadian mobile pastoralists which demonstrated a direct relationship between the retinol levels in the women's blood and in the livestock's milk (Zinsstag, J.*et al.*, 2002b, Bechir, M.*et al.*, 2012b). Such findings serve as indicators for the health status not only of humans but also the ecosystem, as vitamin A levels in milk depend on the pasture quality which is, in turn, dependent on climate (Zinsstag, J.*et al.*, 2002b). The hostile environment where pastoralists live exposes them to wind, dust, humidity, heat and lack of access to safe drinking water. These conditions also favour the prevalence of parasitic infection which is very high at 63% amongst women (95% CI: 55–72) and 60% amongst children (95% CI: 53–77) (Bechir, M.*et al.*, 2012a).

Demography and health surveillance of mobile pastoralists

An estimated 20 million–30 million mobile pastoralists live in the Sahelian belt. In most countries, little demographic data is available and, in particular, the assessment of mobile populations was originally very difficult with previously-existing technologies (Weibel, D.*et al.*, 2011a). Today, communication tools such as mobile phones and the expanding mobile communication network open up completely new possibilities. A study aiming to exploit these new capabilities and their usefulness for the development of demographic and health surveillance systems amongst mobile pastoralists was carried out from 2010 to 2012 in Chad (Jean-Richard, V.*et al.*, 2014c). Groups of mobile pastoralists were enrolled in the study and contacted regularly by mobile phone in order to gather information on the birth, death and health status of the community. About half of the groups already owned mobile phones at the time of enrolment. The study showed that the use of mobile phones for obtaining plausible demographic and health surveillance data is both feasible and well accepted, indicating future promise with regard to expanding the project in Chad and adapting the system to mobile populations in other Sahelian countries. During this study, demographic data on livestock herds was also collected, revealing the potential to combine both human and animal health surveillance using mobile phones (Jean-Richard, V.*et al.*, 2015).

2.4. Continuing 'One Health' projects

Building upon the long-standing relationship with the mobile pastoralist community and the partner institutions in Chad, new projects were developed which aimed to meet the demands that arose from the mobile pastoralists. An early perception of the pastoralists was that although access to veterinary care was limited, human health needs were addressed even less often. This led to further work to assess vitamin A status more broadly in the human population and cattle, as well as to explore the link between pasture and cattle. Subsequently, their priority was the important economic losses as a result of infection of the livestock with *Fasciola gigantica*. This infection weakens the animals, leading to reduced milk and meat production and an increased number of abortions. *F. gigantica* is a parasite which has a freshwater snail as its intermediate host. This is also the case for *Schistosomia haematobium* and *S. mansoni*, two parasite species which infect humans. In Chad, currently, more data is available on livestock fascioliasis than on human schistosomiasis (Massenet, D.*et al.*, 1995, Massenet, D.*et al.*, 2012, Jean-Richard, V.*et al.*, 2014a). An ongoing project will further deepen the understanding of the epidemiology of human schistosomiasis and livestock fascioliasis in mobile pastoralists and their livestock in Chad. Our work expands the 'One Health' approach to 'health in social-ecological systems', addressing the dynamics of both human and animal populations and their ecosystem determinants (Zinsstag, J.*et al.*, 2011, Zinsstag, J., 2012).

Conclusion

The shared objective of all research in the unit is to improve human and animal health in a sustainable way. The research ideally contributes to the development and implementation of locally adapted and accepted health interventions which are supported by the communities as well as the ministries. Applying the 'One Health' approach in challenging settings such as the Sahelian belt has proven to be successful in many of these projects. Involving representatives of the mobile pastoralists, the research community and the ministries in the planning of projects and interventions is crucial and leads to strong engagement for common goals and solutions.

Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

J.Z. (Swiss Tropical and Public Health Institute; University of Basel) was the project leader. E.S. (Swiss Tropical and Public Health Institute; University of Basel), J-R.V. (Swiss Tropical and Public Health Institute; University of Basel), M.B. (Centre de Support en Santé Internationale), L.C. (Swiss Tropical and Public Health Institute; University of Basel) and H.G. (Swiss Tropical and Public Health Institute; University of Basel) were responsible for experimental and project design and performed most of the experiments. E.S., I.O.A. (Institut de Recherche en Elevage pour le Développement) and B.B. (Centre Suisse de Recherches Scientifiques) made conceptual contributions. H.G. wrote the manuscript.

4. Human and animal health surveys among pastoralists

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4.1. Abstract

Good quality and valid human and livestock health surveys, including follow-up, are feasible among mobile pastoralists; however, pastoralists are often excluded from studies, national surveillance systems and development programmes. Preventable and treatable diseases like perinatal tetanus, measles and tuberculosis are indicative of limited access to health providers and information. Services are challenged to include effective outreach with their available financial and human resources. Maternal mortality rates among pastoralists are unacceptably high. Environmental determinants such as water and the pasture ecosystems further influence the morbidity of pastoralists. In addition, there are the common diseases in remote rural zones such as respiratory infections and malaria. The nutritional status of pastoralist children is sometimes better than that of settled children; but pastoralist women tend to have higher under-nutrition rates. Despite mobile technology, securing a customary male chaperone to visit health facilities can be difficult for pastoralist women given the dispersion of families. Simultaneous assessments among people and livestock show the linkages between the health of pastoralists and their animals. Evidence-based control measures, which can be assessed in cluster surveys where data on costs of disease and interventions are also collected, are crucial. These provide important arguments for governmental and non-governmental agencies for interventions. In future, more alternative surveillance systems will be evaluated, for example syndromic surveillance, participatory epidemiology and risk-based joint surveillance systems. The experiences, local concepts and priorities of pastoralist communities, combined with sound field data, are essential to fill information gaps to best provide adapted human and animal health services for mobile pastoralist communities.

Keywords Health, human, joint assessment, livestock, pastoralists, study design

4.2. General introduction to health of pastoralists

Compared with their settled counterparts, mobile or semi-mobile pastoralist groups are less likely to be included in health surveys, assessments and surveillance or in development program designs. However, increasing the economic and environmental sustainability of dryland production is unlikely without improving health and education policies for pastoralists. Adding value to pastoral activities should be facilitated for them and for national economies. By acknowledging the economic and environmental rationale of mobility, the constraints of static health and education services must be overcome. Experiences in various settings show that more progress towards universal health coverage is achieved when coverage among disadvantaged groups is increased first (Gwatkin, D. R. and Ergo, A., 2011). The physically demanding livelihood of pastoralists demands good health.

Livestock keeping confers human health benefits as well as health risks, and the relationships are not always linear (Zinsstag, J.*et al.*, 2011). Only enhanced understanding of mobile groups allows for the design of contextually-appropriate programmes and the ability to monitor and evaluate their actual effectiveness – as, indeed, is true for any other setting. Sound health and socio-economic surveys and follow-up studies are feasible among mobile pastoralists, and this fact should be more widely promoted. However, most studies on general health and demographic in pastoralist communities are older than 20-30 years as shown below.

Highly contagious diseases of livestock such as 'Peste des Petits Ruminants' (PPR) and Rift Valley fever contribute to significant human food and nutritional insecurity (Bechir, M.*et al.*, 2015). The last pockets of rinderpest remained among pastoralists. Only participatory approaches enabled reaching these remote communities for successful disease eradication (Jost, C. C.*et al.*, 2007). Veterinary services successfully controlled many severe cattle diseases and now focus on endemic diseases such as intestinal parasites and zoonoses – also including other livestock than cattle. (OECD, 2012). Some livestock diseases are associated to degradation of resources such as water (see Abakar et al. this volume) and pastures, for leading to physiological diseases due to selen or vitamin E deficiency (Akiyama, T., Kawamura,K., 2007). Pastoralists seek effective livestock vaccines and drugs when available and apply successful herd management practices such as cross-breeding for more trypano-tolerant cattle. Mobility allows them to actively avoid areas with year-round infected vectors or anthrax spore contaminated fields – but flexibility and mobility are constrained by fragmentation of grasslands (Galvin, K. A., 2009) and conflicts (see Bonfoh et al. and Haller et al. this volume).

In the Sahel, the main human health conditions found among mobile pastoralists did not differ substantially from morbidity typical for the Sahelian population. Frequent diarrhea and fevers (Nathan, M. A.*et al.*, 2005), respiratory infections, including lower tract infections in children and tuberculosis in adults, and malaria had more impact on health than food-poisoning and zoonotic diseases such as brucellosis (Chabasse, D.*et al.*, 1985, Ilardi, I.*et al.*, 1987, Schelling, E.*et al.*, 2005). However, pastoralists' mortality and morbidity is affected by barriers of access to health services (see Abakar et al. this volume). Mortality due to infections such as measles and tuberculosis are clear signs of insufficient access to health services and exclusion from vaccination campaigns and appropriate information (Lawson, D. W.*et al.*, 2014), although transmission, for example of measles, was low among Tuareg nomads in Niger due to their dispersion (Loutan, L. and Paillard, S., 1992). Periodic nutritional shortage as well as safety-related issues such as political insecurity impact importantly on human health among pastoralists. Several studies have shown marked differences in nutritional and health status between pastoralists and agro-pastoralists, but no larger scale study assessed demographic parameters including mortalities in the past two decades (Weibel, D.*et al.*, 2011b). In the 1980ie, higher infant mortalities among pastoralists compared to settled, crop-farming

populations were reported from Mali, Kenya and Tanzania (Chabasse, D.*et al.*, 1985, Brainard, J., 1986). The use of un-sterilised instruments during childbirth and female genital mutilation or inability to gather health information partly explain why some pastoral communities struggle with unprecedented HIV/AIDS morbidity (Morton, J., 2006).

Women are particularly vulnerable to political-ecological changes as seen in post-Soviet countries. The decline of professional delivery assistance led to poor reproductive health and increased maternal mortality (Janes, C. R. and Chuluundorj, O., 2004). Besides unavailability of transport, perceived quality of care related to language barriers and not being treated respectfully by health staff, women sometimes cannot visit health centres or outside traditional services unaccompanied and without permission from husbands or fathers. In other cases, women experienced difficulties to access health services because they lack support of their social system and network (Hampshire, K., 2002a). Additionally, women might feel ashamed or embarrassed to ask their husbands, particularly for sexual and reproductive health issues (Grolimund, A., 2010). The absence of a customary male chaperone due to spatial separation may make it impossible for women or their children to receive the needed treatment. Recently, the use of mobile phones showed advantages and disadvantages regarding health care seeking. Pastoralist women could call their husband anytime, but in areas with weak network coverage, this can delay treatment because women no longer asked the most easily accessible male chaperone (Corradi, C. and Schurr, C., 2011, Jennings, L. and Gagliardi, L., 2013). Education initiatives to empower pastoral women (e.g. credit and literacy classes) showed a positive impact on the household member's health, since women tend to reinvest their income, for example, by paying medical fees (Flintan, F., 2008).

It is difficult to draw coherent conclusions on linkages between human and animal health from separated human and livestock health studies. The simultaneous assessment of human and animal health outcomes leads to a better understanding of the specific context of pastoralism, particularly when diverse disciplines such as social sciences, epidemiology and geography are associated. Joint human and animal health surveys are done concurrently in time and/or space and at different levels of aggregation: from individual, household or village level, to communities and their animals, provinces or country. The ideal outcome promotes improved human, animal and ecosystem health (Zinsstag, J.*et al.*, 2011).

The content discussed in this article is derived from the work of the authors in Africa and Central Asia, alongside a review of findings of relevant working groups and literature. We highlight some linkages of the grassland ecosystem with health outcomes such as nutritional status. Where possible, results are shown as comparison of pastoralists to a reference group to better perceive the specificities of pastoralist communities within the remote rural context. Pastoralists are not

necessarily worse off than others in all health aspects. For example, child malnutrition and livestock diseases may occur more frequently in village herds or in intensified peri-urban livestock production systems than in extensively managed vast pastoral lands. We also present methodical considerations for epidemiological human and animal health studies and monitoring and surveillance among pastoralists and discuss potential strategies towards the inclusion and use of health information to develop effective health interventions.

Nutrition in pastoralist settings

In rural Chad, proportions of malnutrition were not higher in pastoral children compared to sedentary children. But both populations showed malnutrition proportions above 10% at the end of the dry season (Schelling, E.*et al.*, 2005, Bechir, M.*et al.*, 2010) (Figure 4.1).

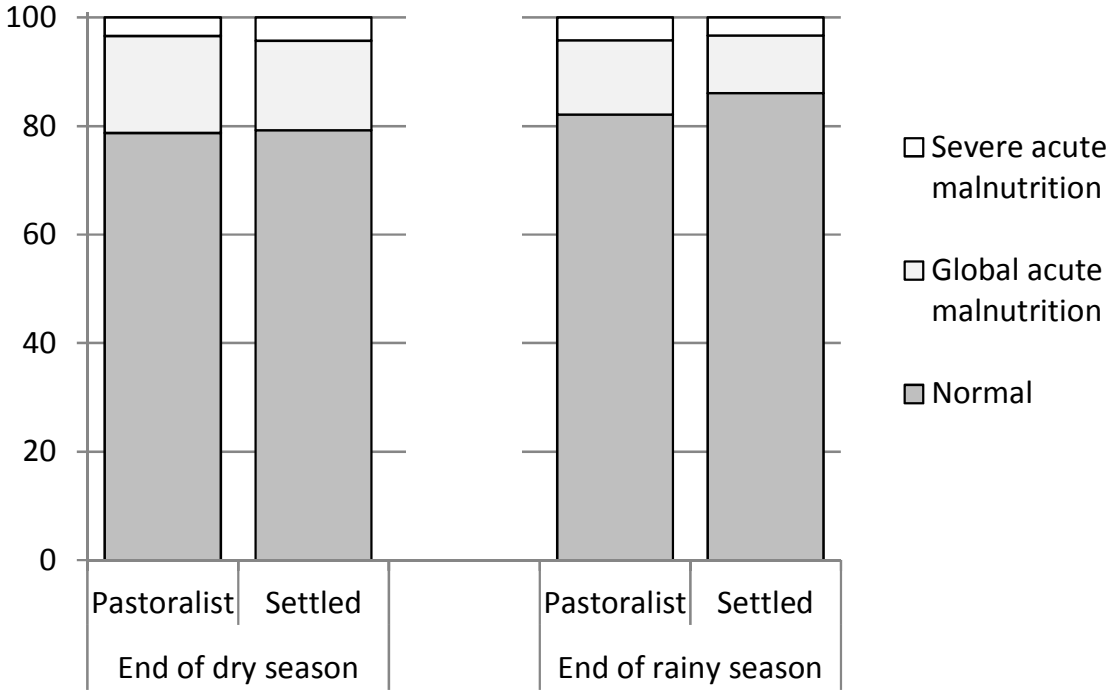

Figure 4.1. Child malnutrion in pastoralist and settled communities of Chad during the end of the dry season and the end of the rainy season. (Source 23)

In contrast, pastoralist women were significantly more under-nourished (up to 48% in the dry season) than settled women, and obesity was only seen among settled women (Bechir, M.*et al.*, 2011b) (Figure 4.2). Malnutrition in children was significantly associated with anaemia and selected intestinal parasites (Bechir, M.*et al.*, 2012a). Other authors have observed that pastoralist mothers deprive themselves when nutrition is in short supply (Shell-Duncan, B., 1995). Among the Ariaal and Rendille in East Africa, children of three settled communities, in a famine-relief based town, showed three times the level of stunting and wasting when compared to the surrounding nomadic and semi-mobile pastoralists. These differences were attributed to greatly reduced

access to milk and higher reliance on cereals in the settled communities (Fratkin, E.*et al.*, 2004). During the wet season, when milk was abundant and grain prices were highest, milk provided almost 90% of dietary energy to Turkana pastoralists and 80% to the Maasai (Nestel, P., 1986, Galvin, K. A., 1992, Lawson, D. W.*et al.*, 2014).

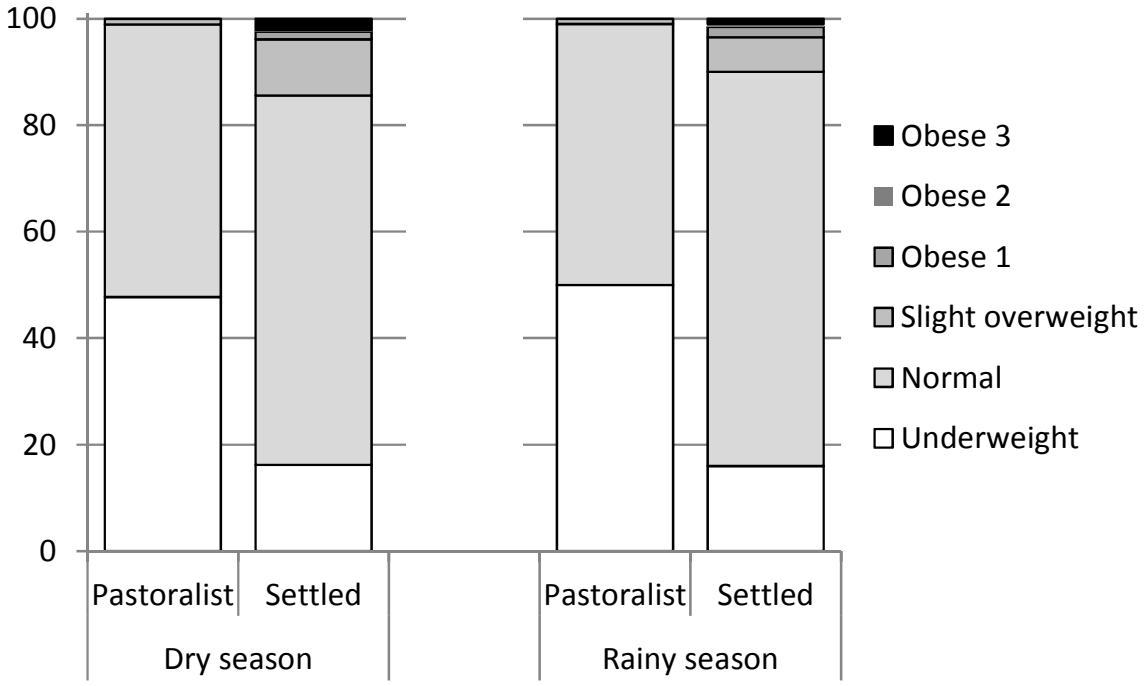

Figure 4.2. Body mass index of pastoralist and settled women during the dry and rainy season of the Lake Chad region (Bechir, M.*et al.*, 2011a). Underweight: BMC of <18. 5; Normal: 18.5-25; Slight overweight: 25-30; Obese 1: 30-35; Obese 2: 35-40; Obese 3: ≥40

Vitamin A levels may indicate the linkages from environment to animals through milk to humans. Milk from cows grazing on green pastures had higher β-carotene levels while the pastoralist consumers of such milk had fewer deficiencies in vitamin A (Zinsstag, J.*et al.*, 2002a). Retinol levels showed strong seasonal variation (Crump, L., 2014). Still, milk as the primary source of vitamin A for pastoralists is insufficient. Serum retinol deficiencies were high among pastoralists, up to 32% in the cold season (Crump, L., 2014). Another study found a high prevalence of moderate serum retinol deficiency in settled children younger than five years at the end of the rainy season, whereas, during the same period, it was as low as 1% among nomadic children (Bechir, M.*et al.*, 2012b). Low fruit and vegetable consumption were seen in several studies in West and East Africa (Holter, U., 1988), which also applies to poorer pastoral households in Central Asia and Mongolia (Children, S. t., 2013). Many pastoralist families have diversified their activities through crop-farming. Diets previously rich in animal protein through milk and meat, though often calorically deficient, are changing to diets based on cereals. Also sugar and oils became new important sources of energy for pastoralists (Schelling, E.*et al.*, 2005) and as in other settings may cause diabetes and hypertension.

In drought situations in the Sahel or winter disasters called Zud in Mongolia, the economic value of livestock drops rapidly. Timely 'commercial destocking', or taking animals off the land before they cause long-term damage to vegetation, benefits the environment. Dried meat provides nutritional energy and protein, while money earned by selling can be used to buy supplemental fodder. Pastoralist communities are commonly willing to sell livestock but often lack access to markets. Interventions that facilitate livestock purchase by providing transport subsidies to traders are successful and cost-effective. Timely replenishment of the livestock economy and continued generation of alternative livelihoods and sources of income are targeted in the post-drought period (Morton, J. and Barton, D., 2002, Simpkin, S. P., 2005, Ericksen, A., 2014).

Human and animal parasitic diseases

In dry areas, natural and man-made water sources are highly frequented by humans, livestock and wildlife, and the density of livestock can be very high. Pastoralists sometimes use surface water rather than safer wells because they have lost traditional access rights. Waterholes and wells rapidly become contagious places and a source of disease (MacPherson, C. N. L.*et al.*, 1987, Foggin, P. M.*et al.*, 1997), for example for cholera and typhus during the rainy season (Schelling, E.*et al.*, 2005, Cummings, M. J.*et al.*, 2012).

In Africa, mainly *Schistosoma haematobium* and *Schistosoma mansoni* in humans and *Schistosoma bovis* in cattle cause schistosomiasis. Highest prevalences in humans and in cattle were found among ethnic groups which pasture their livestock on the shores or islands of Lake Chad where the intermediate hosts - several species of freshwater snails – are present. Other ethnic groups - skilled in building wells - had markedly lower prevalences (Greter, H.*et al.*, 2015). Another study showed that the main source of fascioliasis in ruminants was the lake rather than rain-fed surface water (Jean-Richard, V.*et al.*, 2014b). The degradation of wetlands potentially favours an environment for disease transmission between people, livestock and wildlife populations as they are forced into small isolated areas with limited available water (Mazet, J. A. K.*et al.*, 2009).

In central Asia, Mongolia and the Tibetan plateau, grasslands are populated with rodents - the intermediate host for another parasite, *Echinococcus multilocularis* - that causes the severe human and animal disease alveolar echinococcosis (AE) (Giraudoux, P.*et al.*, 2013). Human cystic echinococcosis is caused by infection with the hydatid cyst or larval stage of the dog tapeworm *Echinococcus granulosus*. The parasite is transmitted between dogs and domestic ungulates, especially sheep. One third of all households in Mongolia keep sheep in extensive pastoral systems. *E. multilocularis* is found in wildlife (Ito, A.*et al.*, 2013) and *E. granulosus* in livestock (e.g. 9.2% seropositivity in goats). Both echinococcosis species are found in people (Ito, A.*et al.*, 2013, Chinchuluun, B.*et al.*, 2014). After a substantial decrease of human echinococcosis, the disease

spread again after the breakdown of health and veterinary services during the transition from socialist planned economy to market economy and privatisation (Davaatseren, N.*et al.*, 1995). In the Turkana region of northwest Kenya, and also among Tibetan nomadic populations, abdominal ultrasound screening surveys have detected hydatid cysts in 5-19% of pastoralists, with 10-20% prevalence in the age group of 20-50 years (MacPherson, C. N. L.*et al.*, 1987).

Furthermore, parasitic diseases considered less important for livestock and people, such as bovine cysticercosis, gain momentum with the advent of export slaughterhouses in pastoral areas. When cattle are infected with bovine cysticercosis, the carcasses are condemned, causing large economic losses to pastoralists and entire regions (Asaava, L. L.*et al.*, 2009).

Bacterial zoonoses

If brucellosis was not present, milk and meat production in traditional cattle production systems in sub-Saharan Africa would increase an estimated 5 - 11% and 12 - 35%, respectively (FAO, 2002). The main human infection routes of several bacterial zoonoses, e.g. brucellosis, Q-fever and bovine tuberculosis, vary between communities: direct contact and particularly contact with abortion by-products dominate in livestock keeping communities, whereas contaminated raw milk products put consumers at risk of infection. Establishing associations between human and livestock zoonotic infections at household level is rarely straight forward in dynamic livestock-keeping communities, because people and livestock do not remain together for long periods. In Kyrgyzstan, human brucellosis seropositivity was related to sheep seropositivity at the district level, but not at the household level (Bonfoh, B.*et al.*, 2011a). Human seroprevalences were very high (up to 30%) among pastoralists in Kyrgyzstan and Mongolia (Bonfoh, B.*et al.*, 2011a, Baljinnyam, Z.*et al.*, 2014). In Mongolia, non-pastoralist communities also showed high prevalence (Tsend, S.*et al.*, 2014). In Togo, human seropositivity was unexpectedly low (below 1%), although cattle seropositivity was high (9% in village and 7% in transhumant cattle) (Dean, A. S.*et al.*, 2013). The Togolese *Brucella abortus* strains from cattle had a large deletion in a gene that might influence virulence and/or host predilection (Dean, A. S.*et al.*, 2014). In Chad, human Q-fever seropositivity was associated with keeping camels but not cattle (Schelling, E.*et al.*, 2003). High Q-fever seropositivities in camels were confirmed in other studies, for example in pastoral settings in Ethiopia (Gumi, B.*et al.*, 2013).

Among pastoralist communities of East Mauritania, the illness 'tuberculosis' had a rich and more complex nomenclature than the biomedical disease. Tuberculosis (TB) was part of different illness concepts according to different stages and perceived causes (hereditary, warm or bitter foods [*Iguindi*], lack of sufficient milk [*Timchi*]). Three types of tuberculosis illness were treated by the healer, two by the faith healer and one by the medical doctor (Ould Taleb, M., 2007). Recently in East-Mauritania, new presumptive TB cases - and later confirmed at health centres – were in a

population-based study not higher among pastoralist than villagers - indicating that the whole rural population is deprived of quality diagnostics and treatment (Lô, A., Dia, A.T., Bonfoh, B., Schelling, E., 2016). Possible cattle-human transmission of tuberculosis was unknown in Mauritania, whereas two-thirds of mycobacterial adenitis patients in Tanzania knew about this possibility (Mfinanga, S. G.*et al.*, 2005). The proportion of human tuberculosis due to *Mycobacterium bovis* was generally lower (<10%) than expected two decades ago. A recent review found a median proportions of 2.8% among human TB patients in Africa and 1.4% in the rest of the world (Muller, B.*et al.*, 2013). A combined field, slaughterhouse and hospital study in Ethiopia showed that *M. bovis* in human TB infection was low – among 1000 *M. tuberculosis* complex isolates from clinical suspects of pulmonary and extra-pulmonary TB, only 4 isolates were *M. bovis* (Berg, S.*et al.*, 2015). Interestingly, *M. tuberculosis* was isolated from several cattle and from one camel, suggesting more comprehensive transmission of TB strains between livestock and people (Gumi, B.*et al.*, 2012). *M. bovis* causes production losses in cattle, especially in African peri-urban dairy farms with 'exotic breeds'. However, it does not seem to be a major economic livestock disease in extensive systems (Tschopp, R.*et al.*, 2012).

4.3. Human and animal health survey designs

It is rarely possible to select a simple random sample among pastoralists because this requires an accurate list of community members. Multi stage cluster sampling is commonly used when complete registries, of humans or animals, do not exist. Herds and pastoralist camps where members move together, represent natural clusters. In contrast to settled communities where a list of villages is either available or can be compiled, a random selection of mobile pastoralist camps from a list is usually not possible. Sampling at water bodies or wells has been suggested, but ethnic and seasonal heterogeneity in migration routes might introduce bias (Kalsbeek, W. D., 1986). Alternatively, random transects have been employed for random selection although smaller camps might risk being overlooked with this approach. Generating random geo-coordinates and sampling all camps within a predefined circumference proved to be useful. However, bias can also be introduced because camps in sparsely populated areas have a higher probability of selection than those in densely populated areas. The use of aerial photography and remote sensing has been considered to estimated population sizes, but might be less useful in the context of sampling (Animales, M. d. l. E. e. d. R., 1993).

Once camps are selected, either all people and/or animals of a group or a random subset are enrolled. If only a single or fixed number of household members or animals within herds are selected, a "household size bias" can be introduced. Therefore, the samples must be weighted according to the number of individuals within each camp for unbiased estimates. If animals are randomly chosen from each herd, the animal prevalence estimate will be an unbiased estimate, but

this is not true for the herd prevalence. There are formulas available to calculate the corresponding herd level prevalence, which are particularly useful where not all animals of a herd were sampled or when estimates need to be corrected for imperfect test sensitivity and specificity (Faes, C.*et al.*, 2011). A combined analysis of human and animal data can be challenging. However, for many research questions – for example, evaluation of the effectiveness of an intervention - a joint statistical analysis would be less important than joining presentation and interpretation of the results (Schelling, E. and Hattendorf, J., 2015).

Health impact assessments of industrial development projects (Winkler, M. S.*et al.*, 2012) should be extended to simultaneously assess livestock health. Construction of dams or mines can also adversely affect the health of the livestock has a direct implication on livelihoods and income of communities and can also indirectly affect human health.

Estimation of achieved intervention coverage, for instance, vaccination, is challenging in the absence of total population size estimates for mobile pastoralist groups, who are chronically underrepresented in censuses, particularly in sub-Saharan Africa. We have estimated that 70% of the total pastoralist population in a Chadian region was no longer present in the same area in the following year (Schelling, E.*et al.*, 2007b). There are pressing needs for baseline demographic and health-related data to plan, implement and evaluate health interventions. A biometric identification system based on the registration and identification of digital fingerprints was acceptable to pastoralist communities in Chad and allowed unique identification of individuals who did not hold governmental identification cards (Weibel, D.*et al.*, 2008). It also reduced the needed minimum number of re-encounters to estimate the total population size. However, re-encounters during random transects were still too few (5%) to derive estimates with a meaningful precision or to establish a retrospective cohort on reported data (Weibel, D.*et al.*, 2011b). More recently, a randomised cohort was equipped with mobile phones, and this pilot study encouraged the further use of mobile phones to plan larger trials (Jean-Richard, V.*et al.*, 2014d) (Abakar et al. this volume). The pilot study led to estimates of densities for pastoralist and sedentary people and livestock over both seasons and years. At the end of the dry season, the densities of pastoralists were almost four times lower than those of villagers, while the pastoralist livestock density was three times higher. The very high livestock densities raised questions about carrying capacity of pastures and ecosystem health (Jean-Richard, V.*et al.*, 2015).

4.4. Conclusions

Field health surveys are central understanding the context and the disease dynamics and for planning of evidence-based testing of control measures. Aggregated data do not capture the complexities of

disease dynamics. Also cost estimates of disease and interventions can be collected – and costs are important arguments for interventions supported by governments or non-governmental organizations. Including pastoralists in surveys is as feasible as other rural communities. The additional inclusion of a non-pastoralist comparison community in the same region sheds light on specific health aspects of pastoralism – and often - more commonalities than differences are found. In future, further alternative surveillance systems will continue to be evaluated; among these syndromic surveillance, participatory epidemiology (Mariner, J. C.*et al.*) and risk-based joint surveillance systems (Abakar et al this volume). Near real-time reporting systems should be established together with ability to respond to reported events. There is a need for consistent, reliable data over a longer term. In developing health interventions and programmes for rural communities, including pastoralists, successful implementation of control measures is further strengthened by the exchange and cooperation between neighbouring countries within regions.

Health of pastoralists is shaped by access to health and veterinary services that are less related to mobility and distance but more to social and institutional relationships and the ecosystem they live in - grasslands, the contamination of water sources and marked seasonal and inter-annual variations of weather and climate. The question of specific factors influencing health of pastoralists, such as mobility and close contact to livestock, remains interesting since pastoralists share basic features with settled communities, like ethnicity, culture and livestock keeping, although in different systems (Grolimund, A., 2010). Essentially, the health of pastoralists and their livestock are within the context of remote rural populations. The experiences, local concepts and priorities of nomadic communities, combined with sound field survey, demographic and surveillance data, however, are essential to better understand how to provide adapted health services to the mobile communities.

5. Prevalence of *Fasciola gigantica* infection in slaughtered animals in south-eastern Lake Chad area in relation to husbandry practices and seasonal water levels

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5.1. Abstract

Background: Fasciolosis has been described in sub-Saharan Africa in many accounts, but the latest reports from Chad are from the 1970s. Mobile pastoralists perceive liver parasites as a significant problem and think that proximity to Lake Chad can lead to infection. This study aimed to assess the importance of liver fluke infections in mobile pastoralists' livestock in the south-eastern Lake Chad region. In 2011, all animals presented at three slaughter slabs near Gredaya in the south-eastern Lake Chad area were examined for infection with *Fasciola* spp. during routine meat inspections.

Results: This study included 616 goats, 132 sheep and 130 cattle. The prevalence of adult *Fasciola gigantica* was 68% (CI 60-76%) in cattle, 12% (CI 10-16%) in goats and 23% (CI 16-30%) in sheep. From all infected animals (n = 200), 53% (n = 106) were classified as lightly infected with 1-10 parasites, 18% (n=36) as moderately infected with 11-100 parasites and 29% (n=58) as heavily infected with more than 100 parasites per animal.

Animals grazing close to the shores of Lake Chad had a much higher risk of infection (prevalence = 38%; n=329) than animals not feeding at the lake (n=353), with only one goat being positive (prevalence = 0.28%).

The ethnic group of the owner was a strong determinant for the risk of infection. Ethnic group likely served as a proxy for husbandry practices. Geospatial distribution showed that animals originating from areas close to the lake were more likely to be infected with *F. gigantica* than those from more distant areas.

Conclusions: Livestock belonging to ethnic groups which traditionally stay near surface water, and which were reported to feed near Lake Chad, have a high risk of infection with *F. gigantica*. Pastoralist perception of fasciolosis as a priority health problem was confirmed. Regular preventive and post-exposure treatment is recommended for animals grazing near the lake. However, further economic analysis is needed.

Keywords: Fasciolosis, Lake Chad, Mobile pastoralists, Slaughter slabs

5.2. Background

Fasciolosis is a parasitic disease of herbivorous mammals caused by trematodes of the genus *Fasciola*. In livestock, it causes severe reductions in milk and meat yield as well as losses due to decreased fertility (Abunna, F.*et al.*, 2010, Sariozkan, S. and YalCin, C., 2011). The host animals become infected with *Fasciola* metacercariae when they ingest contaminated vegetation close to or within water bodies. Swamp areas and seasonally flooded areas at the borders of Lake Chad provide

an optimal habitat for the parasites and the intermediate hosts, which are freshwater snails of the family Lymnaeidae.

In sub-Saharan Africa, infections with *Fasciola gigantica* have often been described (Maiga, Y. I.*et al.*, 1991, Malone, J. B.*et al.*, 1998, Abunna, F.*et al.*, 2010, Amor, N.*et al.*, 2011). In the Lake Chad area, two previous studies from Niger and Cameroon (Graber, M.*et al.*, 1966, Tager-Kagan, P., 1978) have described the disease, but there is no publication from the Chadian side of the lake. *F. gigantica* has been reported in Chadian cattle and small ruminants in a Central African study of wild ruminants (Graber, M. and Thal, J., 1979) and in a treatment study on Chadian cattle (Troncy, P. M. and Vassea-Martin, N., 1976). *F. hepatica* infection has not been reported in Chad.

Fasciolosis is perceived as a significant animal health problem by the mobile pastoralist population in the south-eastern Lake Chad area, particularly since other diseases like bovine pleuropneumonia, against which vaccination is compulsory, are better controlled. Most pastoralist camp leaders expressed concerns about their animals grazing on contaminated pastures in close proximity to water bodies of Lake Chad during a participatory research needs assessment (Jean-Richard, V., 2013). Some pastoralists are aware that it is possible to treat animals for liver flukes with anti-parasitic drugs, but access to quality drugs is difficult in the remote zones (Schelling, E., 2002).

The majority of the income for mobile pastoralists in this area is generated by selling milk and animals at local markets (Wiese, M., 2004), so the adverse economic impact of fasciolosis is of primary importance. The mobile pastoralists observe the parasites when they slaughter animals and are aware that this is a cause of reduced milk production and body weight. This study was initiated to investigate the mobile pastoralists' priority concern of fasciolosis in their livestock.

5.3. Methods

Study zone and population

The south-eastern Lake Chad area is densely populated by sedentary people as well as by mobile communities of different ethnic groups during the dry season, from October to June. In this paper, we describe the ethnic groups using the names as given by the local communities. Kanembou are mainly sedentary, while Arabs are semi-nomadic, moving towards the lake at the end of the dry season when pasture becomes scarce around their villages. Peulh and Gorane communities may be mobile or sedentary, although most large-scale cattle owners are mobile, including the entire family and all of their livestock. Peulh is synonymous with the term Foulbe and Fulani (English). Peulh herders graze their animals in close proximity to the lake shore, with the animals often feeding on grass in shallow water. The Kouri pastoralists utilise pasture areas that partially overlap with the Peulh, primarily herding their cattle on accessible islands within Lake Chad. In contrast, Gorane do

not stay close to the lake, instead capitalising on highly developed well building skills for access to water.

Sampling strategy

Each week from January to December 2011, the livers of all slaughtered animals were examined for the presence of *Fasciola* spp during routine meat inspections at three slaughter slabs (Gredaya, Sidje and Bache Djani) in the administrative district of Gredaya at the south-eastern border of Lake Chad. The parasite burden was established by incising the liver along the bile ducts, according to the usual local meat inspection process. No further pathological assessment was made as the study took place during routine meat inspection by the local veterinary delegate. A semi-quantitative estimation of the number of flukes was made based on the number of parasites counted in the exposed surfaces. Infection was classified as being light (1-10 flukes), moderate (11-100) or heavy (>100) in intensity. Most of the off-take from the local herds was animals which were slaughtered and sold at the weekly markets in Gredaya, Sidje and Bache Djani, and all of these were included in the study. Animals slaughtered in households were not examined. The number of examined animals varied from 1-22 per day of observation. The veterinarian interviewed the owner of each animal presented for slaughter, completing a short questionnaire. Information included the origin of the animal, ethnic group of the current owner, animal breed, history of the animal grazing in or in close proximity to Lake Chad and the semi-quantitative level of flukes counted in the liver.

Figure 5.1. *Fasciola gigantica* from a cow in the south-eastern Lake Chad area.

Morphological analysis and measurement of a sub-sample of the individual fluke specimens collected from infected animals (Figure 5.1) was performed at the Laboratoire de Recherches Vétérinaires et Zootechniques in Chad, based on descriptive criteria (Frank, W., 1976, Sewell, M. and Brocklesby, D., 1990, Mas-Coma, S.*et al.*, 2009), including body size and shape, form of the apical cone and position of the suction cups and ovaries.

The data were double entered in Microsoft® Access 2002 (Microsoft Corp.; Redmond, WA,USA), and compared using Epi Info™ 3.5.1 Data Compare program (Centers for Disease Control and Prevention,

Atlanta, GA, USA). Statistical analysis using descriptive statistics and logistic regression was conducted with Stata IC 10.1 (StataCorp LP, College Station, USA). ArcGIS 9.3 (ESRI Inc. ArcMap™ 9.3, Redlands, CA, USA) and Google Earth (Google Inc., Mountain View, CA, USA) were used for mapping and spatial analysis.

5.4. Results

A total of 880 animals were examined. Two animals were excluded because questionnaires were not completed. Data from the remaining 130 cows, 616 goats and 132 sheep were analysed. The distribution of livestock species by owner ethnic group is shown in Table 5.1.

Table 5.1: Sample size of species and ethnic groups of the owners

	n	%	Arab	%	Peulh	%	Gorane	%	Kanembou	%	Kouri	%
Cattle	130	15%	10	8%	85	65%	28	22%	1	1%	6	5%
Goats	616	70%	278	45%	133	22%	133	22%	72	12%	0	0%
Sheep	132	15%	27	20%	56	42%	47	36%	2	2%	0	0%
Total	878	100%	317	36%	274	31%	208	24%	75	9%	6	1%

Fasciola gigantica specimens (n = 11) measured between 2 and 5 cm, with a mean size of 3.2 cm. The prevalence of *F. gigantica* was 68% (95% CI 60-76%) in cattle, 12% (95% CI 10-16%) for goats and 23% (95% CI 16-30%) for sheep. The analysis revealed a strong relationship ($p < 0.001$) between grazing at the lake and *F. gigantica* infection. Not feeding at the lake was a protective factor, and only one animal reported as not grazing near the lake was infected with *F. gigantica* (0.28 %) (Table 5.2).

Table 5.2: Prevalence for infections with *F. gigantica* by species and stratified for grazing area

	Grazing LC	pos	%	neg	%	Total	Prev	p	OR	CI (95%)
All animals	yes	198	38%	329	62%	527	22.6%	<0.001	212	29.5-1520
	no	1	0%	352	100%	353		Baseline		
Cattle	yes	89	93%	7	7%	96	68.5%	n.a.* perfect prediction		
	no	0	0%	34	100%	34				
Goats	yes	79	22%	277	78%	356	13.0%	<0.001	74	10.2-534.6
	no	1	0%	259	100%	260		Baseline		
Sheep	yes	30	41%	43	59%	73	22.7%	n.a.* perfect prediction		
	no	0	0%	59	100%	59				

The highest prevalence was seen in cattle from the Kouri ethnic group (100%, n = 6), and livestock owned by Peulh also showed high prevalence (95% for cattle, 33% for goats, 48% for sheep). None of the Gorane cattle were reported to have grazed in the lake, and none were positive for *F. gigantica*. Of all Gorane animals (n = 208), only one goat was infected with *F. gigantica*. There was no infection with *F. gigantica* in animals from Kanembou breeders (n = 75). The prevalence in Arab livestock (13%, n = 317) ranged in between those grazing near lake water and those not near the lake (Table 5.3).

Table 5.3: Prevalence of *F. gigantica* in different livestock species by ethnic group of the owner

Ethnic group		**pos**	**%**	**neg**	**%**	**Total**	**p**	**OR**	**CI (95%)**
Gorane		1	0%	207	100%	208	0.001	0.03	0-0.2
Peulh		152	55%	122	45%	274	<0.001	8.6	5.7-12.9
Arab		40	13%	277	87%	317	Baseline		
Kanembou		0	0%	75	100%	75	n.a.* perfect prediction		
Kouri		6	100%	0	0%	6	n.a.* perfect prediction		
Cattle	Gorane	0	0%	28	100%	28	n.a.* perfect prediction		
	Peulh	81	95%	4	5%	85	<0.001	81	12.8-513.2
	Arab	2	20%	8	80%	10	Baseline		
	Kanembou	0	0%	1	100%	1	n.a.* perfect prediction		
	Kouri	6	100%	0	0%	6	n.a.* perfect prediction		
Goats	Gorane	1	1%	132	99%	133	0.04	0.05	0-0.4
	Peulh	44	33%	89	67%	133	<0.001	3.4	2.1-5.7
	Arab	35	13%	243	87%	278	Baseline		
	Kanembou	0	0%	72	100%	72	n.a.* perfect prediction		
Sheep	Gorane	0	0%	47	100%	47	n.a.* perfect prediction		
	Peulh	27	48%	29	52%	56	0.003	7.4	2.0-27.6
	Arab	3	11%	24	89%	27	Baseline		
	Kanembou	0	0%	2	100%	2	n.a.* perfect prediction		

Among all positive animals (n = 200), 53% (n = 106) were classified as lightly infected (1-10 parasites), 18% (n =36) as moderately infected (11-100 parasites) and 29% (n = 58) as heavily infected (> 100 parasites). In cattle, 19% of infections (n = 17) were light, 20% (n = 18) moderate and 61% (n = 54) heavy; in goats, 80% (n = 65) were light, 17% (n = 14) moderate and 2% (n = 2) heavy; and in sheep, 80% (n = 24) light, 13% (n = 4) moderate and 7% (n = 2) heavy. There was a significant difference between the degree of infection in cattle and small ruminants. Sheep and goats had very similar prevalence and burdens. The prevalence in cattle in Sidje, the slaughter slab closest to Lake Chad, was significantly higher than in Gredaya (p = 0.003). Seasonal trends indicate a lower prevalence

between the months of August to October, comprising the rainy season, compared to the rest of the year in all species.

Geospatial distribution

The prevalence rates were plotted according to the coordinates of the villages of origin to show the geospatial distribution of animals and the proportion of positive animals. The size of the circle corresponds to the number of animals originating in each village (Figure 5.2 shows the data for goats, data for cattle are shown in Additional file 1: Figure S1, data for sheep are shown in Additional file 2: Figure S2). There is a notable relationship between proximity to the lake and infection with *F. gigantica* in all three species.

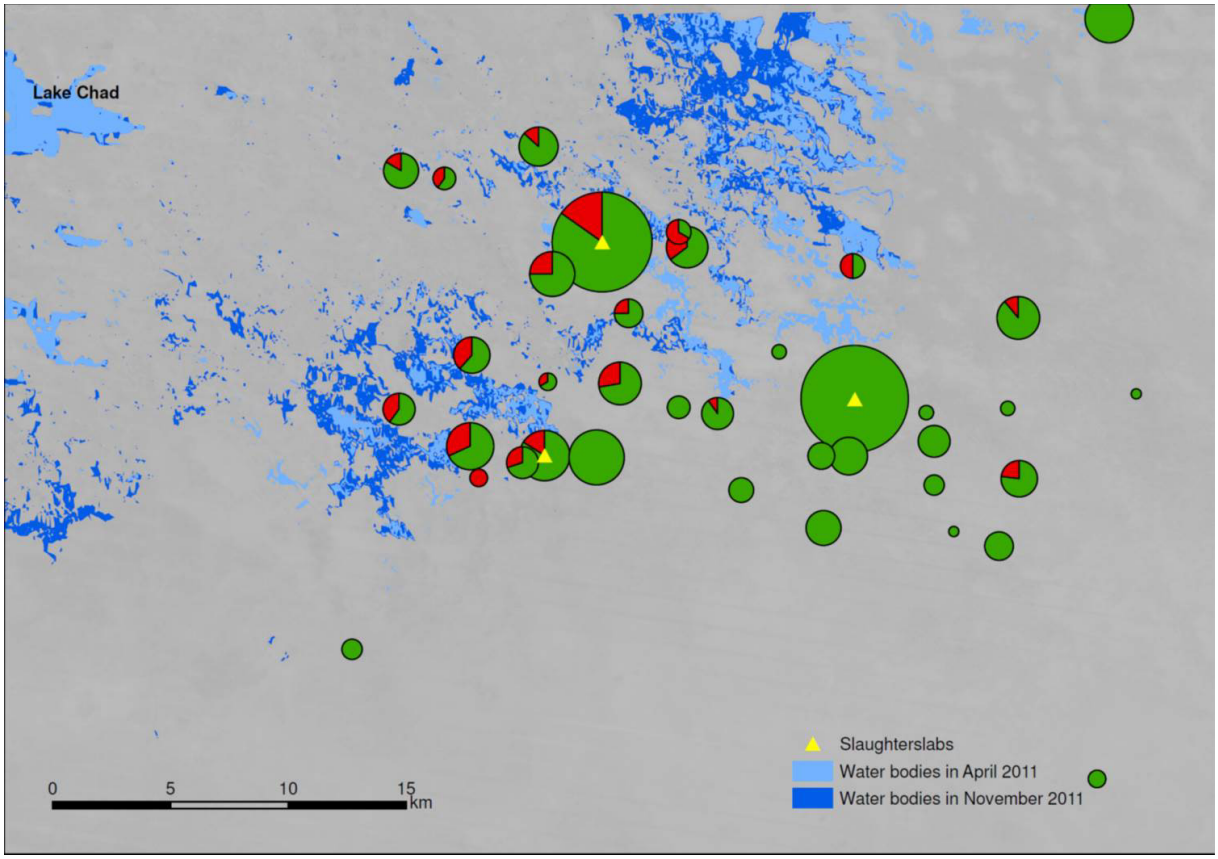

Figure 5.2. Prevalence of *F. gigantica* in slaughtered goats by village of origin.

Legend: prevalence rate according to village of origin coordinates, circle size corresponds to the number of animals, red indicates proportion positive for *Fasciola gigantica.*

5.5. Discussion

This is the first publication on *Fasciola* infection in cattle, sheep and goats in the Lake Chad area of Chad. The results support a relationship between the infection of livestock with *F. gigantica* and the ethnic group of the livestock holder. The ethnic group likely serves as a proxy for the type of animal

husbandry practiced (Jean-Richard, V., 2013). The Kouri cattle, which were kept on islands in the lake, were 100% positive for *F. gigantica*. Although the sample size was very small (n = 6), nonetheless there was clearly a high prevalence in these animals. The livestock kept by Peulh, who utilise pastures close to the lake and its seasonal extensions, also showed a high prevalence (55%.overall, 95% for cattle). In contrast was the low prevalence found in the Gorane and Kanembou livestock. The Gorane pastoralists do not move close to the lake, but stay in drier areas to the east. The Kanembou culture and husbandry practices are, in general, similar to the Gorane, although in the study zone, the majority of Kanembou were sedentary rather than mobile. The prevalence found in Arab livestock ranged in between that found in the other ethnic groups (Kouri/Peulh and Gorane/Kanembou). This finding is supported considering that Arab cattle breeders in the zone were semi-nomadic, only moving their animals towards the lake at the end of the dry season, when the pastures around their villages were depleted. There was a notable relationship between proximity to the lake and infection with *F. gigantica* in all three species. The geospatial distribution and the analysis of grazing patterns strongly suggest that Lake Chad is the source of infection. This would also explain the observed seasonal trend, which is likely due to migration away from the lake during the rainy season when grass is more widely available, potentially reducing exposure to the contaminated areas close to the lake. Further research is currently continuing to establish the seasonal dynamics of *F. gigantica*. The results of this study support a strong recommendation, for Kouri and Peulh livestock, for treatment against *F. gigantica* infection with an initial prophylactic dose when entering the lake region and a second dose at the beginning of the rainy season, or when leaving the area. This type of programme could reduce pasture contamination and the effects on livestock productivity, particularly for Arab livestock that is not continuously grazed near the lake. In contrast, no preventive treatment is necessary for Gorane and Kanembou livestock that are grazed in areas not near the lake. Because they are not in proximity to open water, these animals have a negligible risk of infection. Our recommendation is in line with that of the local veterinarian in the Gredaya administrative district, who recommended treatment every three months as long as animals were kept near the lake. It was noted that prevalence and degree of infection differ between species. This is likely due to feeding patterns as well as specific husbandry practices. Small ruminants avoid wet areas, instead preferring to graze and browse on dry ground. However, grazing dry pastures is not completely protective, as *Fasciola* metacercariae can remain viable for some time on vegetation and in some of the intermediate host snails of the genus *Lymnaea* in previously flooded areas (Frank, W., 1976, Sewell, M. and Brocklesby, D., 1990). Pastoralists in the study area reported that they kept their small ruminants away from the more humid areas near the water as long as possible to decrease the risk of infection, as also noted by Tager-Kagan in the 1970s (Tager-Kagan, P., 1978). In this study, sheep and goats had comparable infection intensities. The similar burden in sheep and

goats could indicate similar susceptibility to infection and/or result from use of comparable feeding areas. Although sheep commonly graze ground cover, while goats typically browse shrubs and trees, the Lake Chad region is now densely populated with herds and subject to increased agricultural cultivation, so there are relatively few shrubs, particularly at the end of the dry season. The higher prevalence noted in cattle at the slaughter slab in Sidje is likely because many Peulh pastoralists pass by this village when leaving the lake or stay nearby during the rainy season. In this study, meat inspection was performed according to the routine local inspection procedure, which consisted of one long transverse cut in the liver along the bile ducts. The method of examination was a limitation to this study in that it provided only a semi-quantitative measurement of the parasite burden. While it would have been ideal to examine the entire organ by cutting it into small pieces to visualise all biliary ducts, the cost to purchase every liver precluded such a method. Using the standard meat inspection approach, it is possible that some animals with few parasites might have been misclassified as not infected. Also, particularly in cattle due to the large liver size, multiple incisions might have shown a higher number of parasites. Therefore, the prevalence and degree of *F. gigantica* infection intensity might have been underestimated using the standard, locally available, semi-quantitative evaluation method employed in this study, but the exposure patterns revealed are nonetheless significant and valid, despite a potentially decreased sensitivity of this method. Although fasciolosis is increasingly being recognised as a human public health issue (WHO, 2007, Mas-Coma, S.*et al.*, 2009), there is very little literature on F. gigantica from Chad or the Lake Chad region, with the most recent dating from the late 1970s (Graber, M.*et al.*, 1966, Tager-Kagan, P., 1978, Graber, M. and Thal, J., 1979). At that time, the recommended control measure was routine deworming treatment of livestock once or preferably twice per year, before and after the rainy season (Troncy, P. M. and Vassea-Martin, N., 1976, Tager-Kagan, P., 1978). Based on the results of the present study, this recommendation should still be implemented for herds grazing near Lake Chad. Further cost-benefit analysis is warranted, as findings would provide evidence for information campaigns and policy development. It is also recommended to assess pastoralist's access to and the quality of available treatments for fasciolosis in remote areas.

5.6. Conclusions

This research quantifies the prevalence of *F. gigantica* in slaughtered livestock in south-eastern Lake Chad area and provides a semi-quantitative assessment of the burden of infection. The results showed that animals which had grazed in close proximity to the lake and its seasonal extensions had a high risk of infection. Cattle of Peulh and Kouri ethnic groups were most affected. These groups keep their animals at the shore of Lake Chad or on islands within the lake. The study confirms the

pastoralists' perceptions of disease priorities with fasciolosis as an important health problem. Treatment against fasciolosis is recommended for animals grazing near or at the lake, and further economic analysis of such treatment is warranted. The data set supporting the results of this article is available in Additional file 3.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

VJR supervised data collection in situ, entered and analysed the data and drafted the manuscript. LC contributed to the realisation of the study and drafted the manuscript. AAA assisted with data entry and coordinated the collection and transport of data. NBN and HG participated in the conceptualisation and realisation of the study and contributed expert opinions to the draft. JH provided statistical knowledge and contributed to statistical analysis. ES added expert knowledge from earlier research in the area. JZ engaged in the conceptualisation of the study and gave general supervision throughout the study. All authors read and approved the final manuscript.

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Additional files

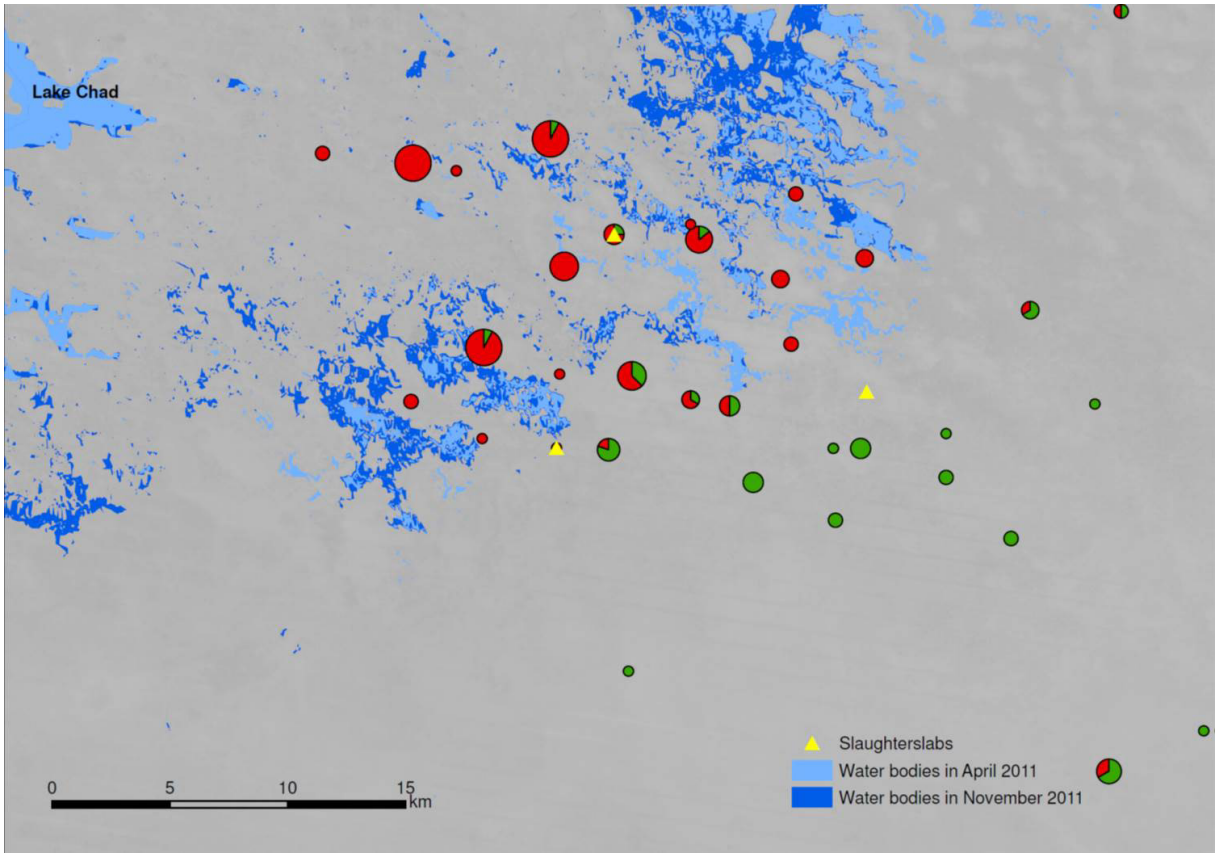

Figure S5.3. Additional file 1: Prevalence of F. gigantica in slaughtered cattle by village of origin. Legend: prevalence rate according to village of origin coordinates, circle size corresponds to the number of animals, red indicates proportion positive for Fasciola gigantica.

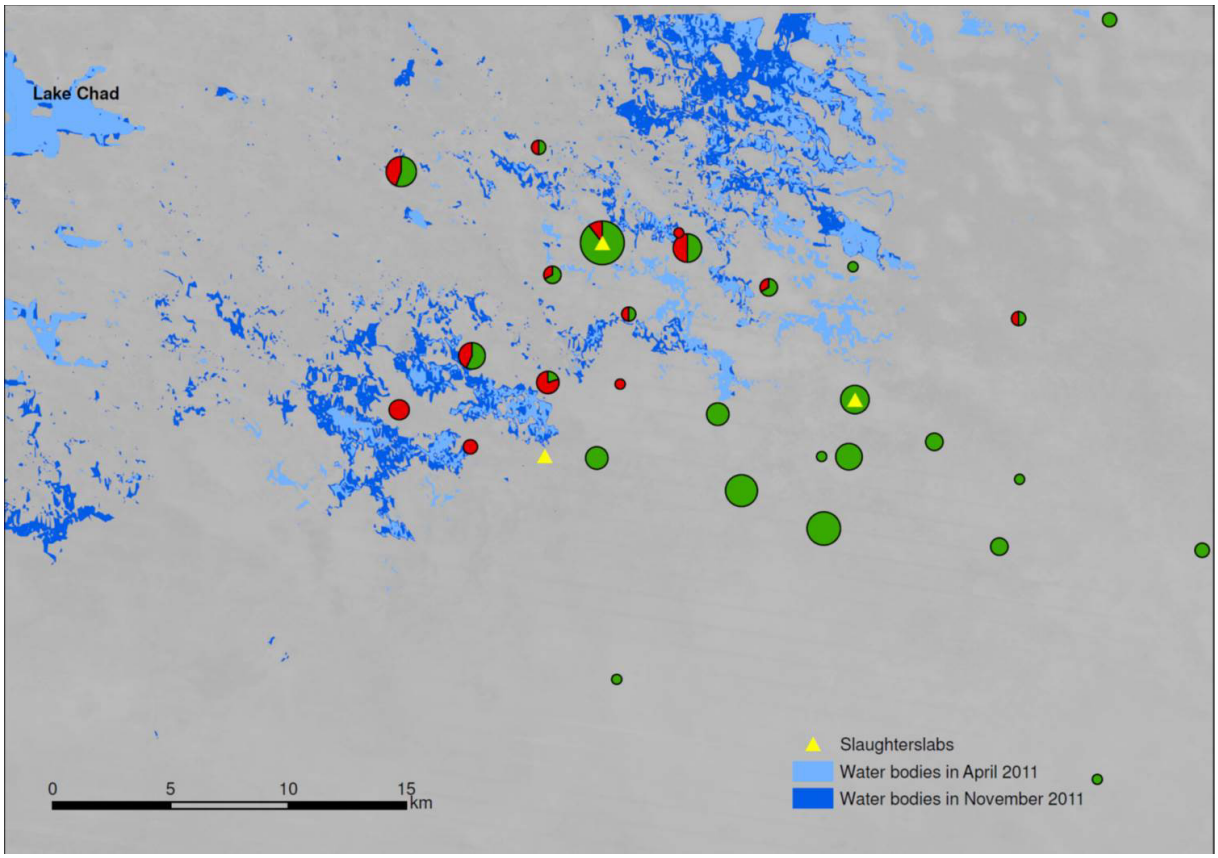

Figure S5.4. Additional file 2: Prevalence of F. gigantica in slaughtered sheep by village of origin. Legend: prevalence rate according to village of origin coordinates, circle size corresponds to the number of animals, red indicates proportion positive for Fasciola gigantica.

Additional file S5.3: Original dataset from questionnaires, −9 always means missing data. Description of columns: ID: ID of record. Date: Date of slaughtering. Abbatoir: ID of slaughterslab 1 = Gredaya, 2 = Sidje, 3 = Bache Djani. Species: Livestock species 1 = cattle 2 = goats 3 = sheep 8 = camels. Origin: Village or well of origin. CoordinatesN: Latitude of the coordinates of the specific village. CoordinatesE: Longitude of the coordinates of the specific village. PastureLake: 1 = the animal has been feeding in close proximity to the lake, 2 = the animal has not been feeding at the lake. Presence_Flukes: 1 = F. gigantica was found in this animal, 2 = F.gigantica was not found in this animal. Infection_Intensity: 0 = no infection; 1 = light infection (1-10 parasites); 2 = medium infection (11-100 parasites); 3 = above 100 parasites (heavy infection). Group_ethn: Ethnic group of livestock owner: 1 = Arab, 2 = Peulh; 3 = Gorane; 4 = Kanembou; 5 = Kouri.

6. Human and animal trematode infections in a mobile pastoralist setting at Lake Chad: added value of a One Health approach beyond zoonotic diseases research

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6.1. Abstract

Background: At Lake Chad in Central Africa mobile pastoralists face economic losses due to livestock trematodiases. *Fasciola gigantica* and *Schistosoma bovis* – trematodes that affect livestock – share transmission ecology traits with *Schistosoma haematobium* and *S. mansoni* that cause human schistosomiasis. This project aimed at assessing treatment strategies and elucidating the predictive potential of human and animal trematode infections.

Methods: Schistosomiasis and fascioliasis were investigated concurrently in humans and cattle by repeated cross-sectional surveys. Urine and stool samples from humans and faecal samples from cattle were examined for trematode eggs. Treatment strategies were assessed by means of focus group discussions and in-depth interviews.

Results: Mobile pastoralists of four ethnic groups participated. Prevalence of human schistosomiasis and livestock trematodiases showed considerable heterogeneity from one ethnic group to another, but correlated within ethnic groups. Effective trematocidal drugs were not available in the study area.

Conclusions: Mutual predictive potential of human schistosomiasis and livestock fascioliasis relates to distinct livestock husbandry practices. Introducing efficacious strategic treatment against human schistosomiasis and livestock fascioliasis might improve human and animal health and well-being. Our research provides evidence for the benefits of a One Health approach targeting diseases that share specific ecological traits.

Keywords

Chad, Disease ecology, Fascioliasis, Mobile pastoralists, One Health, Schistosomiasis

6.2. Introduction

In the frame of a long-standing research partnership built on mutual trust, a multi-stakeholder workshop was held in N'Djamena, Chad in 2011. It brought together pastoralists, representatives of the ministries of health and livestock production, scientists and other constituencies in a trans-disciplinary process. (Montavon, A.*et al.*, 2013). Pastoralists emphasised the importance of fascioliasis as a veterinary health problem affecting livestock productivity and therewith causing economic losses and having a negative impact on their livelihood (Jean-Richard, V.*et al.*, 2014a).

At Lake Chad, livestock fascioliasis is caused by *Fasciola gigantica*, a water-related liver fluke depending on a freshwater snail as intermediate host in its life cycle, similar to the blood flukes of the genus *Schistosoma*. Livestock schistosomiasis is caused by *Schistosoma bovis*, while *S. haematobium* and *S. mansoni* are the causative agents for human schistosomiasis. Infection with

F. gigantica occurs when ruminants are grazing on contaminated pasture in close proximity to open freshwater bodies, whereas *Schistosoma* spp. infect humans and animals by actively penetrating the skin while individuals are in direct contact with contaminated water. Given the shared transmission ecology and similarities in the life cycle of the four trematode species, we concurrently investigated the epidemiology of fascioliasis and schistosomiasis in humans and livestock in a mobile pastoralist setting at Lake Chad. To generate a more holistic picture of the prevailing disease situation in that specific setting, treatment strategies, access to and availability of trematocidal drugs were also assessed.

Throughout the Sahelian belt, mobile pastoralism is the predominant livestock husbandry system providing livelihoods for an estimated 25 million people and producing milk and meat for many more (De Haan, C.*et al.*, 2014). In an arid environment with a limited suitability for agricultural activities, raising livestock by following the annual cycle of pasture growth – known as transhumance – represents a highly adapted and sustainable lifestyle (Krätli, S. and Schareika, N., 2010). The flexibility of the mobility results in a high level of resilience and provides the means to cope with uncertainty and subtle changes in the environment, where rainfall and the consecutive growth of vegetation determine human and livestock movements. The main limiting factor is water and water availability. In that specific ecosystem, human and livestock populations share benefits and risks of water contacts. Indeed, surface water is used for human consumption as well as for watering of livestock, for washing and bathing, and humans and livestock are crossing open water during migration (Jean-Richard, V.*et al.*, 2014c, Krauth, S. J.*et al.*, 2015b).

When looking at the human and animal exposure patterns from an integrated One Health perspective, it is hypothesised that the risk of infection with water-related parasites in humans and animals is comparable. Considering the biological and ecological similarities in the life cycles of *F. gigantica* on one hand, and *S. bovis*, *S. haematobium* and *S. mansoni* on the other hand, this project aimed at testing whether there is a mutual predictive potential of *F. gigantica* infection in livestock and *Schistosoma* infection in humans and *vice versa*. Livestock fascioliasis can be detected in live animals through parasitological methods by performing sedimentation technique and microscopy on faecal samples, although this is rarely practiced and not applied at all in the Lake Chad area. More commonly, the disease is monitored at slaughter; adult *F. gigantica* flukes can reach a body length of up to 7 cm and are therewith easily detectable in contaminated animal livers at meat inspection. In contrast, the diagnosis of human schistosomiasis depends on laboratory diagnosis, which is not available in remote rural settings of the Lake Chad area (Utzinger, J.*et al.*, 2015). If a predictive potential from livestock fascioliasis to human schistosomiasis can be identified, this would present scientific evidence for the use of animal health data (e.g. fascioliasis in cattle) as sentinel for

human schistosomiasis in a setting where no schistosomiasis control programme is in place, as is the current situation at Lake Chad (Scotch, M.*et al.*, 2009).

In this article, we summarise findings from a One Health study on trematode infections in a mobile pastoralist setting at Lake Chad highlighting the potential of a One Health study design beyond direct application to zoonotic diseases, to pathogens sharing similarities in their transmission ecology. We report on the feasibility and efficiency of a joint human and animal research study design for water-based parasitic infection epidemiology. We discuss future perspectives and integrative potential with further innovative approaches for joint human and animal health research for pathogens with similar transmission ecology.

6.3. Methods

Study area

This study was carried out within the semi-arid Sahelian zone on the eastern shores of Lake Chad on Chadian territory (Figure 1).

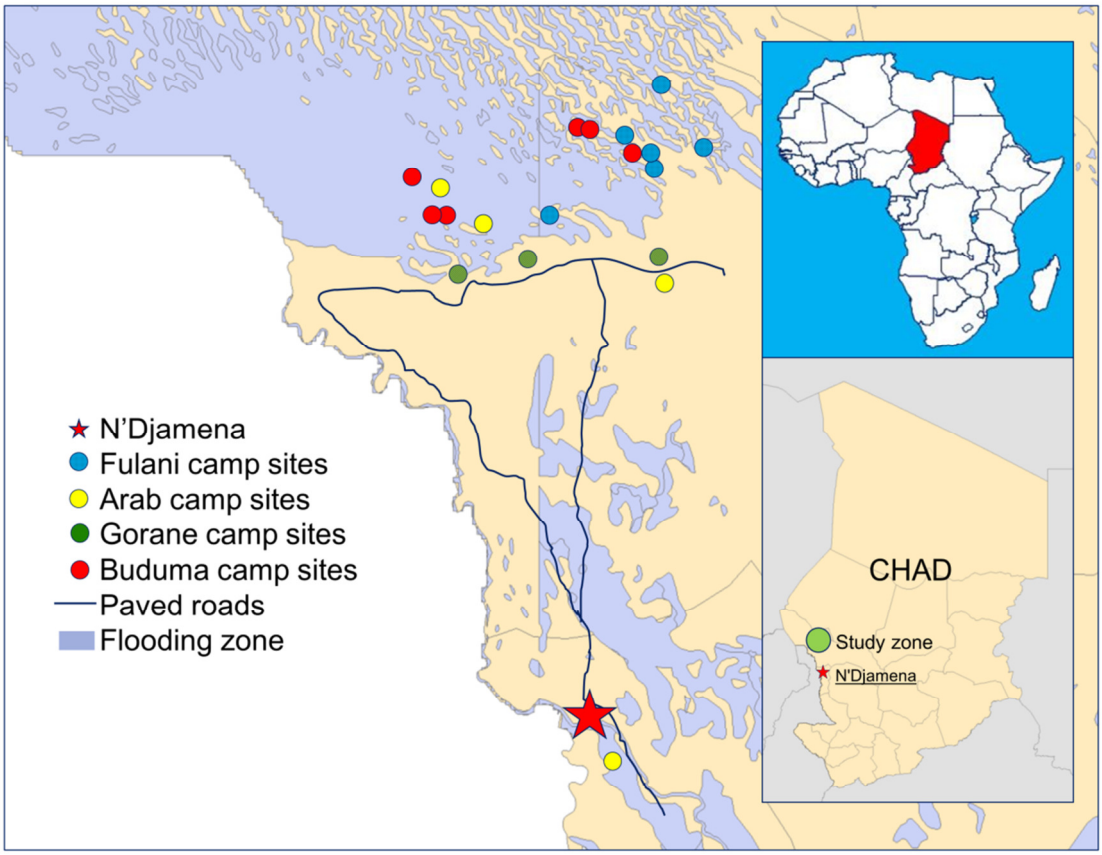

Figure 6.1. Map of the study zone on the eastern shores of Lake Chad, indicating the camp sites of the participating 19 groups of mobile pastoralists. Also displayed is the maximum surface are of Lake Chad, including the flooding zones that can be submerged after the rainy season and dry up during the hot and dry season.

Lake Chad shows seasonally oscillating water levels and is surrounded by a large flooding zone. The seasonal inundations play a crucial role in the evolution of migratory routes of mobile pastoralists. Towards the end of the dry and hot season, pasture remain only on Lake Chad's islands and shores and mobile pastoralists of different ethnic groups together with their flocks gather in the area (Jean-Richard, V.*et al.*, 2015).

Study population

Mobile pastoralists belonging to one of the four predominant ethnic groups in the area were invited to participate. Among the four ethnic groups, the Fulani, Buduma and Gorane are living a fully mobile lifestyle, whilst the Arabs are agro-pastoralists. The four ethnic groups differ in cultural aspects such as language and housing styles but also in transhumance strategy, husbandry system and cattle breed herded (Jean-Richard, V.*et al.*, 2014d). Predominant cattle breeds are the Shewa (Arab Zebu), the Mbororo (Red Fulani) and the Kouri cattle (Quéval, R.*et al.*, 1971, Flury, C.*et al.*, 2009).

Study design

The study consisted of repeated cross-sectional surveys. Data collection took place from September 2013 to December 2014. Due to the lack of demographic data on the mobile pastoralist population in the study area, there was no sampling frame allowing for a sampling proportional to size.

The sample size was determined assuming a prevalence of 5% and an intra-class correlation coefficient of 0.2. Hence, we required 20 clusters with 20 human participants and 20 cattle in each cluster to estimate the prevalence with a precision of the sample estimate (defined as one half-length of the 95% confidence interval (CI)) of 5%-points. The number of clusters and the number of samples per cluster depended also on the prevailing cultural practices and the logistical feasibility in a remote location with poor security standards and threat of incursions of terrorist groups.

Individual participants were randomly selected amongst the group by using the previously published spatial sampling method from the Expanded Programme of Immunization (EPI) of the World Health Organization (WHO). In brief, starting from a central point in the camp site, a direction was randomly selected and followed to the nearest household. From this first household, the second nearest household was selected (Milligan, P.*et al.*, 2004). Within each randomly selected household, two participants were selected by counting out random numbers. This procedure was repeated until 20 participants were enrolled. All individuals aged above 5 years were eligible for participation. Among the cattle herd of the pastoralist group, 20 animals aged 1 year and above were randomly selected by counting out random numbers and were individually ear tagged.

Parasitological survey

Urine filtration, reagent strip testing for microhaematuria, and point-of-care circulating cathodic antigen (POC-CCA) urine cassette test, as well as microscopy of Kato-Katz thick smears from stool

samples were performed in a mobile field laboratory in proximity to the pastoralists' camp sites. Additionally, stool samples were fixed in sodium acetate–acetic acid–formalin (SAF) for transfer and subsequent ether-concentration preparation at the Swiss Tropical and Public Health Institute (Swiss TPH) in Basel. In cattle, a faecal sample was collected from each animal and fixed in SAF for subsequent analysis with the sedimentation technique in laboratories in N'Djamena, Chad and Zurich, Switzerland.

Assessment of treatment availability and access

During questionnaire interviews with individuals infected with Schistosoma, disease perception, treatment strategies, healthseeking behaviour, treatment outcome, and satisfaction were assessed. Livestock fascioliasis treatment strategies and outcome satisfaction were addressed during focus group discussions. Locally available trematocidal drugs were tested for their active ingredient and its quantity using a high pressure liquid chromatography (HPLC)-UV method, combined with tandem mass spectrometry, of which the detailed results are reported elsewhere (Greter, H.*et al.*, 2017).

Statistical analysis

Prevalence estimates were adjusted for correlation within clusters using generalized estimating equations for binary outcomes.

Ethical considerations

The study was approved by the ethics committee in Basel [EKBB; reference no. 64/13]. In Chad, research permission, including ethical approval, was obtained from the 'Direction Générale des Activités Sanitaires' [reference no. 343/MSP/SE/SG/DGAS/2013]. The aim and procedures of the study were explained to each group of mobile pastoralists. Informed consent was signed by group leaders after discussion within the groups. Because of high illiteracy rates, randomly selected individuals within each group provided oral assent.

All participants with a positive diagnostic test from urine filtration, POC-CCA urine cassette test, or Kato–Katz thick smear at the mobile field laboratory received treatment with a single oral dose of praziquantel (40mg/kg) against schistosomiasis and albendazole (400 mg) against soil-transmitted helminthiasis, administered by the teams' medical staff. All selected cattle received a single oral dose of triclabendazole (12mg/kg).

6.4. Results

Parasitological survey

A total of 19 groups of mobile pastoralists belonging to the four ethnic groups of the Fulani, Buduma, Arab and Gorane participated. Of the 415 participants enrolled, 369 were subjected to full urine

analysis (urine filtration, reagent strip testing, and POC-CCA) and 284 for full stool analysis (Kato-Katz thick smear on fresch samples and ether-concentration on SAF-fixed samples). Individuals with complete parasitological data were included in further analyses. Regarding cattle, faecal samples of 534 animals were analysed.

Overall, *S. haematobium* was the most prevalent trematode infection in humans, with a prevalence of 8.1% (95% CI:5.0–12.8%). Stool sample examinations revealed an *S. mansoni* prevalence of 0.4% (95% CI: 0.05–2.4%) with a single participant from the Buduma ethnic group being tested positive, whereas POC-CCA urine cassette tests for *S. mansoni* showed a prevalence of 9.2% (95% CI: 5.1–16.2%). In cattle, *F. gigantica* prevalence was 31.3% (95% CI: 23.3–40.6%) and *S. bovis* prevalence was 20.3% (95% CI: 13.0–30.2%) (Table 6.1).

Table 6.1. Prevalence of human and animal trematode infections, stratified by sex and ethnic group (humans) and by sex and husbandry system (cattle).

Prevalence of human trematode infections								
			Sex		**Ethnic group**			
Urine	**Total**		**Male**	**Female**	**Gorane**	**Arab**	**Buduma**	**Fulani**
N (%)	**369**		**186 (50.5)**	**183 (49.5)**	**61 (16.5)**	**79 (21.4)**	**105 (28.5)**	**124 (33.6)**
Prevalence % (N positive)		**95% CI**						
S. haematobium	8.1(29)	5.0–12.8	8.6(16)	7.1(13)	1.6(1)	8.9(7)	10.4(11)	8.1(10)
Stool	**Total**		**Male**	**Female**	**Gorane**	**Arab**	**Buduma**	**Fulani**
N (%)	**284**		**144 (50.7)**	**140 (49.3)**	**49 (17.2)**	**83 (29.2)**	**59 (20.8)**	**93 (32.8)**
Prevalence % (N positive)		**95% CI**						
S. mansoni	0.4(1)	0.05–2.4	0.7(1)	0	0	0	1.9(1)	0
Prevalence of bovine trematode infections								
			Sex		**Husbandry system**			
	Total		**Male**	**Female**	**Gorane**	**Arab**	**Buduma**	**Fulani**
N (%)	**534**		**81 (15.2)**	**453 (84.8)**	**84 (15.7)**	**126 (23.6)**	**161 (30.2)**	**163 (30.5)**
Prevalence % (N positive)		**95% CI**						
F. gigantica	31.3 (167)	23.3–40.6	28.8 (23)	31.9 (114)	1.1 (1)	24.6 (31)	36.0 (58)	47.3 (77)
S. bovis	20.3 (114)	13.0–30.2	22.5 (18)	21.3 (96)	3.4 (3)	17.5 (22)	29.2 (47)	25.8 (42)

Stratification revealed that trematode prevalence varied significantly between ethnic groups, but showed correlation in humans and cattle within ethnic groups. Whereas in Gorane pastoralists groups there were only single human and cattle schistosomiasis and fascioliasis cases, in Fulani and Buduma pastoralists, both humans and their cattle showed considerable infection rates. Arab

pastoralists and their cattle showed intermediate schistosomiasis and fascioliasis prevalence (Figure 6.2).

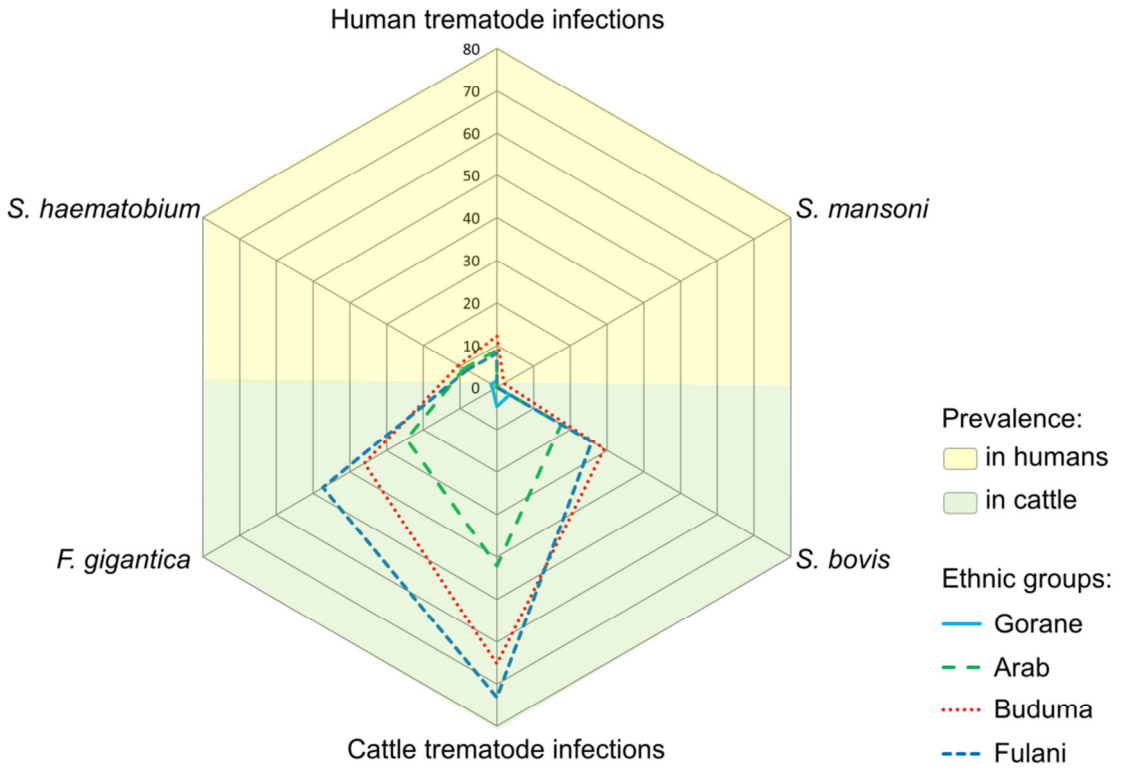

Figure 6.2. Prevalence of human and livestock schistosomiasis and fascioliasis in a mobile pastoralsit setting at Lake Chad, stratified by ethnic group and the respective husbandry system.

Assessment of treatment availability and access

Among the schistosomiasis cases, >60% sought treatment in health centres and from the informal market. Fascioliasis awareness among pastoralists was high and self-mediated therapy using veterinary drugs is the common practice. Mebendazole and albendazole are the drugs locally available, which are used against human parasitic worminfections and for livestock fascioliasis treatment. No praziquantel or triclabendazole—the current treatments of choice against schistosomiasis and fascioliasis, respectively—were among the drug samples obtained on the local markets (Greter, H.*et al.*, 2017).

6.5. Discussion

Our research was embedded in an established partnership and initiated through a trans-disciplinary process, which allowed us to identify health priorities in the population studied and to come forward with specific research questions. Indeed, mobile pastoralists, local authorities, scientists and other

stakeholders engaged in a dialogue that yielded fascioliasis as one of the most important prevailing animal health problems. Knowing about the similarities in the disease ecology and epidemiology of fascioliasis and schistosomiasis, this project investigated the human and livestock diseases concurrently, with the aim to explore potential starting points to develop effective and targeted joint human and animal health interventions.

We found considerable differences in the prevalence of human and cattle schistosomiasis and livestock fascioliasis between the four ethnic groups, whereas distribution patterns of human and animal trematode infections within ethnic groups were similar (Figure 6.2). Traditionally evolved mobility patterns, transhumance practices, and type of livestock husbandry systems differ between the ethnic groups, which might govern distinct exposure risks for acquiring trematode infections for humans and cattle alike. Specifically,access to and use of open freshwater bodies varies between the four ethnic groups, which in turn might explain the prevalence of human and animal trematode infections. For example, the Buduma and Fulani pastoralists grazing their animals inside the flooding zone expose themselves and their livestock to a higher risk of infection with *Schistosoma* spp. and *F. gigantica* than Arab and Gorane pastoralists who remain largely in the drylands surrounding the lake (Figure 6.1). While the Gorane pastoralists avoid grazing and watering animals at open surface water and prefer to use water from traditional wells, the Fulani and Buduma pastoralists use surface water and pastures on the shores and islands of Lake Chad year-round. The Arab agro-pastoralists live in villages where they use traditional wells and pumps, and only approach Lake Chad's shores when the pasture around their villages is fed down towards the end of the dry season. This is also the period when they use surface water for human consumption and watering of livestock (Jean-Richard, V.*et al.*, 2014c).

Interventions for the control of fascioliasis and schistosomiasis should thus focus on Fulani, Buduma, and Arab pastoralists and their cattle. For both human and animal trematode infections, the most effective drugs—praziquantel and triclabendazole—were not available in the study zone (Greter, H.*et al.*, 2017). Introducing efficacious treatments against human schistosomiasis and livestock fascioliasis might not only positively impact human and animal health, but might also result in economic benefits by improving livestock productivity and reducing treatment costs.

The high fascioliasis prevalence in live animals shown here confirms findings of a previous abattoir study (Jean-Richard, V.*et al.*, 2014a). Additionally, *S. bovis* infection in cattle was found at prevalence above 20%. Besides a report from the 1970s, to our knowledge, the current study provides new evidence that bovine schistosomiasis is endemic in the Lake Chad area (Quéval, R.*et al.*, 1971). However, there was limited awareness of the disease among Chadian livestock breeders, veterinarians, and technicians. This might be explained by the fact that clinical manifestations in naturally infected animals are not well documented and are assumed to be subclinical and/or chronic

(Calavas, D. and Martin, P. M., 2014). It therewith receives no veterinary attention and no treatment is administered (Webster, J. P.*et al.*, 2016). This leads to the conclusion that pastoralists erroneously attribute the morbidity caused by bovine schistosomiasis to fascioliasis and the subsequently administered anti fascioliasis treatment fails to improve the animals' condition, which lead to low satisfaction with the available treatment. Given the high prevalence reported here, the epidemiology of livestock schistosomiasis seems largely underappreciated in Chad. Indeed, there is a paucity of prevalence data in the extent literature and most publications solely report on occurrence (Christensen, N. O.*et al.*, 1983, Moné, H.*et al.*, 1999, Stothard, J. R.*et al.*, 2004).

Surprisingly, more data pertaining to molecular biology are available than on the epidemiology of livestock schistosomiasis (Webster, B. L.*et al.*, 2010). In recent years, research on *S. bovis* has gained traction, since molecular investigations revealed hybridization with the human infective species *S. haematobium* (Webster, B. L.et al., 2013). As a prominent example, the 2013 outbreak of schistosomiasis on the French island of Corsica is worth mentioning (Holtfreter, M. C.*et al.*, 2014). As Calvatas and Martin (2014) reminded in a letter, bovine schistosomiasis had been reported from Corsica until the 1960s, which shows that the intermediate host snails are present and the parasite's life cycle could be completed in Corsica.

The findings of our One Health study revealed the spatial and temporal co-occurrence of *S. haematobium, S. mansoni*, and *S. bovis* in the Lake Chad area. Such co-occurrence could potentiall promote hybridization of *S. haematobium* and *S. bovis*, leading to novel phenotypic properties in the hybrid parasites. Research on existing or on-going hybridization is of considerable interest, specifically in light of current efforts to move from morbidity control towards interruption of schistosomiasis, hence aiming at local elimination (Rollinson, D.*et al.*, 2013). A potential associated host switch could change the known epidemiology of human schistosomiasis. Control and elimination programmes would then need to be expanded to include the animal hosts. Targeted interventions can only be developed if the ecology and epidemiology of the disease are well understood (Webster, J. P.*et al.*, 2016). In the specific case of human and animal trematode infections, which additionally have a zoonotic potential, an ecological understanding of the disease is crucial to identifying the level at which transmission interruption could be initiated (Zinsstag, J.*et al.*, 2015b).

For fascioliasis in the social-ecological system of mobile pastoralist husbandry systems, control could, for example, be reached through constant transmission reduction over the long term (Gray, D. J.*et al.*, 2010). Potential leverage points can include decreasing egg contamination of the environment through strategically coordinated temporal treatments of herds. Such efforts should also include behavioural aspects; e.g. adapting water contact patterns and husbandry system could be efficient.

The present study is limited to the report of findings from a mobile pastoralist population encountered in a confined geographical area on the south-eastern Lake Chad area. A larger study would be beneficial to confirm whether or not these findings are generalizable to other parts of the Sahel. Additionally, our study suffers from methodological limitations that are generally associated with studies involving mobile populations. Indeed, designing a proper sampling frame is a formidable challenge among highly dynamic mobile pastoralists and their livestock. Repeating stool and urine sampling, which enhances the diagnostic sensitivity of helminth infections, poses difficulties far away from any laboratory facility (Knopp, S.*et al.*, 2008). Nevertheless, the simultaneous collection and analyses of human and animal health data can reveal linkages that would not be seen in a separate survey design (Rabinowitz, P. M.*et al.*, 2013). The combined statistical analyses of human and animal data are challenging and depend on the scale of analysis (Schelling, E. and Hattendorf, J., 2015). The relationship of human and livestock health can often not be related at household, community and district level but may show a clear relationship, e.g. for human and animal brucellosis sero-prevalence at provincial level (Bonfoh, B.*et al.*, 2012).

These findings highlight the potential of One Health studies for a better understanding of human and animal health in social–ecological systems (Zinsstag, J.*et al.*, 2011). More specifically, the current research contributes to the evaluation of a One Health study design for parasitic diseases. As elements of a systemic understanding of the transmission dynamics of these water-associated parasitic diseases, our results show cultural- and husbandry system-dependent occurrence of schistosomiasis and fascioliasis. The joint interpretation of the results can provide evidence for potential benefits that can be obtained by joint human and animal intervention strategies for human schistosomiasis and livestock fascioliasis aiming at adding value through close collaboration of the human and livestock health sectors (Schelling, E. and Hattendorf, J., 2015, Zinsstag, J.*et al.*, 2015a).

Conclusions

Human schistosomiasis, and bovine fascioliasis and schistosomiasis are endemic in the mobile pastoralist populations and their cattle at Lake Chad, specifically in those entering the flood zone of Lake Chad. When stratified by ethnic groups, the prevalence shows similar patterns for human and cattle trematode infections. This illustrates the strong linkage between the traditionally evolved husbandry system and human and animal exposure to trematode infection risks. For both human and animal trematode infections, the most efficacious drugs—praziquantel and triclabendazole—were not available in the study zone. It is conceivable that introducing efficacious treatment against human schistosomiasis and livestock fascioliasis will not only have a positive impact on human and animal health, but also result in economic benefits by improving livestock productivity and reducing

treatment costs. Additionally, our study provides an example for the expanded application of a One Health study design beyond zoonotic disease, namely on the research on pathogens sharing distinct characteristics in their disease ecology.

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7. Treatment of human and livestock helminth infections in a mobile pastoralist setting at Lake Chad: Attitudes to health and analysis of active pharmaceutical ingredients of locally available anthelminthic drugs

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7.1. Abstract

Mobile pastoralists face challenges in accessing quality health care and medication for managing human and animal diseases. We determined livestock disease priorities, health seeking behaviour of people bearing helminthiases and − placing particular emphasis on trematode infections − treatment strategies and outcome satisfaction among mobile pastoralists of four ethnic groups in the Lake Chad area using focus group discussions. People suffering from schistosomiasis were interviewed about symptoms, health seeking behaviour and their satisfaction with respect to the provided treatment. Anthelminthic drugs for human and veterinary use obtained from various health care structures were analysed for active pharmaceutical ingredients (API) and quantity, using high pressure liquid chromatography-UV and liquid chromatography combined with tandem mass spectrometry. Most people suffering from schistosomiasis sought treatment at health care centres. Yet, they also consulted informal providers without medical training. Regarding animal health, self-mediated therapy was common to manage suspected livestock fascioliasis. Self-reported treatment satisfaction for human schistosomiasis and trematodiasis treatment outcome in livestock were low. Mobile pastoralists perceived the purchased drugs to be of low quality. Among 33 products locally sold as anthelminthic drugs for human or veterinary use, 27 contained albendazole or mebendazole, varying between 91% and 159% of the labelled amount. Six products were sold loosely with incomplete information and their API could not be identified. No counterfeit anthelminthic drugs were detected. None of the samples contained praziquantel or triclabendazole, the drugs of choice against schistosomiasis and fascioliasis, respectively. The perceived unsatisfactory treatment outcomes in humans and animals infected with trematodes are most likely due to empiric diagnosis and the resulting use of inadequate therapy for human schistosomiasis and the lack of efficacious drugs against livestock fascioliasis.

Keywords: Anthelminthic drugs, Chad, Fascioliasis, Focus group discussion, Mobile pastoralists, Schistosomiasis

7.2. Introduction

Parasitic worm infections cause a considerable burden in humans and livestock, particularly in tropical and sub-tropical regions of the world (Hotez, P. J.*et al.*, 2014, Karagiannis-Voules, D. A.*et al.*, 2015, Torgerson, P. R.*et al.*, 2015). In humans, soil-transmitted helminths (*Ascaris lumbricoides*, hookworms and *Trichuris trichiura*) and *Schistosoma* spp. infections are widespread, mainly in settings where open defecation is common (Grimes, J. E.*et al.*, 2014, Strunz, E. C.*et al.*, 2014). Various intestinal helminths (e.g. *Haemonchus* spp.*, Trichostrongylus* spp.*, Ancylostoma* spp.*, Trichuris* spp.*,*

and *Strongyloides* spp.) parasitize ruminant livestock (Zinsstag, J.*et al.*, 1998). Of zoonotic importance are *Echinococcus* spp.*, Taenia* spp. and the trematode species *Fasciola hepatica* and *F. gigantica,* which may accidentally infect humans (Garcia, H. H.*et al.*, 2007, Fürst, T.*et al.*, 2012). Livestock might also suffer from infection with the blood fluke *Schistosoma bovis* (Moné, H.*et al.*, 1999).

Low-intensity infections may remain undiagnosed, explained by the absence or unspecific symptoms, coupled with insensitive diagnostic methods (Bergquist, R.*et al.*, 2009, Becker, S. L.*et al.*, 2015a). Yet, chronic intestinal helminth infection can cause considerable morbidity (Hotez, P. J.*et al.*, 2014). Parasitic infections not only compromise health, but also cause economic losses due to reduced productivity in livestock, weight loss and higher rates of abortion (Charlier, J.*et al.*, 2014). Hence, helminthiases have a double negative impact on populations which depend on livestock, such as settled livestock breeders and mobile pastoralists (Bechir, M.*et al.*, 2011b, Charlier, J.*et al.*, 2014).

Safe and efficacious drugs are available for most intestinal helminth infections (Panic, G.*et al.*, 2014). Regarding human health, the global strategy for the control of major helminthiases is built around preventive chemotherapy, which is the periodic deworming of at-risk populations coordinated by the World Health Organization (WHO). For example, 600 million tablets of albendazole and mebendazole are donated yearly to deworm school-aged children. Schistosomiasis is on the WHO agenda for elimination as a public health problem by 2025 (WHO, 2013), and praziquantel is the drug of choice (WHO, 2002, Doenhoff, M. J.*et al.*, 2008). The aforementioned drugs are also used in veterinary medicine (Mehlhorn, H., 2008). For fascioliasis in livestock and for humans, triclabendazole is the recommended treatment (Keiser, J.*et al.*, 2005, Villegas, F.*et al.*, 2012).

In grassland ecosystems such as the Sahelian belt in Africa, mobile pastoralism is a highly adapted lifestyle that largely depends on livestock and allows maintaining human populations on only marginally productive land. Mobility is key, driven by the constant search for water and pasture (Krätli, S. and Schareika, N., 2010). Camels, cattle, goats and sheep provide milk and meat for consumption and trade and are often referred to as “a bank on four legs” by the pastoralists. Hence, animal health is crucial, since the health of the people is directly dependent on healthy and productive animals (Bechir, M.*et al.*, 2012c). However, to date, access to health care is often limited for rural populations in low- and middle-income countries, particularly for marginalized and mobile populations (Sheik-Mohamed, A. and Velema, J. P., 1999, Wiese, M.*et al.*, 2004a, Zinsstag, J.*et al.*, 2006, Obrist, B.*et al.*, 2007, Sy, I.*et al.*, 2010). In resource-constrained settings, health systems often lack adequate governmental funding and sufficient qualified personnel. Consequently, health posts in rural areas are sparse and a single health post may represent the only official health provider for more than 10’000 people. Additionally, health centers in rural areas are characterized by a lack of basic infrastructure, clean water and sufficient space for inpatients. Shortages in life saving

medications are common. With regard to veterinary health, the situation is comparable, and governmental veterinary services infrequently cover vaccination services (Schelling, E.*et al.*, 2007a). In Chad, the private sector stepped in to fill the gap and developed business-oriented health services. An informal market for drugs has evolved and medical products are available in city and village markets and from street vendors (Videau, J. Y., 2006), as shown in Fig. 7.1.

Figure 7.1. Drugs for human and veterinary use on display in a village market in the Lake Chad area in May 2014 (photo: Helena Greter).

However, sales persons are untrained, thus a purchase from these sources is not accompanied by any kind of guidance on the treatment. The origin and quality of the sold drugs are often unknown since these products might be imported into Chad via unofficial routes, presumably without maintaining quality guidelines of the pharmaceuticals, such as storage temperature. This practice has established in the 1990s in Chad and – despite its non-authorized status – continues to build inroads, since purchase from these sources is relatively straightforward and inexpensive (Djimouko, S. and Mbairo, P., 2014). In the Lake Chad area, *doctor choukou*, (i.e. unqualified sales persons who offer drugs for human and veterinary use and services such as infusions and injections), travel from camp to camp to serve the needs of the mobile pastoralists. Since *doctor choukou* lack medical training, the drugs and treatments provided by them might cause harm in humans and animals (Wiese, M., 2000, Hampshire, K., 2002b, Schelling, E., 2002, Gauthier, B. and Wane, W., 2011, Djimouko, S. and Mbairo, P., 2014).

The aim of the current study was to elucidate mobilepastoralists' perception of schistosomiasis, livestock disease priorities and access to, and common practice of, treatment of human and animal helminth infections on the south-eastern shores of Lake Chad. Additionally, medications locally sold as anthelminthic drugs for human or veterinary use were purchased and tested for the presence and quantity of active pharmaceutical ingredients (API) as a measure for anthelminthic drug quality.

7.3. Materials and methods

Ethical considerations

The study was approved by the ethics committee of Basel, Switzerland (reference no. EKBB 64/13) and the 'Direction Générale des Activités Sanitaires' in N'Djamena, Chad (reference no. 343/MSP/SE/SG/DGAS/2013). At the first contact with each group of mobile pastoralists, the objectives and procedures of the study were explained. The group leader, together with other local authorities, had time to discuss and decide about the groups' participation. Once a collective decision had been taken, written informed consent was obtained from the group leader. Due to high illiteracy rates, individual participants consented orally. These consent procedures were approved by the respective ethics committees.

Study site and study population

The study was carried out between September 2013 and May 2014 and focused on 19 individual groups of mobile pastoralists, located in the south-eastern shores of Lake Chad. Participating pastoralists were of four different ethnic groups; Gorane, Arab, Fulani and Buduma. Due to the longstanding research partnership with pastoralist community in the study area (Montavon, A.*et al.*, 2013), the sampling of the groups was partly convenience (10 groups) and partly random (nine groups), by contacts on transects.

Qualitative data collection

Within each of the 19 participating groups, 20 individuals aged 5 years and above were randomly selected for a parasitological survey, examining urine and stool samples. All human participants diagnosed with helminths received treatment administered by a study nurse, following national guidelines. Individuals identified with a *Schistosoma* infection were interviewed using a short pre-tested questionnaire addressing standard questions on disease perception and duration, health seeking behavior, and treatment outcomes (Lengeler, C.*et al.*, 2002b, 2002a). For children below the age of 10 years, parents or legal guardians were interviewed. Additionally, seven individuals with symptoms that might be due to schistosomiasis who had contacted the study nurse were identified as positive for *Schistosoma* infection and participated in the questionnaire interview.

Focus group discussions (FGDs) were conducted to address animal health topics. People responsible for livestock were eligible for participation, depending on their availability at the time of the visit. These FGDs comprised three to six adult individuals and opics addressed included animal health priorities and health seeking behavior. In groups reporting fascioliasis as a priority, treatment strategies and outcome satisfaction were discussed. Languages used for the questionnaire interviews and the FGDs were Chadian Arabic, Kanembou and Fulbe. An interpreter translated questions and answers into French for the investigator. The content of these translations were validated with a second independent interpreter.

Purchase of locally available anthelminthic drugs

Between September 2014 and March 2015, two Chadian collaborators purchased locally available anthelmintic drugs from various outlets, namely village markets, health centres and informal drug sellers (*doctor choukou*) in the study area, as well as in human and veterinary pharmacies and on markets in N'Djamena. Collaborators asked for drugs to treat human helminthiasis and animal fascioliasis, using the local language terms (Table 3). Medications were purchased from village markets, health centers, and informal drug sellers (*doctor choukou*) in the study area, as well as in human and veterinary pharmacies, and on markets in N'Djamena. Collaborators asked for drugs to treat human helminthiasis and animal fascioliasis, using local language terms.

API content analysis of anthelminthic drugs

Reagents and apparatus

Albendazole, mebendazole, thiabendazole, flubendazole (Sigma-Aldrich; Buchs, Switzerland), and triclabendazole (Carbogen Amics; Bubendorf, Switzerland) were of analytical standard. Methanol, acetonitrile, and 25% hydrochloric acid were of high pressure liquid chromatography (HPLC)-grade. An Agilent Series 1100 HPLC-UV/vis system with computer control (ChemStation) was used for the chromatographic analysis of albendazole, mebendazole, thiabendazole and flubendazole. For the tandem mass spectrometric measurements of triclabendazole and praziquantel, an API 3000 triple quadrupole mass spectrometer (MS) (AB Sciex; Framingham, MA, USA) with a turbo inspray interface was used.

Standard solutions and calibration line

The preparation of the drug solution was adapted from Kulik et al. (2011). Briefly, albendazole and mebendazole were dissolved in 0.25% hydrochloric acid in methanol to a concentration of 0.5 mg/ml using 50 ml-volumetric flasks. The solutions were ultrasonicated for 5 min. Thiabendazole, flubendazole, triclabendazole, and praziquantel were dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Buchs, Switzerland) to 10 mg/ml stock solutions. For the calibration line, drug solutions were

diluted in ammonium formate buffer (25 mM, pH 4.0) in a 2-fold serial dilution with concentrations between 0.31 and 10 µg/ml.

Sample solutions and quantification of active ingredient

Five tablets, or less if five were not available, were weighed and pulverized. The tablet powder was dissolved in 0.25% hydrochloric acid in methanol, corresponding to 0.25 mg/ml albendazole or mebendazole using 50 ml-volumetric flasks. The solutions were ultrasonicated for 10 min and filtered through a mesh of 0.45 µm. The samples were diluted in ammonium formate buffer (25 mM, pH 4.0) to a concentration corresponding to 5 µg/ml and analyzed. The calculated amount of active ingredient of each sample was compared with the nominal amount. We applied an acceptance range of 90-110% of active ingredient in the tablets, which was adapted from WHO guidelines (WHO, 2011).

Analytical parameters

For chromatographic measurements, a reversed-phase XB-C18 column (150 x 4.6 mm; 2.6 µm; Phenomenex; Torrance, CA, USA) was used as solid phase. Mobile phase A consisted of ammonium formate buffer (25 mM, pH 4.0) and mobile phase B of acetonitrile. A gradient from 10% to 100% B and back to 10% B, over 6 min at a flow rate of 1 ml/min was used for drug elution. An injection volume of 50 µl was chosen.

Liquid chromatography combined with tandem MS (LC-MS/MS) measurements for praziquantel was performed according to published protocols (Meister, I.*et al.*, 2016). The mass spectrometric parameters of triclabendazole were optimized by direct infusion of 1µg/ml drug in methanol into MS/MS.

7.4. Results

Human schistosomiasis: perception, health seeking behavior and treatment

The total number of randomly selected participants was 401. Out of this number, 63 were diagnosed with a *Schistosoma* infection and 50 of them were interviewed. Additionally, seven non-randomly selected individuals sought care from the study nurse after suspecting that they had schistosomiasis. They tested positive and also took part in the questionnaire interview.

An infection with *S. haematobium* was diagnosed in 35 individuals, whereas the remaining 22 individuals were infected with *S. mansoni*. No co-infection was observed.

Table 7.1. Self-reported symptoms of 57 schistosomiasis patients; overall, and stratified by infection with *S. haematobium* and *S. mansoni*.

Symptom	All schistosomiasis patients, n (%)	Infection with *S. haematobium,* n (%)	Infection with *S. mansoni,* n (%)
Abdominal pain	45 (79)	27 (77)	18 (82)
Pain when urinating	29 (51)	18 (51)	11 (51)
Headache	20 (35)	10 (29)	10 (45)
Blood in urine	15 (26)	11 (31)	4 (18)
Joint pain	11 (19)	5 (14)	6 (27)
Chest pain	8 (14)	4 (11)	4 (18)
Back pain	8 (14)	5 (14)	3 (14)
Colored urine	6 (10)	4 (11)	2 (9)
Itching	5 (9)	3 (9)	2 (9)
Renal pain	4 (7)	1 (3)	3 (14)
Constipation	3 (5)	2 (6)	1 (5)
Weight loss	1 (2)	1 (3)	0
Asymptomatic	5 (9)	3 (9)	2 (9)

Abdominal pain (79%) was the predominant symptom, regardless of whether the causative agent was *S. haematobium* or *S. mansoni*. Additionally, patients identified with *S. haematobium* reported pain when urinating (51%) and blood in urine (31%). Individuals infected with *S. mansoni* also reported pain when urinating (51%), headache (45%) and joint pain (27%) (Table 7.1). None of the patients reported blood in stool, diarrhea or bloody diarrhea. Five individuals did not report any kind of symptoms; hence the data reported here refer to spontaneous responses.

Among the 52 schistosomiasis patients who reported one or several symptoms, 22 (42%) did not seek treatment. Health centers were visited by 17 individuals (32%), with twice as many male patients (n=11 (65%)). *Doctor choukou* were consulted by 14 of the patients (26%) with equal numbers of male and seven female patients. Traditional treatment procedures or self-medication was rarely undertaken and exclusively reported from male participants (Figure 7.2).

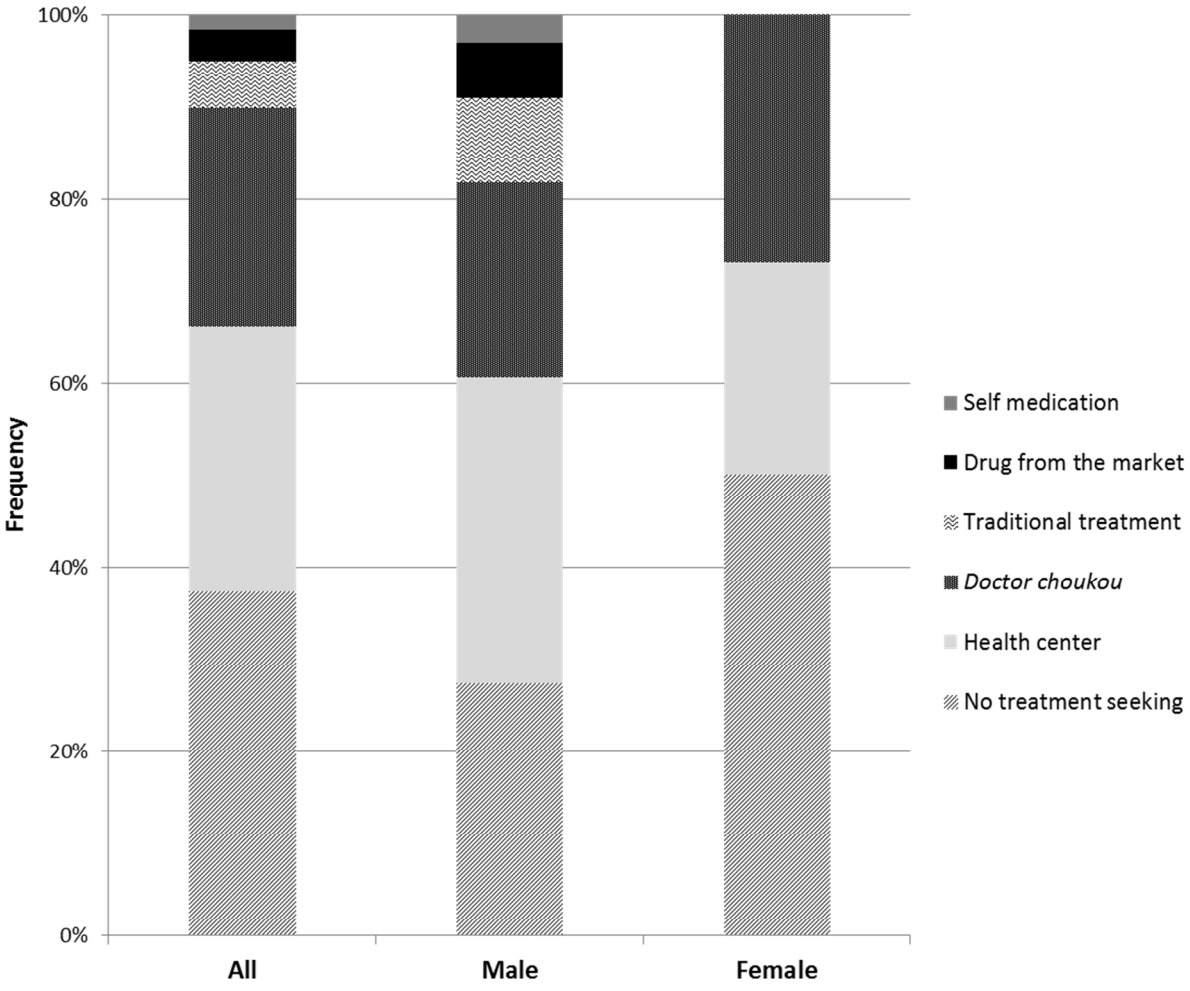

Figure 7.2. Self-reported health seeking behavior from 52 schistosomiasis patients from the south-eastern Lake Chad area, interviewed between September 2013 and May 2014 (stratified by sex).

Medication received at health centers or from *doctor choukou* was reported as pills or injection (Table 3). A product name or API of a drug was not known by any patient. If asked about the perceived treatment outcome, a slight improvement for a short 2- to 3-days was reported, before the symptoms appeared again.

FGDs on animal health: priorities, access to treatment and outcome satisfaction

A total of 17 FGDs were conducted (Table 7.2). Among the livestock diseases that were discussed as priorities, fascioliasis was mentioned as highly important by all participating groups of Arab, Fulani and Buduma pastoralists (n=14). For Gorane pastoralists (n=3), urinary infection in livestock was of highest importance. Other diseases put forth as priorities were anthrax, foot and mouth disease, and respiratory infections. A summary of these disease names in all languages used during the FGDs is presented in Table 3. Also the presence of the tsetse fly was reported as a livestock health concern.

Table 7.2. Findings from 17 FGDs on livestock health priorities, treatment seeking behavior and drug sources, fascioliasis treatment and satisfaction with treatment outcome, stratified by ethnic groups.

	Ethnic group **Gorane**	**Arab**	**Fulani**	**Buduma and Kouri**
Priority livestock diseases	Urinary tract disease (piroplasmosis) Anthrax Foot and mouth disease Diarrhea Lung disease (PPCB)	Fascioliasis Urinary infection (piroplasmosis) Foot and mouth disease Tsetse fly Lung disease (PPCB)	Fascioliasis Anthrax Urinary infection (piroplasmosis) Foot and mouth disease Lung disease (PPCB)	Fascioliasis Lung disease (PPCB) Foot and mouth disease Tsetse fly Anthrax
Treatment source	Market *Doctor choukou*	Veterinary pharmacy in N'Djamena Market *Doctor choukou*	Market Veterinary pharmacy in N'Djamena *Doctor choukou*	Market
Drugs used to treat fascioliasis	n.a.	Benzal Colors of tablets* Disto Vermita	Benzal Disto Ivomec Vermita Vermitol	Colors of tablets* Disto
Satisfaction with treatment outcome	n.a.	Low (for market drugs)	Low (for market drugs) Arbitrary Better (for drugs from vet. pharm. N'Djamena)	Arbitrary

(*: participants referred to the drugs used by reporting the colors of the bolus (white, green, and red); n.a.: Gorane pastoralists did not prioritize fascioliasis; hence, these questions were not addressed with these groups).

Mobile pastoralists obtained veterinary drugs for use against fascioliasis from markets, veterinary pharmacies in N'Djamena and *doctor choukou*. Those who emphasized fascioliasis as a priority consistently named at least one drug product. Satisfaction with fascioliasis treatment outcome was reported as low, particularly when drugs purchased from the market were administered. Others stated that treatment efficacy was arbitrary: a drug might have a positive effect on animal health, while subsequent treatments with the same drug showed no effect. It was also stated that drugs from veterinary pharmacies in N'Djamena might lead to a better health outcome (Table 7.2). However, because travel to the city is time consuming and expensive, and prices at veterinary pharmacies are higher, the pastoralists continue to use drugs from the market or from *doctor choukou*. Besides these reports, mobile pastoralists were concerned about unsatisfactory treatment outcomes for suspected livestock fascioliasis. Several FGD participants reported to feel the unsatisfactory treatment outcome could be due to the low quality of drugs. One group leader brought this concern to the point by stating: «*We simply have to believe what the drug sellers tell us*

and use the products they sell us. But we think we get betrayed with bad quality drugs, or even falsified drugs, due to our illiteracy.»

Table 7.3. Summary of terms used to describe medical treatments and human and animal disease in all languages used during questionnaire interviews and FGDs, and in English.

English / medical term	French	Chadian Arabic	Kanembou	Fulbe
Intestinal helminths	*Vers*	*Hanish* / Dut*[+]	*Guli liu*	*Djildii*
Fascioliasis	*Douve*	*Dut djibte*	*Guli matum*	*Babare*
Medication (Pill)	*Comprimé*	*Quinin*	*Quinitum*	*Quinin*
Injection	*Injection*	*Ibre*	*Lira*	*Baatel*
Bovine piroplasmosis	*Piroplasmose bovine*	*Ouadja bol*	-	-
Anthrax	*Maladie du charbon*	*Abou-tchabaga*	*Bantu ndjulum*	*Da-tewa*
Blackleg disease	*Charbon symptomatique*	*Bièdre*	-	-
Foot and mouth disease	*Fièvre aphteuse*	*Ablissem*	*Nglam*	*Mboru*
Contagious bovine pleuropneumonia (CBPP)	*Pleuropneumonie contagieuse bovine (PPCB)*	*Fashfash*	*Gurufu*	*Hendu*

*refers to intestinal parasites in humans; [+]refers to intestinal parasites in animals

Anthelminthic Drug Analysis

In total, 33 different products sold as anthelminthic drugs were purchased from different sources (in N'djamena: 8 from markets, 1 from a licensed pharmacy, 4 from veterinary pharmacies; At Lake Chad: 11 from markets, 3 from health centers, 6 from *doctor choukou*). At all outlets, drugs were stored at local ambient temperatures (18-48°C). Upon arrival at the analytical facilities in Switzerland, the products were stored at room temperature (22°C). No drugs obtained in the original packaging had passed the expiry date when purchased. For the benzimidazoles albendazole, mebendazole, flubendazole, thiabendazole and triclabendazole, we determined the quantity of drug in each sample, and compared it with the nominal amount (Figures 7.3 and 7.4). The calibration line was linear with a goodness of fit (R^2) of >0.995.

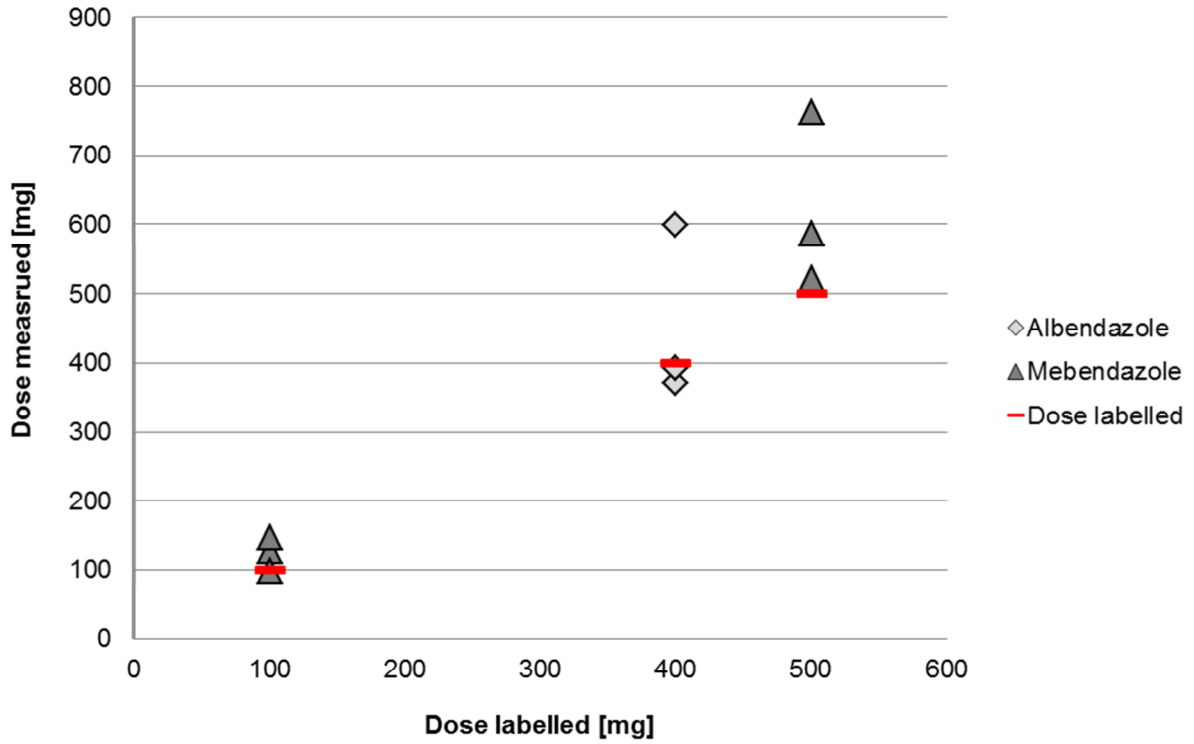

Figure 7.3. Amount of albendazole and mebendazole in the analyzed tablets for human use.

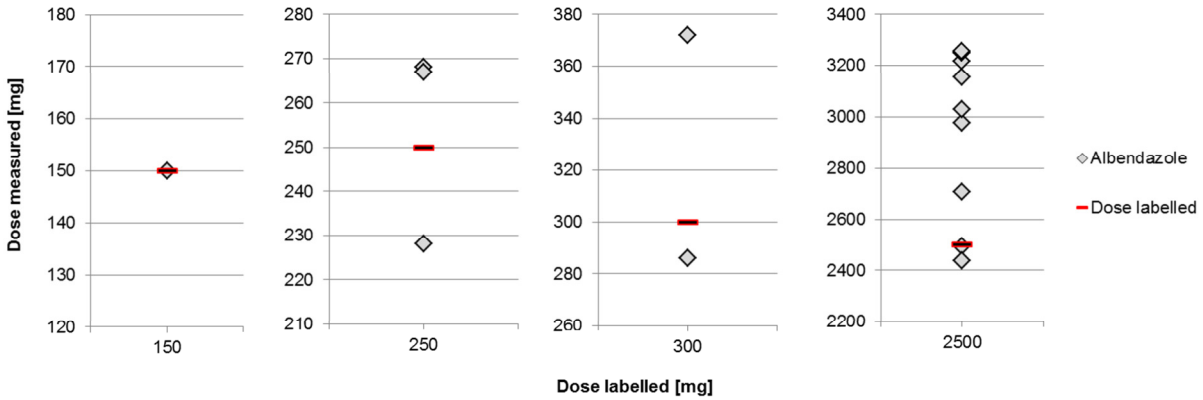

Figure 7.4. Amount of albendazole in the veterinary use tablets analyzed.

Among all products, nine were designated for human use (Table 7.4) and 22 for veterinary use (Tables7. 4, 7.5 and 7.6). For two samples sold without the original packaging, the target species could not be clarified; however, one of them contained mebendazole at 159% of the labeled amount, while the other contained albendazole (107mg) but had no amount labeled (Table 7.5). All tablets for human use contained albendazole (n=3) or mebendazole (n=6), with the amounts of active ingredient ranging from 91% to 152% of the labeled amount (Table 7.4). Among the 22 veterinary use samples, 15 contained albendazole at levels of 91 - 130% of the labeled amount (Table 7.5). For one product with no dosage labeled, an amount of 173 mg of albendazole was calculated (Table 7.6). Five

products were sold loose with a hand written notation of the product name. Tests performed revealed that they did not contain albendazole, mebendazole, flubendazole, thiabendazole, triclabendazole or praziquantel. Among these, the two products sold under the name *Disto* were reported to contain bithionol sulfoxide, but we did not perform analysis for this substance. Another veterinary use product sold as an anthelminthic was reported as an antibacterial tetracycline (Table 7.7).

Table 7.4. Drugs sold as anthelmintic treatment in humans, purchased in Chad between September 2014 and March 2015.

Sample (name as labeled)	Country of origin (as labeled)	Drug labeled	Drug detected	Nominal drug amount [mg]	Percentage of labeled amount [%]
Zentel 400 mg	South Africa	ABZ	ABZ	400	150
Zenlee 400 mg	India	ABZ	ABZ	400	93
INERSA Albendazole 400 mg	France	ABZ	ABZ	400	98
Vermox 500 mg	Belgium	MBZ	MBZ	500	152
Wormex 500 mg	India	MBZ	MBZ	500	104
Mebendazole 500 mg	n.l.	MBZ	MBZ	500	117
Mebendazole 100 mg	Denmark	MBZ	MBZ	100	98
Diameb 100 mg	India	MBZ	MBZ	100	147
Lokel`s Mebendazole 100 mg	India	MBZ	MBZ	100	127

ABZ; albendazole; MBZ: mebendazole; n.l.: not labeled

Table 7.5. Veterinary use drugs sold as fascioliasis treatment, purchased in Chad between September 2014 and March 2015

Sample (name as labeled)	Country of origin (as labeled)	Drug labeled	Drug detected	Nominal drug amount [mg]	Percentage of labeled amount [%]
Benzal 2500 mg	France	ABZ	ABZ	2500	129
Alben 2500 (white)	n.l.	ABZ	ABZ	2500	130
Alben 2500 (green)	n.l.	ABZ	ABZ	2500	100
Albendazole 2500 mg	n.l.	ABZ	ABZ	2500	126
LOBS Alben 2500	France	ABZ	ABZ	2500	108
Ceva Vermitan 2500 mg	Romania	ABZ	ABZ	2500	119
Benzal 2500 mg	France	ABZ	ABZ	2500	130
Albendazole 2500 mg	n.l.	ABZ	ABZ	2500	121
Benzal 2500 mg	France	ABZ	ABZ	2500	98
Benzal 300 mg	France	ABZ	ABZ	300	124
LOBS Alben 300	France	ABZ	ABZ	300	95
Albaneet 250 mg	China	ABZ	ABZ	250	107
NB 250 mg	n.l.	ABZ	ABZ	250	91
Albenor 250 mg	Nigeria	ABZ	ABZ	250	107
Albenor 150 mg	Nigeria	ABZ	ABZ	150	109

ABZ; albendazole; MBZ: mebendazole; n.l.: not labeled

Table 7.6. Mebendazole or albendazole containing drugs with incompleteinformation, purchased in Chad between September 2014 and March 2015

Sample (name as labeled)	Country of origin (as labeled)	Drug labeled	Drug detected	Nominal drug amount [mg]	Detected amount [mg]	Percentage of labeled amount [%]
Mebendazole 500 mg[x]	n.l.	MBZ	MBZ	500	794	159
not labeled[x]	n.l.	n.l.	ABZ	n.l.	107	na
Vermita[v]	n.l.	n.l.	ABZ	n.l.	173	na

[v] Veterinary drug; [x] Target species not known

ABZ; albendazole; MBZ: mebendazole; n.l.: not labeled; na: not applicable

Table 7.7. Veterinary use drugs for the treatment of fascioliasis with incomplete information. The active ingredient could not be determined. Drugs were purchased in Chad between September 2014 and March 2015.

Sample (name as reported)	Drug labeled	Drug information from veterinary pharmacists	Tested for ABZ, MBZ, FBZ, TBZ, TCZ, PZQ	ABZ, MBZ, FBZ, TBZ, TCZ, PZQ detected
Disto	n.l.	Bithionol sufloxide	Yes	No
Disto	n.l.	Bithionol sulfoxide	Yes	No
Balmizole 3	n.l.	No	Yes	No
Vermizol	n.l.	No	Yes	No
Volimizol	n.l.	No	Yes	No
PSE Terravet	n.l.	Oxytetracycline HCl	Yes	No

ABZ: albendazole; MBZ: mebendazole; FBZ: flubendazole; TBZ: thiabendazole;
TCZ: triclabendazole; PZQ: Praziquantel; n.l.: not labeled

7.5. Discussion

Among mobile pastoralists living in the Lake Chad, we assessed access to, common practice of, and drugs used for the treatment of human and animal trematode infections in a setting with poorly developed human and veterinary health systems.

Awareness of livestock fascioliasis was high, confirming findings from earlier studies at Lake Chad (Jean-Richard, V.*et al.*, 2014a), which indicates the significance of the disease. Although the disease is not diagnosed by a veterinarian, earlier studies have shown that experience based diagnosis and traditional knowledge of symptoms of fascioliasis is quite accurate in Fulani pastoralists in the area (Schillhorn van Veen, T. W., 1997). Indeed, mobile pastoralists frequently undertake fascioliasis treatment with locally available products, showing that the willingness to pay for treatment is generally high. In contrast to other livestock diseases, no traditional treatment is practiced for fascioliasis. Self-mediated therapy with drugs obtained from markets, *doctor choukou*, and veterinary pharmacies is the common practice to treat livestock with suspected fascioliasis. Most of these drugs tested contained albendazole. Albendazole is still used as a flukicide in livestock in resource-constrained settings, since it is cheap and safe. Its efficacy is, however, limited to adult *Fasciola*

flukes and therewith generally low (Buchanan, J. F.*et al.*, 2003). Two samples were reported to contain bithionol sulfoxide, an old treatment rarely used today as a flukicide (Mehlhorn, H., 2008). At present, the most efficacious drug available to treat fascioliasis is triclabendazole, which has a high margin of safety and, additionally, kills young developing stages of *Fasciola* (Keiser, J.*et al.*, 2005). Triclabendazole was not detected among the samples obtained, neither at Lake Chad nor in N'Djamena. To our knowledge, triclabendazole is not yet licensed in Chad (pers. comm., Idriss O. Alfaroukh, 2014). Additionally, about half of the drugs tested were of substandard quality due to over concentration of the active ingredient, exceeding the labeled amount of albendazole by more than the accepted threshold of 110% (Caudron, J. M.*et al.*, 2008). Of notice, here, we present a snap-shot of anthelminthic drug research from the Lake Chad area, and a larger study is required to confirm our results. Also, it is worth stating that in the present study, we did not examine the physicochemical quality of the drugs such as the dissolution profile. We exclusively focused on the type and amount of active compound as quality proxies. However, a recent comprehensive study from Ethiopia investigated the quality of anthelminthic drugs, including all pharmaceutical parameters and found about 8% of the tested samples of albendazole and over 40% of mebendazole to be overdosed (Suleman, S.*et al.*, 2014).

Among the human schistosomiasis cases, about 60% of patients reported seeking treatment, in equal parts at governmental health centers and with *doctor choukou*. Male patients had better access to health centers than their female counterparts. This finding reflects the well-known custom that among the ethnic groups of mobile pastoralists in the study area, women can only attend a health center with the permission of their father, husband or brother (Hampshire, K., 2002b). Regarding human schistosomiasis and human helminth infections in general, knowledge remained non-specific, and no distinction could be made between different parasitic diseases. None of the patients was aware of a drug name or drug ingredient to treat human worm diseases. All drugs purchased and tested in our study to treat human helminthiases contained exclusively albendazole and mebendazole. These were available and accessible at health centers, in markets and from *doctor choukou*. The lack of laboratory diagnostics for helminth infections at health centers, thus dictating exclusively empiric diagnoses may explain the selection of this therapy. Still, patients reported a slight improvement for 2 to 3 days after the treatment, before the recurrence of the symptoms. This may also point at an antibiotic treatment administered by health center staff or *doctor choukou*, who may interpret the most frequently stated symptoms of schistosomiasis patients as a bacterial urinary infection.

Similarly to the veterinary drugs, half of the tested drugs for human use were found to contain an over concentration of albendazole or mebendazole, as observed earlier in Chad and elsewhere in resource-poor settings (ReMeD, 1995, Caudron, J. M.*et al.*, 2008). Nevertheless, all products

contained more than 90% of the labeled drug. These results do not support the pastoralists' hypothesis of products circulating with too low or no pharmaceutical compound at all. Nonetheless, albendazole or mebendazole are not effective against *S. haematobium, S. mansoni* and *S. bovis*, and have very limited activity against *F. gigantica.* Praziquantel, the drug of choice to treat schistosomiasis, was not found among the samples tested. Although it is available from licensed pharmacies in N'Djamena, our findings indicate that praziquantel is not available at rural health centers in the study area and on the informal drug market.

The present study is limited to the south-eastern Lake Chad area and the mobile pastoralist population, the picture drawn represents therewith a narrow insight to the topic addressed. A study including the sedentary population but also the health sector in the study area, together with a nationwide study on drug quality and availability, should be performed to complete the picture.

7.6. Conclusion

In regard to livestock fascioliasis, awareness, willingness to treat and pay for treatment was high. Access to human and veterinary anthelminthic treatment exists, predominantly through the informal sector. The tests performed on the available drugs reveal that some products may not meet all quality standards and that needs certainly to be addressed. Still, the reported low treatment success is most likely not due to the low quality of drugs, but rather caused by the lacking availability of reliable diagnostic tools and efficacious drugs, i.e. praziquantel and triclabendazole. Regarding livestock health, the non-availability of triclabendazole for fascioliasis treatment leads to an additional economical loss for the mobile pastoralists, since financial means are spent on ineffective treatment. Introducing efficacious treatment against livestock fascioliasis will therewith not only impact on animal health, but also result in economic benefits by improving productivity and reducing treatment costs.

Regarding human health, more than half of the interviewed individuals infected with schistosomiasis sought for treatment. Rapid diagnostic tests for the diagnosis of schistosomiasis that are of low costs, easy to perform and of high sensitivity are now available for the diagnosis of *S. mansoni* and promote hopes that such RDTs will also be available soon for *S. haematobium* diagnosis. In the meantime, reagent strip testing for microhaematuria as an indicator of urinary schistosomiasis is still widely used since it is easy to perform. Although its sensitivity is about 75% and its use as sole diagnostic tool is currently under discussion (Krauth, S. J.*et al.*, 2015a), it has the potential to distinguish an active *S. haematobium* infection from a bacterial urinary infection and can therewith guide health staff to the selection of the appropriate treatment. Promoting these diagnostic tools in health centers and introducing praziquantel, accompanied with an awareness campaign among the general

public and training for health personnel is the way forward to reduce burden of schistosomiasis in Chad and to put the country on the map as a further candidate for the schistosomiasis elimination goal.

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8. Rapid re-infection with *Fasciola gigantica* in cattle 6 months after triclabendazole treatment in a mobile pastoralist setting at Lake Chad

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8.1. Abstract

At Lake Chad in central Africa, livestock fascioliasis caused by *Fasciola gigantica* represents a major animal health problem, specifically in cattle. To test the effect of a single-dose triclabendazole treatment on the rate of re-infection of bovine fascioliasis in naturally infected cattle a single-arm intervention study was conducted. Within the herds of 14 groups of mobile pastoralists, a total of 375 cattle (1-14 years of age) were randomly selected. Selected animals were individually marked with an ear tag and a faecal sample was obtained from each animal to determine baseline prevalence. Age, sex, breed and body condition was recorded. All animals received a single oral dose of triclabendazole (12mg/kg), according to the manufacturer's guidelines. Faecal samples were obtained at baseline and follow-up 6 month later, when the cattle returned from tha annual migration cycle. Samples were conserved in sodium-acetate formalin (SAF) solution and transferred to a laboratory. A sedimentation method followed by microscopy was applied for *F. gigantica* egg detection.

From the 375 cattle enrolled at baseline, 198 cattle (52%) in 12 groups of mobile pastoralists were sampled at follow-up. At baseline, bovine fascioliasis prevalence in cattle with follow-up data was 41.9% (95% CI 35.2 – 48.9). Six month after the treatment, 46% (95% CI 39.2 – 52.9) of all cattle were found *F. gigantica* egg positive. Prevalence varied between different herds (0%-88%) and half of the groups showed prevalence above 60%. Faecal egg counts were higher at follow-up compared to baseline.

The observed rapid re-infection with *F. gigantica* in cattle shows that a single pre-rainy season treatment leads to a rapid re-infection despite the partial migration away from Lake Chad into drier areas. A more detailed understanding of the transmission of *F. gigantica* in relation to the seasonal migration is necessary to develop an effective strategic control package for cattle in the Lake Chad area.

Keywords Cattle, *Fasciola gigantica*, Lake Chad, mobile pastoralism, prevalence, re-infection, triclabendazole

8.2. Introduction

Worldwide, livestock fascioliasis caused by *Fasciola hepatica* and *F. gigantica* is of high veterinary importance due to productivity losses (Wamae, L. W.*et al.*, 1998, Kaplan, R. M., 2001). Infected animals show reduced productivity and fertility. Alongside the negative health outcomes, treatment expenses and condemnation of infected carcasses at slaughter may impact negatively on the

livelihoods of people depending on livestock (Suleiman, A.*et al.*, 2015). Although no estimates of global burden or economic losses are depicted in numbers today, the impact is assumed to be significant (WHO, 2015a). In the Sahelian belt of Africa, livestock is traditionally managed in mobile pastoralism husbandry systems and provides existence for an estimated 20 million people (De Haan, C.*et al.*, 2014). According to the Chadian "Plan national de Devéloppement Sanitaitre II (PNDS2) 2013-2015, livestock production represents the second important economic factor to the country with 24% gross domestic product (GDP), after the export of crude oil (40% GDP). The Lake Chad area and its seasonal flood plains is home to mobile pastoralists with a seasonally high population density of indigenous livestock (Jean-Richard, V.*et al.*, 2015). The basin is long-known to be a trematode-endemic area, including *F. gigantica* (Bouchet, A.*et al.*, 1969, Tager-Kagan, P., 1978). A recent abattoir study in the south-eastern Lake Chad area revealed that close to 70% of indigenous cattle was fascioliasis positive (Jean-Richard, V.*et al.*, 2014a). A study among mobile pastoralists on knowledge, attitude and practice on fascioliasis, and the use of anthelmintic drugs in the region showed that, despite a high awareness of the disease, use of anthelmintic drugs was depending on access and drug availability as well as on financial capacity. Treatment was non-strategic and the available drugs, albendazole and bithionol sulfoxide, were administered only to those animals showing severe illness (Greter, H.*et al.*, 2017).

In Africa and elsewhere, strategic anthelmintic treatments of livestock have been demonstrated to be effective and beneficial (Zinsstag, J.*et al.*, 1997, Zinsstag, J.*et al.*, 2000). However, there is a gap in knowledge on strategic anthelmintic treatment experience in mobile pastoralist husbandry systems, specifically from the Sahel. There exists no plan for strategic control of fascioliasis in cattle in Chad.

Today, triclabendazole is the recommended treatment for fascioliasis, yet, to our knowledge, it has not been tested in naturally infected cattle in a mobile pastoralist setting in Africa. Triclabendazole has been synthesized in 1978 and is available for veterinary use since 1983 (Keiser, J.*et al.*, 2005). It is administered to ruminants for the treatment of fascioliasis caused by *Fasciola hepatica* and *F. gigantica.* In controlled studies in cattle, efficacy has been shown to be as high as 96.5% and 97.8% (Rapic, D.*et al.*, 1988, Richards, R. J.*et al.*, 1990). An efficacy of 90% (Lecuyer, B.*et al.*, 1985) and 100% has been achieved in naturally infected cattle (Craig, T. M. and Huey, R. L., 1984, Lecuyer, B.*et al.*, 1985, Stansfield, D. G.*et al.*, 1987). Although resistance has been described in the meantime, triclabendazole remains the most effective available drug for the treatment of livestock fascioliasis today (Moll, L.*et al.*, 2000, Mehlhorn, H., 2008). However, triclabendazole is currently not commercially available in Chad and, to our knowledge, in none of the neighbouring countries (Keiser, J.*et al.*, 2005, Keyyu, J. D.*et al.*, 2009, Greter, H.*et al.*, 2017). Triclabendazole is also the treatment of choice in human fascioliasis cases (WHO, 2007). In contrast to older flukicides whose activity is limited to adult liver flukes, triclabendazole is effective against all live stages of *Fasciola* flukes in the

final host (Keiser, J.*et al.*, 2005). Here, we report on the results of a single-arm intervention trial of triclabendazole treatment on bovine fascioliasis in naturally infected Chadian cattle in a mobile pastoralist husbandry system at on the eastern shores of Lake Chad. The rationale for the choice of treatment date at the end of the dry season was that cattle would be cleared from infection prior to migrating away from the wetlands of Lake Chad towards semi-arid pasture East of Lake Chad with much lower risk of re-infection during the rainy season (Jean-Richard, V.*et al.*, 2014a).

8.3. Methods

Study area and local husbandry practices

This study was conducted in the regions of Chari-Baguirmi, Hadjer-Lamis and Lac on the eastern shores of Lake Chad in Chad. A recent abattoir study had revealed high prevalence of fascioliasis in cattle in the area (Jean-Richard, V.*et al.*, 2014a). All participating animals originated from herds owned by one of the four locally predominant ethnic groups of mobile pastoralists. The regions are located in the Sahelian ecological zone and border to Cameroon and Nigeria. The area in inhabited by fishermen, a sedentary population that predominantly lives of agriculture and different ethnic groups of mobile pastoralists with large numbers of livestock (Jean-Richard, V.*et al.*, 2015). The locally predominant ethnic groups of mobile pastoralists belong to the Arabs, Gorane, Fulani, and Buduma. The climate consists of a rainy season from June to October, a cool and dry season from November to February and a hot and dry season from March to May. The areas' vegetation cover consists of savannah grassland. In the traditional livestock management system, a variety of cattle breeds are raised (*Bos indicus*), with a well-developed dewlap and short horns, and the Mbororo (red Fulani) breed (*Bos indicus*), a reddish brown cattle with long, whitish lyre-shaped horns (Flury, C.*et al.*, 2009). A worldwide unique breed to the Lake Chad is the Kouri cattle (*Bos taurus*) (Quéval, R.*et al.*, 1971). It is highly adapted to the shallow waters of the lake and swims from island to island in search for pasture (Joshi, N. R. and Phillips, R. W., 1957). Although all cattle are kept in grazing systems, husbandry systems practiced by different mobile pastoralists show individual differences. The Gorane pastoralists breed Arab cattle and can avoid watering animals on surface water due to their excellent well building skills. The Arabs breed Arab cattle and are semi-nomadic. They leave their villages when pasture for their livestock gets scarce towards the end of the dry season, when they approach closer to the Lake Chads' shores. In contrast, the Fulani with their Mbororo cattle and the Buduma with their Kouri cattle live a pure nomadic lifestyle (Loutan, L. and Lamotte, J. M., 1984, MERA, 2008). They fully depend on surface water and use pastures on the shores of and on island within Lake Chad.

Study design, animals and treatment

This study was designed as a single-arm intervention trial to evaluate disease prevalence at baseline and 6 month after treatment with triclabendazole in a typical Sahelian mobile pastoralist setting. The timing was set in a way that the cattle are treated prior to moving away from the Lake Chaud wetlands towards drier areas with a presumably lower infection risk for a duration of five to six months. In order to simulate mixed husbandry practices and to include all cattle breeds, animals from herds raised by all four groups of pastoralists were included in the study. Participating groups of pastoralists were partly selected randomly and partly through convenience sampling. Within the herds of each group, 25 cattle older than 1 year of age were randomly selected, individually marked with an ear tag and age, sex and body condition were recorded. A faecal sample was collected from each animal. All selected animals received a single-dose treatment with triclabendazole (12mg/kg). For the following six month, marked animals (ear tag) were kept together with non-tagged cattle and the pastoralists continued with their normal husbandry practices, except for that they were asked to not sell or slaughter tagged animals and to not administer anthelmintic treatment to marked animals during this period.

Sample collection and parasitological analyses

The baseline survey was carried out in April and May 2014, the follow up survey took place from October to December 2014. At baseline and follow-up, faecal samples were collected from each individual animal. Approximately 3 gr of faeces was conserved in a vial containing 30 ml of sodium-acetate formalin solution (SAF) and marked with the individual ID of each animal. Samples were transferred to the parasitology laboratory at the Institute of Parasitology, Vetsuisse Faculty, University of Zurich, in Switzerland. For detection of *Fasciola gigantica* eggs a sedimentation technique followed by microscopy was applied. The amount of faecal matter was not accurately weighted when prepared for SAF conservation in the field. For microscopy, a standardized container was used and eggs were counted. Therefore, the faecal egg count (FEC) reported in the result section does not corresponds to egg-per-gram (EPG). EPG were determined for 18 samples to calculate the proportion of the FEC with respect to EPG. Linear regression revealed a high R2 value of 0.986 and estimated a correction factor of 1.95. The mean residual, i.e. the absolute difference between observed and estimated EPG value, was 3.6 eggs per gram.

Statistical analysis

Data were entered into Microsoft Access version 14 (Microsoft Corp.; Redmond,WA) and internal consistency checks were done. Descriptive statistics and statistical modelling was performed using STATA Version 13 (StataCorp, College Station, Texas, USA) and R v 3.2.3. Generalized estimate equation (GEE) models for correlated binary outcomes and independent correlation structure were

applied to account for correlation within herds, with the individual pastoralist groups defined as cluster. The parasitological status of the tested cattle was described in terms of prevalence and egg shedding intensity.

8.4. Results

Study flow

During baseline in April and May 2014, in 14 mobile pastoralist groups 375 cattle were enrolled and sampled. At follow-up in October and December 2014, 12 groups and 198 animals were successfully traced, resulting in a follow-up rate of 53%. Two pastoralist groups (1 Buduma and 1 Kouri) were lost to follow up because they had entered deep into Lake Chad onto islands which were not accessible for the study team. The main reason for loss to follow up (124 animals) was related to separation of herds. One animal had died and one animal was sold by the owner during the study (Figure 8.1).

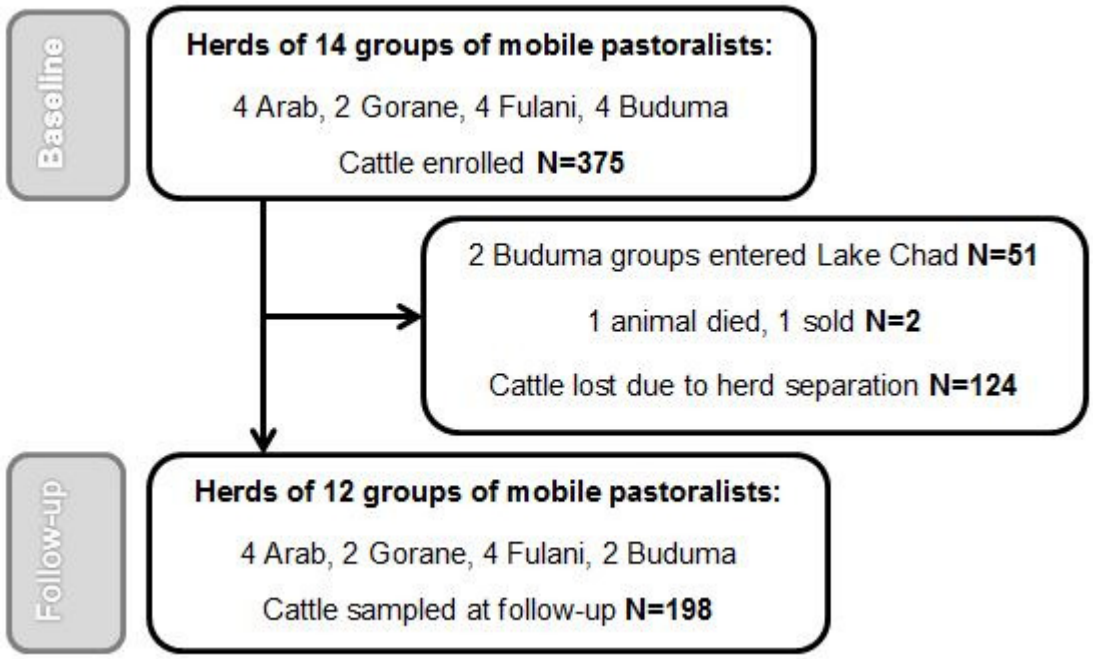

Figure 8.1 Trial flow diagram of a single-arm intervention trial conducted on the eastern shores of Lake Chad in Chad from April until December 2014.

Baseline characteristics

The apparent baseline prevalence of *F. gigantica* infection among the 198 cattle with follow-up data was 41.9% (83/198, 95% CI 35.2 – 48.9%) and differed only slightly from the observed prevalence in animals lost to follow-up (36.7%). Within the 12 groups of mobile pastoralists with follow-up data, the number of sampled cattle varied from 2 to 28 animals. In two of the 12 mobile pastoralist groups, all animals were negative. Prevalence among animals from herds in positive groups ranged from 3% to 88%. The 12 groups were composed of 4 Arab, 2 Gorane, 2 Buduma and 4 Fulani pastoralist. Both

Gorane pastoralists and 3 Arab pastoralist groups kept exclusively cattle of the Arab zebu breed. All Fulani and one Arab pastoralist groups kept Mbororo cattle and both Buduma groups kept the Kouri breed cattle. At baseline, cattle from the four Fulani herds were most affected with a mean prevalence was 68%. Least affected were cattle from the herds of the Gorane pastoralists, where only one animal was found to shed *F. gigantica* eggs (3%). The Mbororo and Kouri breed showed higher prevalence when compared to the Arab breed (63%, 52% and 17%, respectively). Among the 198 cattle, 90% were female. The age of animals ranged from 1 – 14 years, mean age 7 years and interquartile range 4 – 9 years. Two age categories were defined grouping heifers and young bulls (1-3 years old) and cows and bulls (4 – 14 years old). There was no significant difference in fascioliasis prevalence between sexes (42% vs 38%) and between age groups (35% vs 44%). Basic characteristics of the cattle of the final cohort are provided in table 8.1.

Table 8.1 Baseline characteristics and prevalence of 198 cattle on the eastern shores of Lake Chad.

	N (%)	Prevalence (%)
Sex		
Female	177 (90%)	42%
Male	21 (10%)	38%
Age categories		
Heifers and young bulls	48 (25%)	35%
Cows and bulls	150 (75%)	44%
Breed		
Arab	84 (42%)	17%
Kouri	21 (11%)	52%
Mbororo	93 (47%)	63%
Husbandry system		
Arab	66 (33%)	30%
Gorane	36 (18%)	3%
Fulani	75 (38%)	68%
Buduma / Kouri	21 (11%)	52%

Prevalence at follow up

Half a year after treatment, 46% (95%-CI: 39%-46%) of the cattle were shedding *F. gigantica* eggs in faeces. In three mobile pastoralist groups, all animals were negative. Prevalence among animals from herds in positive groups ranged from 29% to 75%, with half of the herds (6/12) showed prevalences above 60%. While at baseline, cattle from the Fulani pastoralists where most affected, highest prevalence was seen in cattle herded by Arab pastoralists at follow-up (figure 8.2).

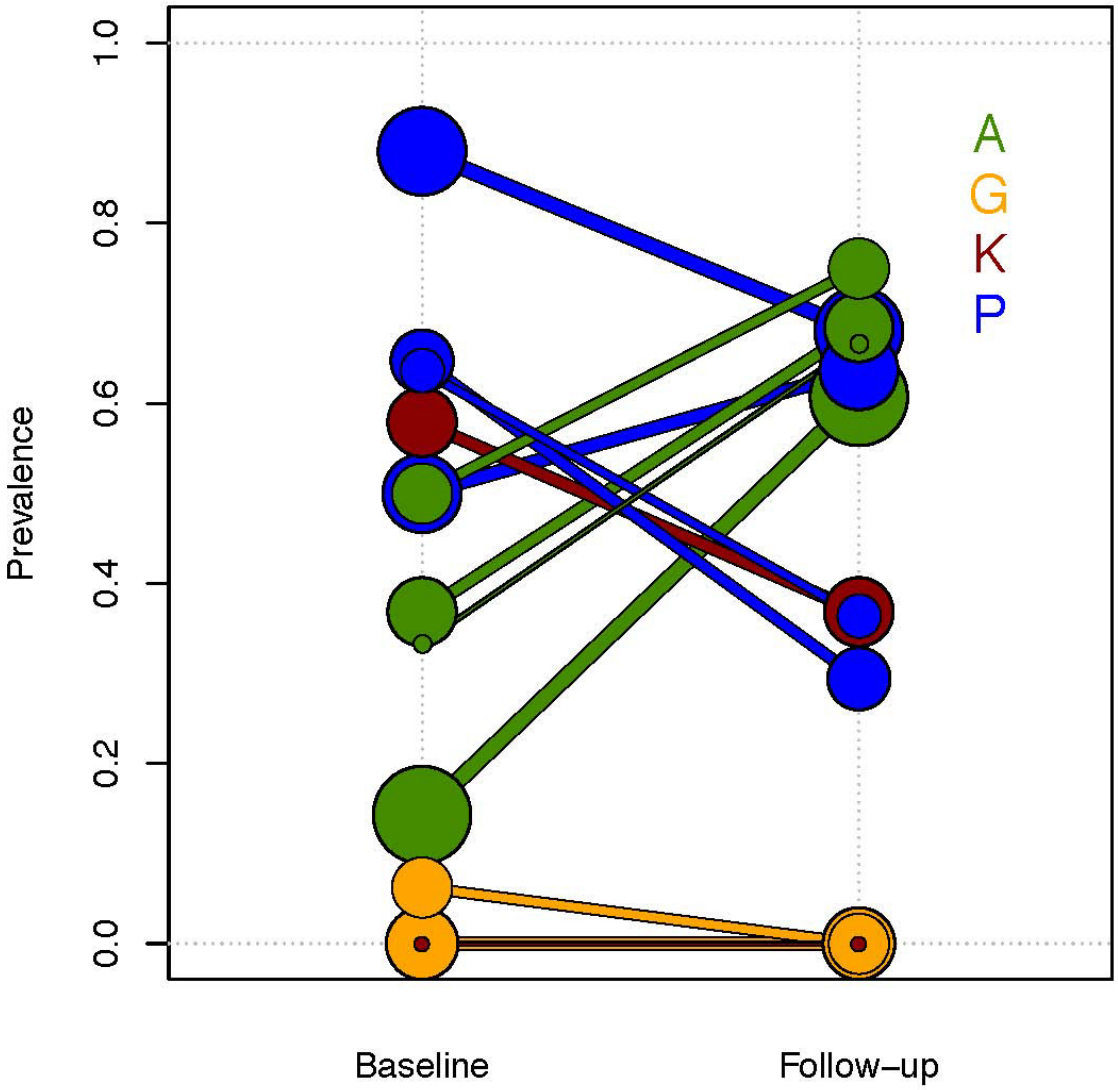

Figure 8.2 Prevalence of bovine fascioliasis in 198 cattle from Arab (A), Gorane (G), Buduma (K), and Fulani (P) pastoralist husbandry system at baseline and follow-up. The sizes of the individual points reflect the number of animals sampled per mobile pastoralist group.

At follow-up, the prevalence in cattle infected at baseline was significantly higher compared to cattle not infected at baseline (56% vs 38%, OR 2.1, 95% CI 1.2-3.7, p=0.01). Egg shedding intensity was slightly lower at baseline (median 4 EPG, IQR 2-6) compared to follow up (median 8 EPG, IQR: 4-16) (figure 8.3). No noteworthy difference in egg shedding intensity at follow up was detected by baseline infection status.

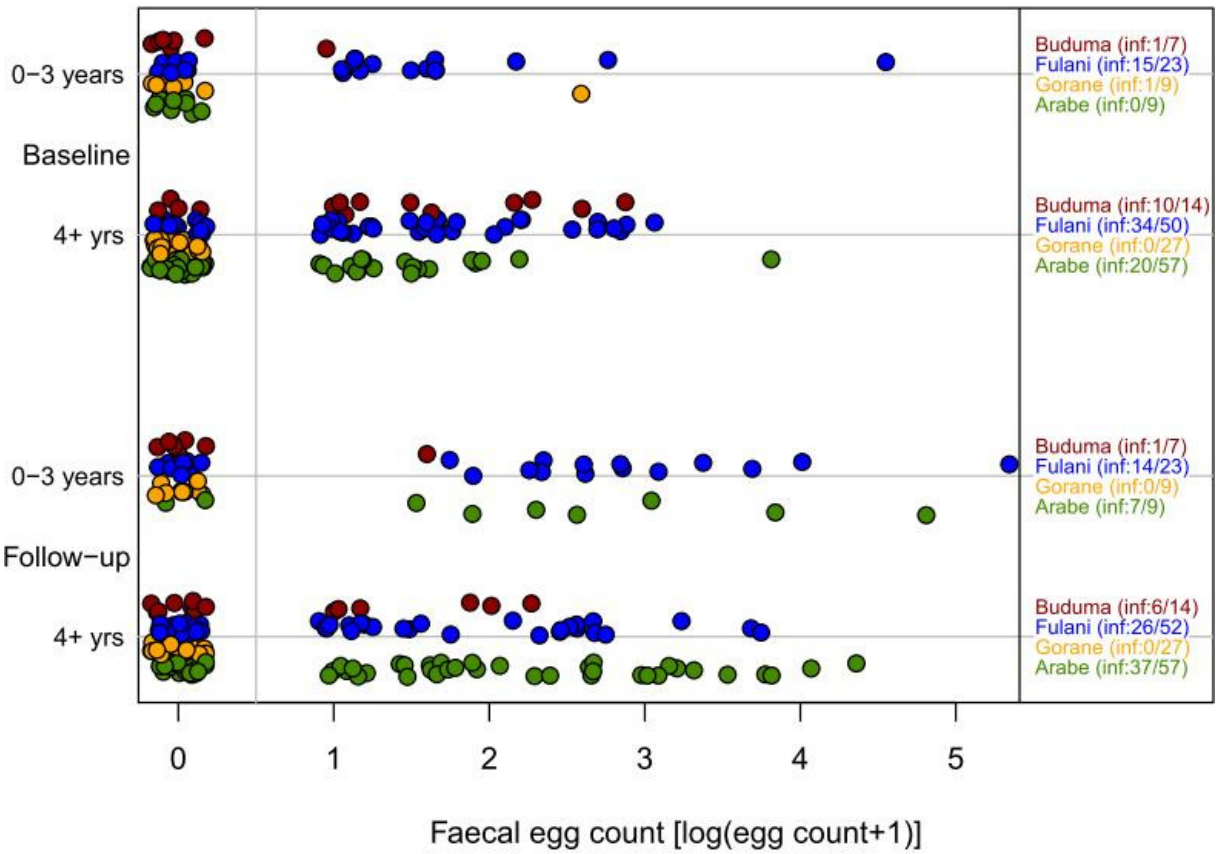

Figure 8.3 Faecal egg count in 198 cattle from 4 different husbandry systems before and 6 month after treatment with triclabendazole (points jittered to avoid overplotting).

Risk factors for infection and re-infection

Prevalence was balanced in heifers and young bulls and adult animals (46% vs 46%, OR=1.0, 95% cI 0.5-1.9) (table 2). Although there was no evidence that the prevalence increased or decreased with increasing age (Fig 4). Body condition at baseline and follow-up did not differ between surveys, at baseline and at follow-up approx. 26% of the animals were in good body condition and 73% were in poor body condition. Among the different cattle breeds, the Mbororo cattle had significantly higher odds to for fascioliasis infection after six month, compared to Arab and Kouri cattle (Mbororo: odds ratio (OR) = 2.8, 95% CI: 1.5 – 5.0; Kouri: OR = 1.1, 95% CI: 0.3 – 2.6). Analysis per husbandry system is only descriptive because groups represent clusters and might cause model instability. The husbandry system of the Arab pastoralists seems to encompass the highest risk, since 67% of the cattle kept in Arab husbandry system were re-infected, followed by the Fulani husbandry system with 53% and the Buduma and Kouri with 33%. In the husbandry system of the Gorane pastoralists, nill fascioliasis case was detected at follow-up, being one case at baseline.

Table 8.2. Fascioliasis prevalence in cattle 6 month after triclabendazole treatment, stratified by risk factors.

	Positive [% (N)]	Negative [% (N)]	OR	95% CI
Age category				
Heifers and young bulls	46% (22)	54% (26)	Ref	
Cows and bulls	46% (69)	54% (81)	1.0	0.5-1.9
Sex				
Female	46% (81)	54% (96)	Ref	
Male	48% (10)	52% (11)	1.0	0.4-2.7
Breed				
Arab	34% (29)	66% (55)	Ref	
Mbororo	59% (55)	41% (38)	2.8	1.5-5.0
Kouri	33% (7)	67% (14)	1.1	0.3-2.6
Husbandry system[a]				
Gorane	0	100% (36)		
Arab	67% (44)	33% (22)		
Fulani	53% (40)	47% (35)		
Buduma / Kouri	33% (7)	67% (14)		

[a] Statistical analysis omitted because husbandry system is nested within herd (cluster).

8.5. Discussion

This study has assessed post-triclabendazole treatment infection patterns with bovine fascioliasis in cattle from mobile pastoralist husbandry systems at Lake Chad. To our knowledge, this is the first data on triclabendazole treatment in naturally infected cattle from central Africa. The single-arm intervention trial was carried out in herds including naturally infected cattle on the eastern Lake Chad area. The baseline survey and initial triclabendazole treatment took place during the dry season and follow-up after the rainy season. Although there was a relatively high loss to follow-up, baseline prevalence did not differ much between animals followed and lost (41.9% vs 38.6%). A recent abattoir study on the south-eastern shores of Lake Chad revealed a bovine fascioliasis prevalence of 68% and confirmed the importance the disease in this very zone. Among cattle originating from five different husbandry systems, the variations in prevalence and intensity of *F. gigantica* ranged from 0% to 100% (Jean-Richard, V.*et al.*, 2014a). This study reports a prevalence at baseline of 41.9%, with a variance between four different husbandry systems of 0 – 88%, supporting the findings from the abattoir study. Variation in infection may be characterized by the social-ecological contexts of different ethnic groups of mobile pastoralistm that translates to the individual husbandry systems. Cattle herded by Gorane pastoralists have a minimal risk of *F. gigantica* infection, in contrast to cattle herded by Arab, Fulani and Buduma pastoralists. Six month after single-dose triclabendazole treatment, a prevalence of 46% was observed. In fenced livestock, it has been shown that post-treatment re-infection patterns are largely dependent on pasture infestation (Brunsdon, R. V., 1980).

The here presented study was carried out in cattle herded in a highly mobile way and re-infection was rapid regarding prevalence and egg shedding intensity. Prevalence at follow-up exceeded baseline prevalence and was even above the initial level. This may partly be explained due to suspected high pasture contamination, resulting from high livestock numbers on the shores of Lake Chad towards the end of the dry season, feeding on the last remaining pasture prior to moving out of the Lake Chad wetlands (Jean-Richard, V.*et al.*, 2015). Our results are not conclusive whether re-infection occurred still in the Lake Chad area or during migration outside the wetlands. A further study could be planned in a way that treatment takes place at the very moment of leaving the wetlands. This could be planned by mobile communication with livestock holders. A treatment at the end of the rainy season, when the cattle return to the wetland of Lake Chad would likely lead to a rapid re-infection again. A more detailed understanding of the transmission of *Fasciolia gigantica* in relation to the seasonal migration between wetlands and dryland pasture is warranted to develop an effective strategic control package for cattle in the Lake Chad area.

Baseline prevalence might be an underestimate because about 40% (158/375) of the cattle have been treated with albendazole in late 2013. Yet, given the limited effectiveness of a single albendazole treatment and the observed rapid reinfection the eventual effect can be neglected (Keyyu, J. D.*et al.*, 2009). Studies report the specificity of the sedimentation method being 100%, though its sensitivity level lies between 63 – 82.5% (Anderson, N.*et al.*, 1999, Duthaler, U.*et al.*, 2010). Additionally, fasciolaisis has a prepatency phase of 8 – 13 weeks, where no eggs are shed in faeces (Mehlhorn, H., 2008). These factors may further point out that the prevalence reported here represents an underestimate and the 'true' prevalence may be higher. Additionally, as mentioned in the introduction, triclabendazole has shown efficacy of 90 – 100% in naturally infected cattle (Craig, T. M. and Huey, R. L., 1984, Lecuyer, B.*et al.*, 1985, Stansfield, D. G.*et al.*, 1987). Still, we cannot exclude that some animals where not cured and might rather be "still infected" than re-infected. The slight difference in prevalence in age groups at baseline and follow-up may be explained by the fact that exposure time was only synchronized after the initial treatment.

To establish an adapted, effective treatment plan for the control of bovine fascioliasis, a more holistic approach is needed.

Limitations

We cannot rule out with absolute certainty that animals have been treated in the meanwhile. However, our estimate is rather conservative. GEE models have been reported to provide too narrow confidence intervals if the numbers of clusters is low. However, the alternative – namely random

effect models – rely on distributional assumptions which might be violated if zero prevalence have been observed in some clusters.

Acknowledgements

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9. Validation of a point-of-care circulating cathodic antigen urine cassette test for *Schistosoma mansoni* diagnosis in the Sahel, and potential cross-reaction in pregnancy

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9.1. Abstract

On the shores of Lake Chad, schistosomiasis among mobile pastoralists was investigated in a field laboratory. Point-of-care circulating cathodic antigen (POC-CCA) cassette test, reagent strips, and filtration were conducted on urine samples. Fresh stool samples were subjected to the Kato-Katz technique, and fixed samples were examined with an ether-concentration method at a reference laboratory. POC-CCA urine cassette tests revealed a *Schistosoma mansoni* prevalence of 6.9%, compared to only 0.5% by stool microscopy. Three pregnant women with negative urine and stool testing had positive POC-CCA. This observation raises concern of cross-reactivity in pregnancy. Hence, two pregnant women in Switzerland with no history of schistosomiasis were subjected to POC-CCA and one tested positive. Our data suggest that POC-CCA can be performed under extreme Sahelian conditions (e.g., temperatures > 40°C), and it is more sensitive than stool microscopy for *S. mansoni* diagnosis. However, potential cross-reactivity in pregnancy needs further investigation.

9.2. Introduction

Schistosomiasis is listed among the neglected tropical diseases (NTDs) (Hotez, P. J.*et al.*, 2014) and specifically mentioned in the World Health Assembly resolution WHA 65.21a that calls for global elimination by 2025 (WHO, 2013). In Africa, schistosomiasis is mainly caused by chronic infection with *Schistosoma haematobium* and *Schistosoma mansoni* (Colley, D. G.*et al.*, 2014). The most widely used diagnostic methods for *S. haematobium* are urine filtration with microscopy and reagent strip testing for microhematuria, whereas the diagnosis of *S. mansoni* heavily relies on Kato-Katz thick smear examination under a microscope (Utzinger, J.*et al.*, 2015). In rural areas of Africa, where most of the worldwide *Schistosoma* infections occur, the capacity of health centers is insufficient to provide even these basic laboratory diagnostics. Hence, the World Health Organization (WHO)'s plan for global elimination of schistosomiasis will require additional diagnostic tools, characterized by high sensitivity, high specificity, ease of use at the point-of-care (POC), and low costs (Utzinger, J.*et al.*, 2015). First products developed within this spirit are already available on the market. One of them is a POC circulating cathodic antigen (CCA) urine cassette test for the diagnosis of *S. mansoni*. The test is based on a lateral flow principle and detects adult *Schistosoma* worm CCA in the hosts' urine when a colloid carbon conjugate of a monoclonal antibody is added to the sample (van Dam, G. J.*et al.*, 2004). Although CCA is excreted by adult worms of different *Schistosoma* species, the test is most sensitive to CCA excreted from adult *S. mansoni.* The test also allows detection of *S. mansoni* infection before the excretion of parasite eggs in the stool. According to the manufacturer's guidelines, heavy infections with *S. haematobium* might also be detected. Hence, the test reveals *Schistosoma* infections by indirect depiction of the presence of adult worms (or young developing stages of the parasite) in the host body and has therewith a higher sensitivity than stool microscopy,

since S. mansoni egg excretion shows considerable intraspecimen and day-to-day variation (Colley, D. G.*et al.*, 2013). Of note, the POC-CCA urine cassette test has been successfully validated in a multi-country study and showed an overall specificity of 94 % (Colley, D. G.*et al.*, 2013). The POC-CCA is now being recommended by WHO for the rapid mapping of community prevalence in endemic settings (Utzinger, J.*et al.*, 2015), and might be used for POC diagnosis of migrants from endemic regions and returning travelers to Europe (Becker, S. L.*et al.*, 2015b).

With regard to the WHO goal of schistosomiasis elimination, it is important to gain a deeper insight into the disease situation in endemic areas. In Chad, nationwide data on schistosomiasis date back to the 1970s (Becquet, R.*et al.*, 1970, Delpy, P.*et al.*, 1972). More recent studies reflect the infection status of the urban population in the vicinity of the capital city of N'Djamena (Massenet, D.*et al.*, 1995, Hamit, M. A.*et al.*, 2008, Massenet, D.*et al.*, 2012). Yet, to attempt elimination, a more detailed picture of the infection status in the rural Chadian population is required and specific at-risk groups must be identified.

Here, we report findings from a cross-sectional survey among mobile pastoralists from the Lake Chad region, placing particular emphasis on the use of the POC-CCA urine cassette test under extreme environmental conditions (e.g., aridness and air temperature up to 48°C). Our findings are compared to the standard Kato-Katz technique and an ether-concentration method of fixed stool samples for *S. mansoni* diagnosis. As we found a potential cross-reaction of the POC-CCA test among pregnant women, we invited two pregnant women from Switzerland with no history of schistosomiasis to participate.

9.3. Methods

Ethical considerations. The validation of the POC-CCA urine cassette test for the diagnosis of *S. mansoni* was embedded in a larger epidemiologic study pertaining to helminth infections in mobile pastoralists and their livestock in the south-eastern Lake Chad area in Chad. The study was approved by the ethics committee in Basel, Switzerland (EKBB; reference no. 64/13). In Chad, research permission, including ethical approval, was obtained from the 'Direction Générale des Activités Sanitaires' (reference no. 343/MSP/SE/SG/DGAS/2013). The aim and procedures of the study were explained to each group of mobile pastoralists. Informed consent was signed by group leaders after discussion within the groups. Because of high illiteracy rates, randomly selected individuals within each group provided oral consent. All participants found with a positive test result for *S. haematobium* by urine filtration and *S. mansoni* on the spot (Kato-Katz thick smear and POC-CCA urine cassette test) were treated with a single 40 mg/kg oral dose of praziquantel (WHO, 2002). In addition, two pregnant women in Switzerland participated on a voluntary basis. Both provided written informed consent.

Procedures. The data were collected between April and May 2014 in 13 camps of mobile pastoralists on the south-eastern shores of Lake Chad. A mobile field laboratory was set up in the shade of a tree in close proximity to camps. Study participants were given two collection containers; one for urine and one for stool. Urine samples were collected between 10 a.m. and 9 p.m., with 60% of the urine specimens sampled between 10 a.m. and 2 p.m. Urine samples were first tested for microhematuria, a proxy for *S. haematobium* infection (Savioli, L.*et al.*, 1990), using reagent strips (Hemastix, Siemens Healthcare Diagnostics GmbH; Eschborn, Germany). Second, a POC-CCA cassette test (Rapid Medical Diagnostics; Pretoria, South Africa) was used for *S. mansoni* diagnosis. Third, 10 mL of urine was filtered using a syringe pressed through a 13-mm diameter filter holder containing a 20 µm wire-mesh filter (Sefar AG; Heiden, Switzerland). Filters were examined under a solar-powered light microscope by a trained laboratory technician and the first author (Helena Greter).

From each stool sample, a single Kato-Katz thick smear using 41.7 mg standard templates was prepared and examined under a microscope on the spot by a trained laboratory technician (Katz, N.*et al.*, 1972). In addition, approximately 1 g of stool was fixed in a Falcon tube containing 20 mL of sodium acetate-acetic acid-formalin (SAF) solution. The SAF-fixed stool samples were forwarded to the Swiss National Reference Laboratory for Imported Parasitic Infections at the Swiss Tropical and Public Health Institute (Basel, Switzerland) and subjected to an ether-concentration method for the diagnosis of *S. mansoni* and other helminths (Utzinger, J.*et al.*, 2010).

9.4. Results

Urine and stool samples were obtained from a random sample of 193 individuals in 13 mobile camps. Complete parasitological data (i.e., reagent strip, urine filtration, POC-CCA urine cassette test, Kato-Katz thick smear, and SAF-fixed stool samples examined by an ether-concentration technique) were available from 187 participants (96 females (47 girls aged <14 years) and 91 males (51 boys aged <14 years)). There were 13 positive POC-CCA urine cassette tests, owing to a *S. mansoni* prevalence of 6.9%. Stool microscopy, using Kato-Katz and ether-concentration, detected eggs of *S. mansoni* in only one individual (0.5%). There were more than twice as many positive POC-CCA urine cassette tests in females (N = 9) compared to males (N = 4), whereas the only positive stool microscopy was in a male participant. Among the 13 positive POC-CCA urine cassette tests, 8 showed a faintly positive test line, whereas the remaining 5 showed a strong positive test line (Figure 9.1) (Coulibaly, J. T.*et al.*, 2013).

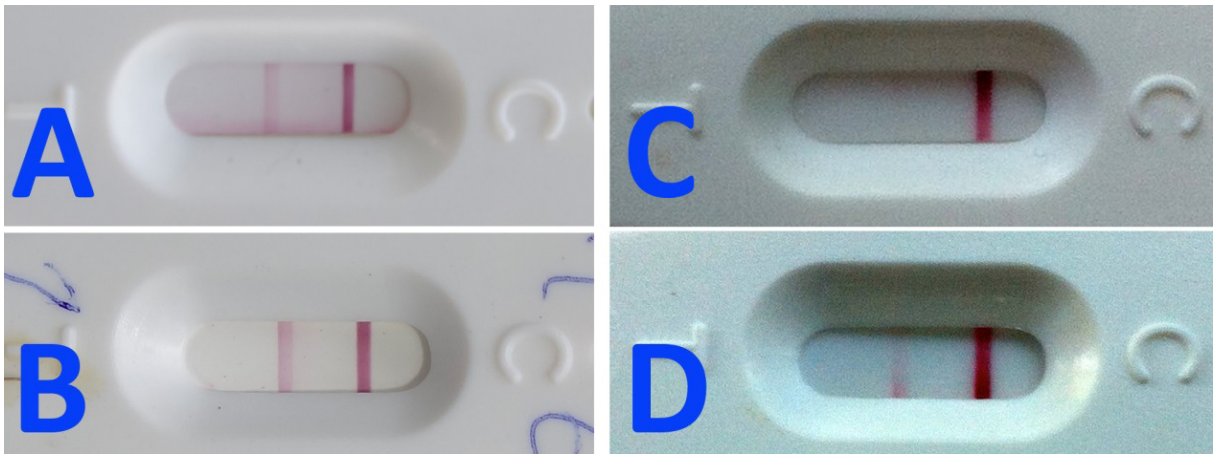

Figure 9.1. Point-of-Care Circulating Cathodic Antigen (POC-CCA) urine cassette test results obtained from samples in Chad (A, B) and from pregnant women in Switzerland (C, D). A: faintly positive test band from Chad. B: strong positive test band from Chad. C: negative test result from Swiss pregnant woman x. D: positive test result from Swiss pregnant woman y.

According to the manufacturer's guidelines for the POC-CCA assay, the intensity of the test line is correlated with the intensity of *S. mansoni* infection. It is also mentioned in the guidelines that heavy infections with *S. haematobium* and microhematuria may produce positive test results in the POC-CCA cassette test. Positive reagent strip or urine filtration results were present in all four male participants who were POC-CCA cassette test positive, but coprology-negative. In females, on the other hand, six individuals with positive POC-CCA cassette tests were found negative with all other tests used (Kato-Katz, ether-concentration, reagent strip, and urine filtration) (Table 9.1).

Table 9.1. Comparison between POC-CCA urine cassette test and stool microscopy, urine filtration, and reagent strip results for total number of participants, stratified by sex.

	Overall	**Males**	**Females**
Participants with complete data	**187**	**91**	**96**
POC-CCA urine cassette test negative	174	87	87
POC-CCA urine cassette test positive	13	4	9
S. mansoni egg-positive (Kato-Katz and/or ether-concentration)	1	1	0
S. haematobium egg-positive (urine filtration) and microhematuria positive (reagent strip)	3	2	1
Microhematuria positive (reagent strip) and *S. haematobium* egg-negative (urine filtration)	3	1	2
POC-CCA urine cassette test positive alone	6	0	6

POC-CCA = point-of care circulating cathodic antigen.

Those six females who had a positive POC-CCA urine cassette test that could not be explained due to a *S. haematobium* infection or microhematuria were adults at reproductive age (19-39 years) (Table 9.2). We found that 3 out of the 6 women were pregnant. For all pregnant women, POC-CCA urine cassette tests were repeated and positive tests were confirmed.

Table 9.2. Comparison between positive POC-CCA urine cassette test, stool microscopy, urine filtration, and reagent strip results for female participants, stratified by age group and pregnancy status. For the category "females, > 14 years" pregnancy status was not assessed.

	Females, all ages	**Females, <14 years**	**Females, >14 years**	**Females, >14 years and pregnant**
POC-CCA urine cassette test positive	9	1	4	4
S. mansoni egg-positive (Kato-Katz and/or ether-concentration)	0	0	0	0
S. haematobium egg-positive (urine filtration) and microhematuria positive (reagent strip)	1	0	1	0
Microhematuria positive (reagent strip) and *S. haematobium* egg-negative (urine filtration)	2	1	0	1
POC-CCA urine cassette test positive alone	6	0	3	3

The aforementioned positive POC-CCA urine cassette test results, coupled with negative coprologic examinations, raised concern about a potential cross-reaction of POC-CCA in pregnancy. Hence, we invited two pregnant women from Switzerland without any history of schistosomiasis to provide urine samples that were subjected to duplicate POC-CCA urine cassette testing. As shown in Figure 9.1, in one of the two pregnant Swiss women, the POC-CCA consistently revealed a faintly positive test line.

9.5. Discussion

POC-CCA urine cassette tests were employed among 187 mobile pastoralists, aged 5-77 years, under extreme environmental conditions in Chad. In contrast, most of the previously published studies that validated the POC-CCA urine cassette test for the diagnosis of *S. mansoni* in schistosomiasis-endemic areas focused on school-aged children (van Dam, G. J.*et al.*, 2004, Stothard, J. R.*et al.*, 2006, Legesse, M. and Erko, B., 2007, Ayele, B.*et al.*, 2008, Midzi, N.*et al.*, 2009, Coulibaly, J. T.*et al.*, 2011, Tchuem Tchuente, L. A.*et al.*, 2012, Adriko, M.*et al.*, 2014, Foo, K. T.*et al.*, 2015,

Mwinzi, P. N.*et al.*, 2015). Positive POC-CCA urine cassette test results in males and females with negative stool microscopy may be explained by the fact that we only examined a single stool sample per participant, and hence, we might have missed individuals with low infection intensity (Utzinger, J.*et al.*, 2001). Interestingly, we found three positive POC-CCA urine cassette tests among pregnant women with negative stool microscopy. These results were confirmed when repeating POC-CCA urine cassette tests. Moreover, one of two pregnant Swiss women without history of schistosomiasis had a positive POC-CCA result. Hence, our results raise concern about a potential cross-reaction with certain non-*Schistosoma*-related metabolites in pregnant women's urine. These observations warrant further research to assess the reliability and accuracy of POC-CCA urine cassette tests before wider use in health care practice.

Treatment with praziquantel – be it in the frame of preventive chemotherapy or targeted to positive individuals – is safe (Utzinger, J. and Keiser, J., 2004), and WHO guidelines established in 2002 explicitly recommend its administration also to pregnant or lactating women (Allen, H. E.*et al.*, 2002). Yet, most guidelines discourage intake of medicines during pregnancy to minimize risk of adverse events. In the endgame of reaching the goal of schistosomiasis elimination, a test-and-treat strategy is likely to gain traction. It will be important to dispose of rapid, easy-to-handle diagnostic tools at the POC that are highly accurate and whose performance remains reliable even under extreme environmental conditions such as in the Sahel.

Acknowledgments: We thank Dr Neels van Rooyen from Rapid Medical Diagnostics (Pretoria, South Africa) for the generous donation of the POC-CCA urine cassette tests. Without the willingness of the Chadian field team to work under trees in extreme heat and dust, this study would not have been possible. We thank all study participants in Chad and the two pregnant women in Switzerland.
Funding for this study was provided by the Swiss National Science Foundation (Bern, Switzerland; grant no. 320030 141246). Training of the Chadian laboratory technicians was supported by the Rudolf Geigy Foundation (Basel, Switzerland).

10. All that is blood is not schistosomiasis: experiences with reagent strip testing for urogenital schistosomiasis with special consideration to very-low prevalence settings

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10.1. Abstract

Background: Reagent strip testing for microhaematuria has long been used for community diagnosis of *Schistosoma haematobium*. Sensitivities and specificities are reasonable, and hence, microhaematuria can serve as a proxy for *S. haematobium* infection. However, assessment of test performance in the context of the underlying *S. haematobium* prevalence is rare and test parameters other than sensitivity and specificity have been neglected.

Methods: Data about the association between microhaematuria and urine filtration results from three studies were compared and put into context with findings from a recent Cochrane review. Data were stratified by *S. haematobium* prevalence to identify prevalence-related differences in test performance. Kappa agreement and regression models were employed to compare data for different *S. haematobium* prevalence categories.

Results: We found a "background" prevalence of microhaematuria (13%, on average) which does not seem to be associated with schistosomiasis in most settings, irrespective of the prevalence of *S. haematobium*. This background level of microhaematuria might be due to cases missed with urine filtration, or alternative causes apart from *S. haematobium*. Especially in very-low prevalence settings, positive results for microhaematuria likely give an inaccurate picture of the extent of *S. haematobium*, whereas negative results are a sound indicator for the absence of infection.

Conclusions: Reagent strip testing for microhaematuria remains a good proxy for urogenital schistosomiasis, but implications of test results and scope of application differ depending on the setting in which reagent strips are employed. In very-low prevalence settings, microhaematuria is an unstable proxy for urogenital schistosomiasis and treatment decision should not be based on reagent strip results alone. Our findings underscore the need for highly accurate diagnostic tools for settings targeted for elimination of urogenital schistosomiasis.

Keywords: Chad, Côte d'Ivoire, Diagnosis, Microhaematuria, Reagent strip testing, *Schistosoma haematobium*, Schistosomiasis

10.2. Background

Since the early 1980s, reagent strip testing for microhaematuria has been used as an indirect diagnostic assay for *Schistosoma haematobium* (Mott, K. E.*et al.*, 1983, Mott, K. E.*et al.*, 1985). Indeed, various studies validated reagent strips against standard urine filtration and concluded that the detection of microhaematuria is a valid proxy for urogenital schistosomiasis and related morbidity (Stephenson, L. S.*et al.*, 1984, Lengeler, C.*et al.*, 1993, King, C. H. and Bertsch, D., 2013, Ochodo, E. A.*et al.*, 2015). In a recent Cochrane systematic review it has been summarised that

reagent strip testing for *S. haematobium* diagnosis has an overall sensitivity and specificity of 75% and 87%, respectively (Ochodo, E. A.*et al.*, 2015). For specific settings it was suggested that reagent strip testing can be used for individual diagnosis and treatment decision (Mott, K. E.*et al.*, 1983, Taylor, P.*et al.*, 1990, Lengeler, C.*et al.*, 2002a), while other groups described reagent strip testing more conservatively as a useful tool for estimating community prevalence (Lengeler, C.*et al.*, 1993, Mtasiwa, D.*et al.*, 1996, Robinson, E.*et al.*, 2009).

Interestingly, in most settings, there was some proportion of 'false positive' (FP) reagent strip results where microhaematuria could not be associated with *S. haematobium* through reference microscopy (King, C. H. and Bertsch, D., 2013, Ochodo, E. A.*et al.*, 2015). Even after the administration of praziquantel, the prevalence of microhaematuria rarely goes to zero and authors have suggested several explanations for this observation. One suggestion is that some *S. haematobium* infections were missed by microscopy. Especially in low-prevalence settings, *S. haematobium* egg output is generally low and thus hard to be detected by a single filtration of only 10 ml of urine (Mott, K. E.*et al.*, 1985, Kosinski, K. C.*et al.*, 2011). Repeated urine sampling and use of more sensitive diagnostic assays might remedy this issue (Taylor, P.*et al.*, 1990, Kosinski, K. C.*et al.*, 2011, King, C. H. and Bertsch, D., 2013). Another explanation is that bladder lesions and associated microhaematuria persisted longer than the actual excretion of eggs into the bladder (Doehring, E.*et al.*, 1985). A third reason why some microhaematuria is unrelated to urogenital schistosomiasis is that residual menstrual blood or pregnancy in females results in positive reagent strip results (Brown, M. A.*et al.*, 2005). Fourth, it has been noted that tests from different producers performed differently in their ability to detect microhaematuria. For example, the Hemastix® (Bayer Diagnostics; Basingstoke, United Kingdom) proved less sensitive than the Combur 9 Test® (Roche Diagnostics; Basel, Switzerland) for microhaematuria and *S. haematobium* infection as confirmed by microscopy (Lengeler, C.*et al.*, 1993, Ugbomoiko, U. S.*et al.*, 2009). Finally, *S. haematobium* infection is not the only aetiology of microhaematuria (Hatz, C.*et al.*, 1990, McDonald, M. M.*et al.*, 2006).

The purpose of this study was to assess the diagnostic accuracy of reagent strips for microhaematuria with particular consideration of settings characterised by low levels of *S. haematobium* prevalence. We addressed three specific research questions. First, does the level of microhaematuria correspondent to the level of *S. haematobium* infection in low-prevalence settings? Second, is microhaematuria - that seems unrelated to *S. haematobium* - merely due to missed cases? Third, can microhaematuria be used as a proxy for *S. haematobium* in low-prevalence (<20%) areas or settings with very low-prevalence that are targeted for elimination (<5%)?

10.3. Methods

Ethical considerations

The three study protocols from which original data were obtained for the current investigation (two studies in Côte d'Ivoire, one in Chad) were approved by the institutional research commission of the Swiss Tropical and Public Health Institute (Swiss TPH; Basel, Switzerland) and received clearance from the ethics committees of Basel (EKBB, reference nos. 377/09 and 64/13) and the national ethics committee in Côte d'Ivoire (reference no. 32-MSLS/CNERdkn and 1993 MSHP/CNER). In Chad, research authorization including ethical approval was granted by the Direction Générale des Activités Sanitaires in N'Djamena (reference no. 343/MSP/SE/SG/DGAS/2013).

District, regional and local authorities, village chiefs, study participants and parents/guardians of individuals aged below 18 years were informed about the purpose, procedures and potential risks and benefits of the study. Information was provided in the national language (French), as well as common languages spoken in south-central and northern Côte d'Ivoire (Baoulé, Dioula/Peulh/Fula and Senoufo) and the Lake Chad area (Arab, Dioula/Peulh/Fula and Kanembou). All authorities and camp/village chiefs were asked for their written or oral consent for the conduct of the study in their administrative area. In Côte d'Ivoire, written informed consent was obtained from all participants and the parents/guardians of minors. In case of illiteracy, consent was given in front of an impartial witness of the participant's choosing who signed in the name of the participant. In Chad, informed consent was signed by the camp leader in front of an impartial witness after discussion within the group. Due to high illiteracy rates, participating individuals consented orally. These consent procedures had been approved by the respective ethics committees.

Participation was voluntary and there were no further obligations for those who withdrew from the study. All results were coded and treated confidentially. At the end of the studies, all positive individuals were offered a single 40 mg/kg oral dose of praziquantel free of charge.

Data

Côte d'Ivoire

During the course of a relatively large study performed in 2014/2015 in the Tchologo region in northern Côte d'Ivoire (Krauth, S. J.*et al.*, 2015b), participants from 28 randomly selected villages, including one to two unofficial settlements in close proximity to the villages, were asked to provide a urine sample. Sample collection was performed throughout the day with 47% of all samples collected between 10 a.m. and 2 p.m., 83% before 4 p.m. and 98% before 6 p.m. Urine samples were transferred to nearby laboratories in Korhogo and Ouangolodougou, where they were subjected to reagent strip testing (Hemastix®, Bayer Diagnostics; Basingstoke, United Kingdom) and the standard urine filtration method (Plouvier, S.*et al.*, 1975). In brief, reagent strips were performed according to

the manufacturer's instructions and results recorded as negative, trace, 1+, 2+ and 3+. With regard to the urine filtration method, 10 ml of a vigorously shaken specimen were pressed through a 13 mm diameter Nytrel filter with a mesh size of 20 µm (Sefar AG; Heiden, Switzerland), placed on a microscope slide, stained with a drop of Lugol's iodine and then examined under a microscope enumerating *S. haematobium* eggs. All parasitological examinations were performed by the same technician and laboratory assistant. 15% of the slides were subjected to quality control. In case of discrepancies between the two readings, all slides of the respective day were read a second time.

Additionally, we re-examined data from a study conducted in 2010 in Grand Moutcho in south Côte d'Ivoire that assessed the dynamics of *S. haematobium* egg output following oral administration of a single dose of praziquantel (40 mg/kg). Details of this study have been published elsewhere (Stete, K.*et al.*, 2012). In brief, urine samples of two consecutive days were collected from 124 children aged 7-15 years during a baseline survey. Each sample was tested using urine filtration with Lugol's iodine staining and reagent strip tests (Combur-7-Test®, Roche Diagnostics; Basel, Switzerland) for microhaematuria, proteinuria and leukocyturia. All *S. haematobium*-positive children (n = 90) were treated. Subsequently, single urine samples were collected from all treated children on each school day (four times per week) for the first 2 weeks and then twice a week up to 8 weeks post-treatment. All samples were subjected to urine filtration and reagent strip testing for microhaematuria, proteinuria and leukocyturia (Combur 7 Test®).

Chad

Urine filtration of 10 ml or whole urine samples (without Lugol's iodine staining) and reagent strip testing (Hemastix, Bayer Diagnostics; Basingstoke, United Kingdom) were likewise performed in the Lake Chad area, where 19 randomly selected groups of mobile pastoralists from four ethnic groups were enrolled in 2013 and 2014. Participants were followed up twice, 6 and 12 months after the baseline survey. Participants found positive for schistosomiasis with urine filtration and/or with a point-of-care cathodic circulating antigen (POC-CCA) urine cassette test for the detection of *Schistosoma mansoni* infection, were treated with a single dose of praziquantel (40 mg/kg). All parasitological tests in Chad were performed on the spot in a mobile laboratory by one of the authors (HG) with assistance from experienced laboratory technicians.

Published data from recent Cochrane review

Relevant data from a recent Cochrane systematic review entitled "Circulating antigen tests and urine reagent strips for diagnosis of active schistosomiasis in endemic areas" (Ochodo, E. A.*et al.*, 2015) were extracted and re-organised by prevalence to put our data in the context of the extant literature.

Statistical analysis

Data were analysed using Stata/IC version 12.1 (StataCorp; College Station, TX, United States of America). A random effects logit regression was employed on our original data with village/camp included as random effect to calculate the relationship between microhaematuria and *S. haematobium* prevalence, tha latter confirmed by urine filtration. Reagent strips were read qualitatively (positive or negative). Trace-positive reagent strips ere considered as positive. Of note, distinguishing between reagent strip read-out intensities did not change the results notably, except reducing the sample size.

Data from the recent Cochrane systematic review were entered as the number of 'true positives' (TP), FP, 'false negatives' (FN) and 'true negatives' (TN), as reported in the Cochrane systematic review. Subsequent percentages were calculated from these numbers and compared to our data. To examine test performance for different prevalence levels, all baseline and follow-up survey results were grouped according to prevalence categories (0-5%, 5-10%, 10-20%, 20-50% and 50-100%).

10.4. Results

Study participants and prevalence

In the Tchologo region of northern Côte d'Ivoire, 8-33 individuals per village (including nearby Fcamps) were included in the study. Overall, there were 831 participants and among them, 809 provided a urine sample. Single reagent strip reading and urine filtration were available from 805 and 802 of the participants, respectively, and 801 participants (493 females and 308 males) had complete data. The prevalence of *S. haematobium* based on single urine filtration was 2.2%, whereas a positive reagent strip test result was noted in 19.5% of participants.

In Grand Moutcho, south Côte d'Ivoire, 124 school-aged children (62 females and 62 males) participated in the baseline survey. The prevalence of *S. haematobium* and microhaematuria was 74% and 62%, respectively at day 1 of the baseline survey and 70% and 66%, respectively at day 2 of the baseline. The overall prevalence of *S. haematobium* and microhaematuria for both baseline days combined was 79% and 71%, respectively (Stete, K.*et al.*, 2012).

In Chad, a total of 402 participants provided a urine sample and 369 of them (181 females, 188 males) were tested with reagent strips and urine filtration. 214 individuals (62.9%) provided a subsequent sample for the first follow-up and 75 (22.1%) provided a sample for the second follow-up. A total of 60 individuals were treated with praziquantel at baseline as they had a positive test result (either urine filtration, or POC-CCA for *S. mansoni*) or because health personnel suggested treatment based on clinical assessment. Urine filtration revealed prevalence of *S. haematobium* of 7.9% at baseline, 2.7% after the first, and 2.6% after the second follow-up. Microhaematuria was

found in 21.1% at baseline and in 12.7% and 10.4% after the first and second follow-up, respectively (Figure 1). In all settings, individuals with light intensity infections (egg excretion <50 egg per 10 ml of urine) had a negative reagent strip test result significantly more often ($p < 0.005$) than individuals with heavy infection intensity (egg excretion ≥50 egg per 10 ml of urine).

Of note, the prevalence of *S. mansoni* as assessed with duplicate Kato-Katz thick smears from a single stool sample in northern Côte d'Ivoire was 0.8%. In Chad, single Kato-Katz thick smears and an ether-concentration method revealed a prevalence of *S. mansoni* of 0.3%.

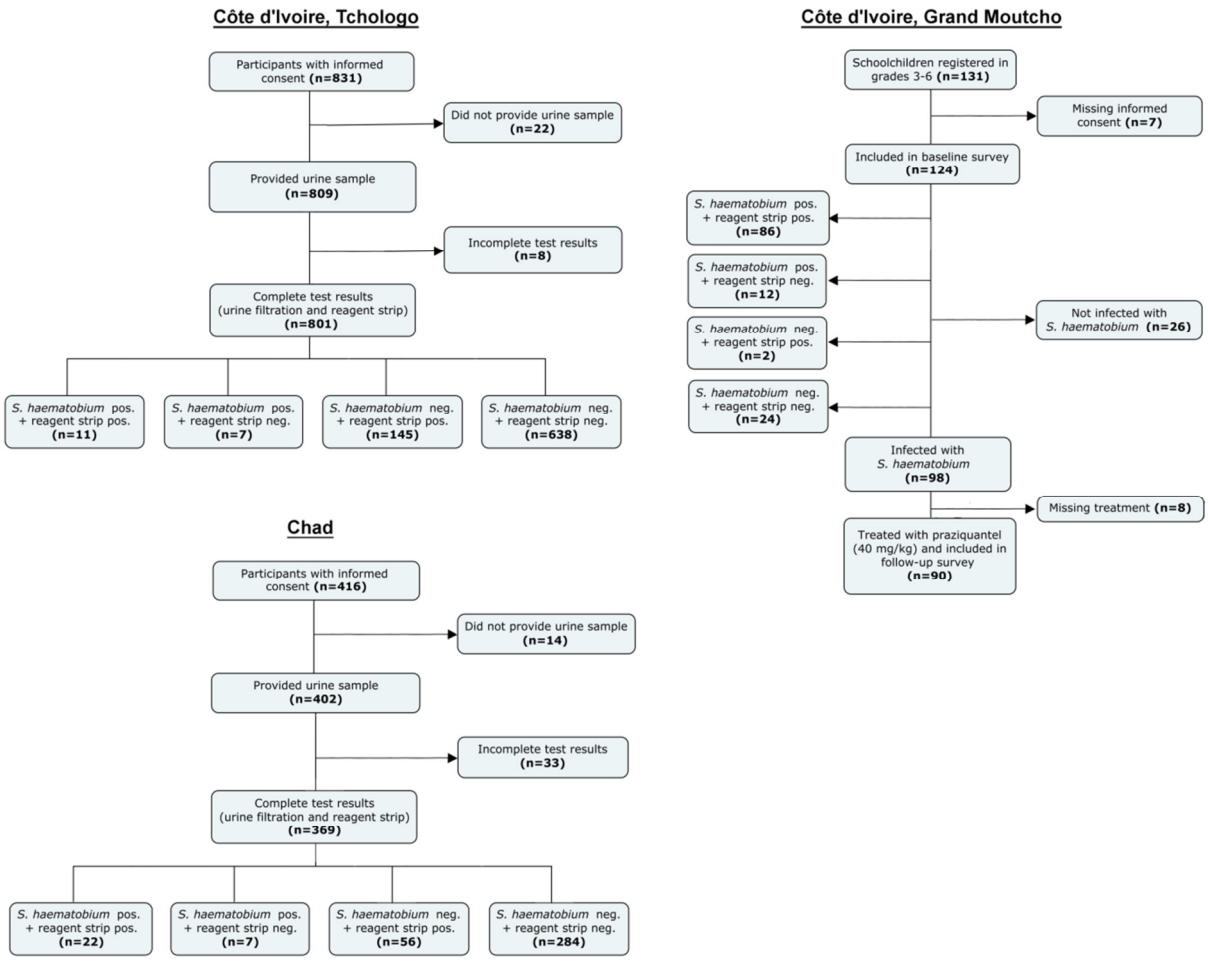

Figure 10.1. Flowchart of participation, sample provision, and diagnostic tests performed in the three study sites Côte d'Ivoire (Tchologo and Grand Moutcho) and Chad.

Performance of reagent strip testing compared to urine filtration

In our studies, reagent strip testing resulted in reasonable sensitivities of 61.1% (north Côte d'Ivoire), 75.9% (Chad) and 87.8% (south Côte d'Ivoire). Specificities were somewhat higher; 81.5% (north Côte d'Ivoire), 83.5% (Lake Chad) and 92.3% (south Côte d'Ivoire).

A random effects logit regression between reagent strip tests and urine filtration outcome with village included as random effect, revealed an odds of having a positive filtration when reagent strip

was positive of 7.4 (95% confidence interval (CI): 2.3-23.8) in northern Côte d'Ivoire, 86.0 (95% CI: 18.0-410.8) in southern Côte d'Ivoire and 20.7 (95% CI: 7.5-57.3) in Chad. The respective Kappa agreements between the filtration results and the reagent strip results all showed nearly perfect agreement; 0.81 (northern Côte d'Ivoire), 0.88 (southern Côte d'Ivoire) and 0.83 (Chad) (Landis, J. R. and Koch, G. G., 1977).

The positive predictive value (PPV), which indicates the likelihood (in %) of being infected with *S. haematobium* if tested positive for microhaematuria, differed greatly from one study to another (7.1% in north Côte d'Ivoire, 28.2% in Lake Chad area and 97.7% in south Côte d'Ivoire). The negative predictive value (NPV; likelihood of not being infected with *S. haematobium* if tested negative with reagent strip), on the other hand, was very high in all of our surveys. A detailed description of test performance in each of the three study sites including the follow-up surveys at Lake Chad are summarised in Table 10.1. The model predicted odds for having microhaematuria despite a negative urine filtration result at baseline were 7.1 in northern Côte d'Ivoire, 28.2 at Lake Chad and 79.0 in southern Côte d'Ivoire.

Table 10.1. Reagent strip test performance in the three study sites

Study	TP	FP	FN	TN	egg pos	MH	Sens.	Spec.	PPV	NPV
Côte d'Ivoire, Tchologo	11	145	7	638	2.2%	19.5%	61.1%	81.5%	7.1%	98.9%
Chad baseline	22	56	7	284	7.9%	21.1%	75.9%	83.5%	28.2%	97.6%
Chad 1st follow-up	4	24	2	190	2.7%	12.7%	66.7%	88.8%	14.3%	99.0%
Chad 2nd follow-up	1	7	1	68	2.6%	10.4%	50.0%	90.7%	12.5%	98.6%
Côte d'Ivoire, Grand Moutcho	86	2	12	24	79.0%	71.0%	87.8%	92.3%	97.7%	66.7%

TP, true positive (positive with reagent strip and filtration); FP, false positive (positive with reagent strip, negative with filtration); FN, false negative (negative with reagent strip, positive with filtration); TN, true negative (negative with both, reagent strip and filtration); egg pos, egg positive in filtration method; MH, microhaematuria positive; Sens., sensitivity; Spec., specificity; PPV, positive predictive value; NPV, negative predictive value.

Test performance by prevalence

Data obtained in our studies and data from the Cochrane systematic review showed that, regardless of the setting, around 15-20 % of the subjects had microhaematuria whenever the prevalence of *S. haematobium* was below 21 %. Above this prevalence level, microhaematuria increased in parallel to *S. haematobium* (Figure 10.2).

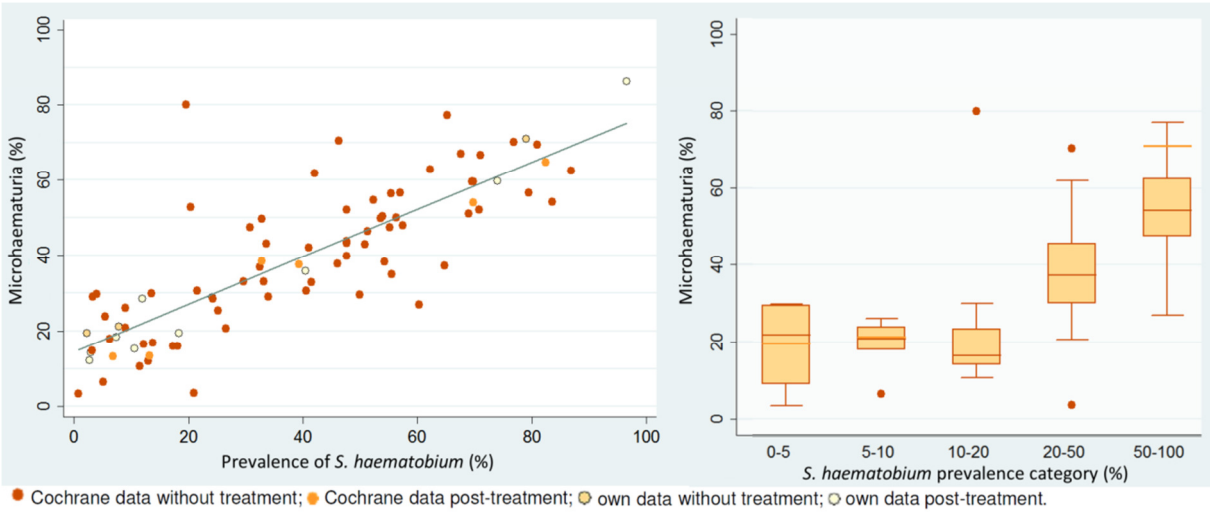

Figure 10.2. Average microhaematuria over *S. haematobium* prevalence from all surveys as scatter plot and box plot. Light-orange lines in the box plot refer to data from our three surveys.

While sensitivity and specificity were relatively stable over various prevalence levels, PPV and NPV are inherently dependent on prevalence (Figures 10.3 and 10.4). However, the percentage of microhaematuria seemingly unrelated to *S. haematobium* was stable over different prevalence ranges when taken into account that it will be hidden for higher prevalences.

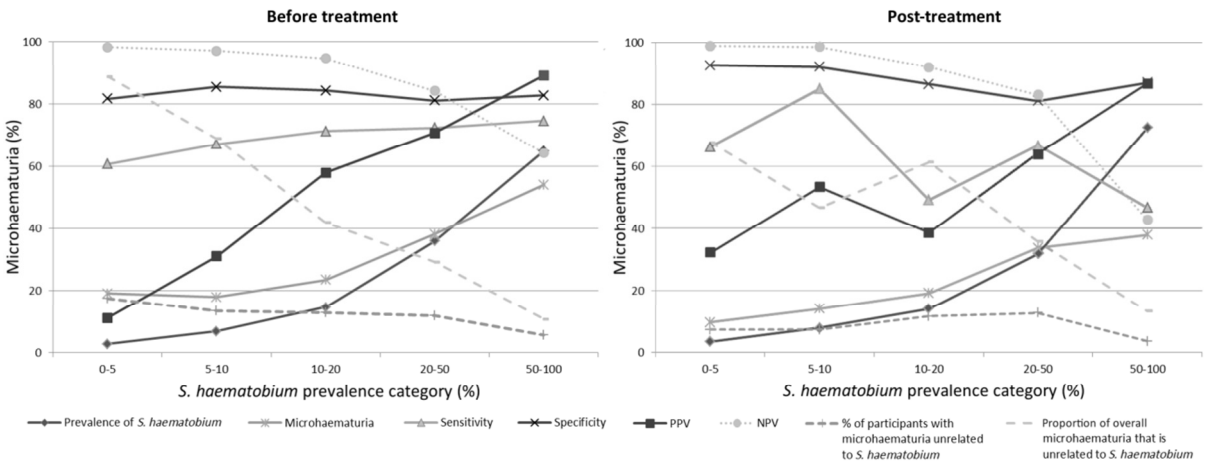

Figure 10.3. Test parameters (arithmetic mean of all studies) at different prevalence categories before and after treatment. Each of follow-up survey in our studies was counted as separate survey.

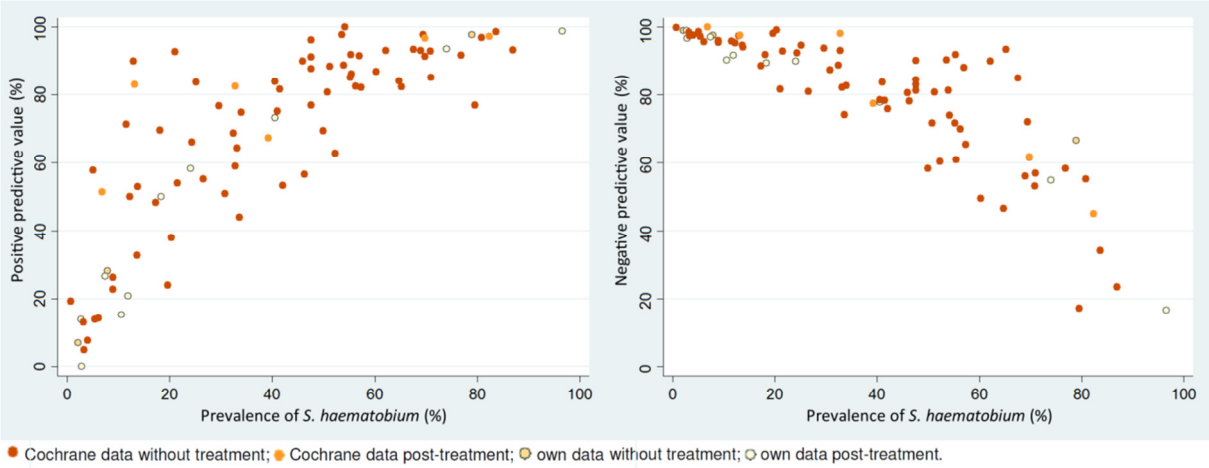

Figure 10.4. Positive and negative predictive values over *S. haematobium* prevalence.

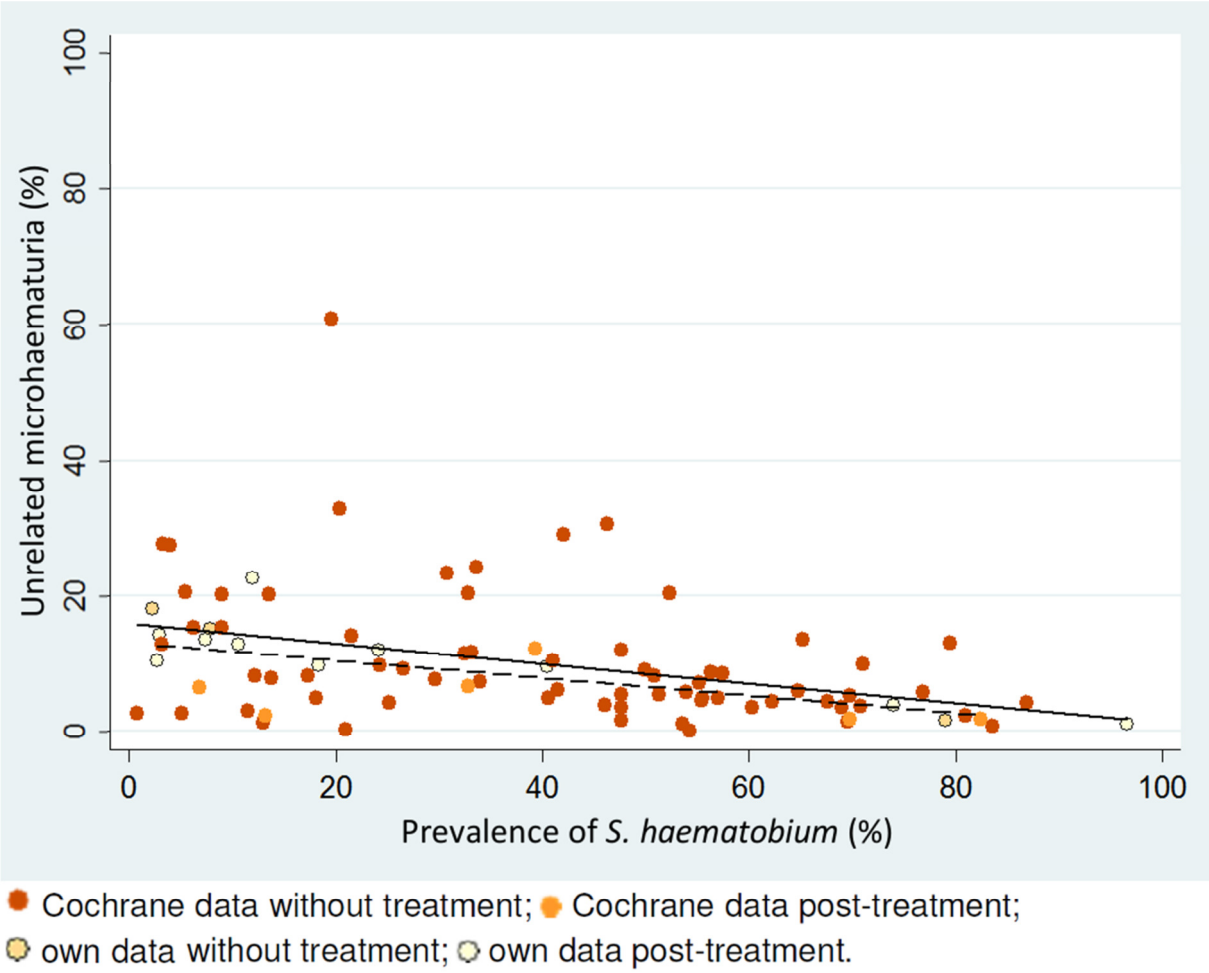

Figure 10.5. Microhaematuria not associated with *S. haematobium*, stratified by *S. haematobium* prevalence in the study. Solid regression line, all studies; dashed regression line studies post-treatment.

Treatment prior to testing did not substantially alter the picture and seemingly unrelated microhaematuria, although slightly less, shows the same pattern across prevalence ranges (Figure 10.5).

When the dynamics of microhaematuria was examined over an 8-week period post-treatment for children who tested positive for *S. haematobium* at baseline and were given a single 40 mg/kg oral dose of praziquantel, it is revealed that, while the prevalence of microhaematuria drops about similarly as the egg output over time, the level of microhaematuria seemingly unrelated to *S. haematobium* egg output quickly reached the same overall background level as found in other studies. The model-predicted odds for having microhaematuria despite a negative filtration result, reached a stable level after the first week post-treatment (Figure 10.6).

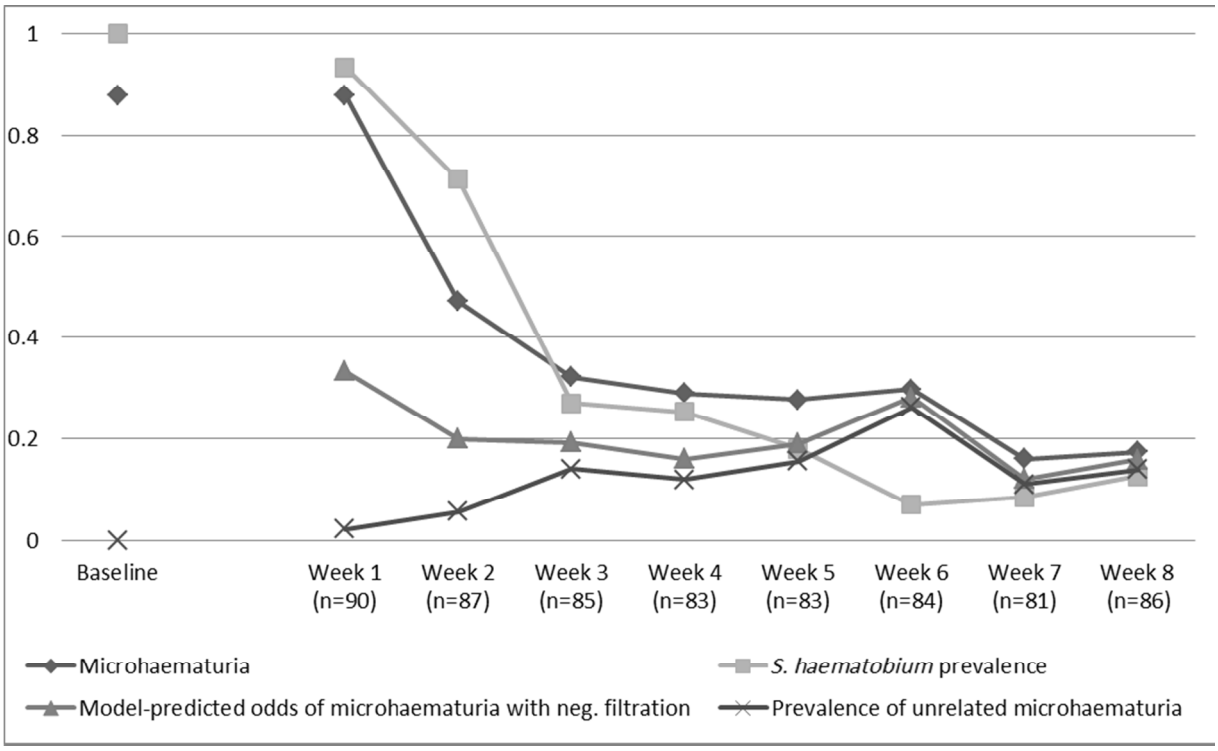

Figure 10.6. Dynamics of microhaematuria and model-predicted odds of microhaematuria unrelated to *S. haematobium* over an 8-week period post-treatment of all positive participants.

Furthermore, our data indicate that this seemingly unrelated microhaematuria is mostly independent of gender. Although females consistently showed slightly higher levels of microhaematuria unrelated to *S. haematobium* than males in our studies from northern Côte d'Ivoire and Chad, this gender difference was only marginal over all age groups with the exception of women and men aged 45 years and above (Figure 10.7).

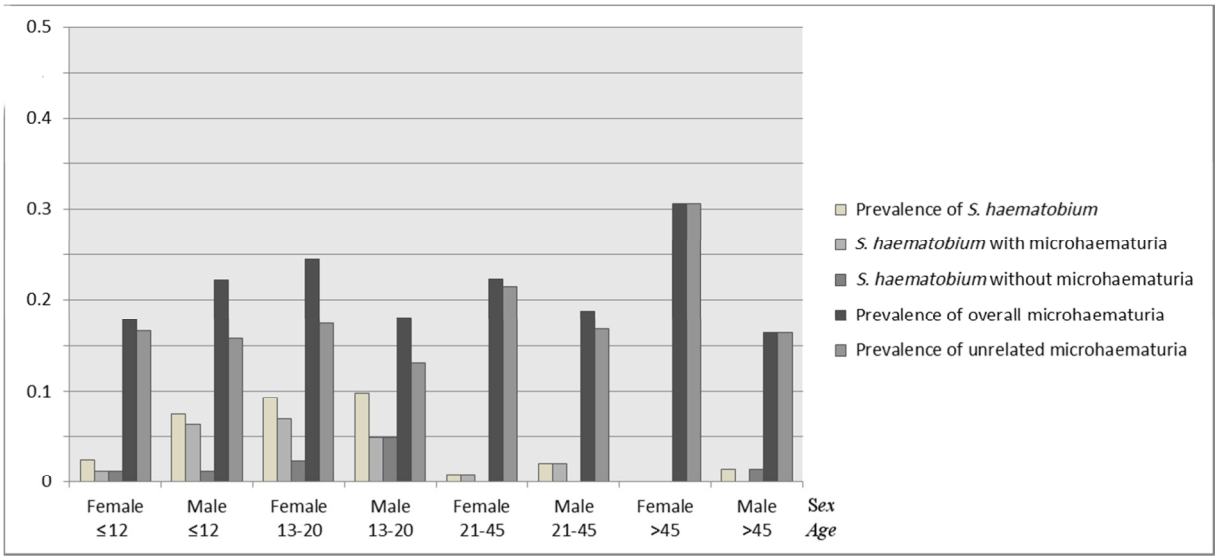

Figure 10.7. Unrelated microhaematuria, *S. haematobium* prevalence with and without associated microhaematuria and overall prevalence of microhematuria by sex and age-group in northern Côte d'Ivoire and in the baseline survey in Chad.

Theoretical assessment of the likelihood of missed cases as explanation for seemingly unrelated microhaematuria

If we assume that all cases of "unrelated" microhaematuria can be explained by true *S. haematobium* cases that were missed with urine filtration, the expected prevalence of "unrelated" microhaematuria could be calculated as follows:

Expected true prevalence of *S. haematobium* times the probability to miss a remaining infection.

One way to control for the true prevalence is to consider post-treatment studies only. Yet, although it reduces infection intensity, treatment with praziquantel does not always completely cure an infected individual. Hence, the following assumptions were considered:

① 18 % of treated individuals are not fully cured (Keiser, J.*et al.*, 2010);

② 20 % of low intensity *S. haematobium* cases are missed by urine filtration (note, the 20% difference in case-detection stems from a single compared to triplicate urine filtrations in a lightly infected study group) (Kosinski, K. C.*et al.*, 2011); and

③ 5.7 % *S. haematobium* cases present without microhaematuria (note, arithmetic mean of *S. haematobuim* infections without microhaematuria from all post-treatment studies from the Cochrane systematic review and our own data, excluding one study of the Cochrane review with an exceptionally high number of *S. haematobium* cases without microhaematuria.

The expected prevalence of FP reagent strips would consist of the uncured *S. haematobium* cases (presenting with microhaematuria) missed by urine filtration. Hence: ① + ② + (1 - ③) = 0.18 * 0.2

* (1-0.057) = 3.4%. The remaining FP reagent strip results cannot rationally be attributed to missed cases of *S. haematobium*.

If we assume that half of the treatments do not completely cure *S. haematobium* infections, the same rationale would lead to an expected 9.4 % (0.2*0.5*(1-0.057)) of seemingly unrelated microhaematuria which could be explained by missed *S. haematobium* cases. Re-infection can of course increase the prevalence of *S. haematobium* after treatments. The level of re-infection in the included studies is unknown, but it is unlikely to change the numbers to a big extent.

10.5. Discussion

The current comparison of findings of *S. haematobium* eggs in urine and microhaematuria detected by reagent strips confirmed that the usefulness of microhaematuria as a proxy for estimating the community prevalence of urogenital schistosomiasis is influenced by the overall prevalence of *S. haematobium* (Lengeler, C.*et al.*, 1993, Birrie, H.*et al.*, 1995, van der Werf, M. J. and de Vlas, S. J., 2004, Kosinski, K. C.*et al.*, 2011, King, C. H. and Bertsch, D., 2013, Ochodo, E. A.*et al.*, 2015). The Kappa agreement between the two tests revealed very good agreement (>0.80). Moreover, the sensitivity and specificity of microhaematuria have repeatedly been found to be high enough for the reagent strip testing to be a valid diagnostic tool for *S. haematobium* at the community level. These parameters are considerably influenced by the association of *S. haematobium* infection with microhaematuria (94.3%). However, PPV was very low in low-prevalence settings indicating that either large fractions of microhaematuria in such settings are unrelated to schistosomiasis or that the true prevalence of *S. haematobium* in these settings is grossly underestimated. NPV and PPV are well known to be prevalence-dependent with lower PPV and higher NPV the lower the prevalence of the disease in a given setting (Altman, D. G. and Bland, J. M., 1994, Heston, T. F., 2011). In this sense our findings on the NPV and PPV are not novel, yet, they imply that a positive reagent strip test would not necessarily relate to a positive *S. haematobium* result in very-low prevalence settings.

Our findings have several implications that are offered for discussion. First, regardless of the study settings, there seems to be some level of “background microhaematuria”, which is, at first glance, not directly related to *S. haematobium* infection. The average level of this seemingly unrelated microhaematuria was around 13% across settings and reported *S. haematobium* prevalences. The observation that background microhaematuria tends to decline with higher prevalence of *S. haematobium* (visualized in Figure 5) can be explained by the increasing probability to be infected with *S. haematobium*, which will hide any unrelated microhaematuria either because it is being attributed to schistosomiasis or because it co-occurs with *S. haematobium*-induced microhaematuria.

Some authors have argued that most of this background microhaematuria is due to undetected *S. haematobium* explained by the lack of sensitivity of widely used diagnostic tools (Bergquist, R.*et al.*, 2009, Utzinger, J.*et al.*, 2015). However, data from studies performed after praziquantel administration challenge this hypothesis. Indeed, after praziquantel administration, the number of seemingly unrelated microhaematuria attributable to missed *S. haematobium* cases consist of individuals where treatment failed to completely clear infection (or perhaps explained by the presence of immature *S. haematobium* flukes that are only marginally affected by praziquantel or the occurrence of rapid reinfection). If 20 % of *S. haematobium* cases are missed by urine filtration (Kosinski, K. C.*et al.*, 2011) and 18 % of positive *S. haematobium* cases treated with praziquantel are not fully cured (Keiser, J.*et al.*, 2010) and 5.7 % of *S. haematobium* infections present without microhaematuria, only 3.4 % (0.18 * 0.2 * (1-0.057)) of seemingly unrelated microhaematuria cases would be attributable to missed cases in post-treatment studies. The remaining unrelated microhaematuria of, say at least 10%, would remain unexplained and further studies are warranted to assess the cause of this microhaematuria. Potential aetiologies include bladder-stones or urinary tract infections and sickle cell disease as well as persistent bladder lesions from cured *S. haematobium* infections (Benbassat, J.*et al.*, 1996, Hatz, C. F.*et al.*, 1998, Tomson, C. and Porter, T., 2002, McDonald, M. M.*et al.*, 2006). Truly FP reagent strip results have also been reported, and are thought to be caused by the presence of semen in urine (Mazouz, B. and Almagor, M., 2003). However, some studies suggest that cure rates of *S. haematobium* infections after praziquantel vary greatly depending on the study and the diagnostic effort and can be as low as 50 % (King, C. H.*et al.*, 2000, Liu, R.*et al.*, 2011) which would indicate that the majority of the background prevalence that we found throughout settings would indeed be explained by *S. haematobium* infections missed by urine filtration. If this assessment holds, it would mean that there is a persisting *S. haematobium* prevalence of around 10-15% in settings which were characterised to have lower prevalences. In turn, this would change the current picture about schistosomiasis burden of disease as well as the evaluation of the success of schistosomiasis control programmes. Indeed, a recent study on a promising high-sensitivity diagnostic tool based on the detection of a circulating anodic antigen (UCAA) did find a prevalence of 13.3 % of *S. haematobium* with the antigen test when urine filtration found a prevalence of only 3.3 % and reagent strip testing found a prevalence of 4.1 % (Knopp, S.*et al.*, 2015).

Due to the lack of a 'gold' standard in *S. haematobium* diagnosis, it cannot be ascertained 100 % whether this background haematuria results from missed *S. haematobium* cases or from alternative causes of microhaematuria. The level of seemingly unrelated microhaematuria did not differ much between males and females for different age groups, excluding the explanation of unrelated microhaematuria caused by pregnancy or menstruation. If the observed background haematuria is

due to alternate causes such as bladder-stones or urinary tract infections (Benbassat, J.*et al.*, 1996, Tomson, C. and Porter, T., 2002, McDonald, M. M.*et al.*, 2006), it follows that individual treatment-decision targeting *S. haematobium* should not merely be based on reagent strip results, particularly in low-prevalence settings.

In either case, researchers, health care personnel and disease control managers need to be aware that in settings with a *S. haematobium* prevalence below 20 %, especially in settings targeted for elimination, a positive reagent strip test should always be followed up with urine filtration or better with other, more sensitive, diagnostic assays. Moreover, in view of the likelihood to miss an infection, which is, in addition, higher in low-intensity infections, and the fact that egg output shows a considerable day-to-day fluctuation (Lengeler, C.*et al.*, 1993, Lwambo, N. J.*et al.*, 1997, Vinkeles Melchers, N. V.*et al.*, 2014), the follow-up diagnosis should contain multiple samples over consecutive days.

Taken together, while reagent strip testing remains a valid tool for rapid assessment of community prevalence of *S. haematobium* (Lengeler, C.*et al.*, 1993, Mtasiwa, D.*et al.*, 1996, Robinson, E.*et al.*, 2009) or even for individual diagnosis (Mott, K. E.*et al.*, 1985, Taylor, P.*et al.*, 1990, Ochodo, E. A.*et al.*, 2015), one has to consider the epidemiological setting in which the test is executed as well as the goals of a schistosomiasis control and elimination programme or the specific research questions.

Praziquantel is a safe and efficacious drug that is recommended for preventive chemotherapy against schistosomiasis (WHO, 2002, Knopp, S.*et al.*, 2013). As preventive chemotherapy is escalating and schistosomiasis elimination is the new goal (Rollinson, D.*et al.*, 2013, WHO, 2015b), settings characterized by very-low prevalence and intensity of *Schistosoma* infection are becoming the norm rather than the exception and this has important ramifications for the use of diagnostic assays.

Conclusion

Irrespective of the true cause of the persisting background prevalence of microhaematuria – be it missed *S. haematobium* cases or alternate causes for microhaematuria – the overwhelming implication of our findings and those of other researchers, is that there is a need for more accurate diagnostic tools (higher sensitivity and higher specificity) if we indeed want to aim for elimination of schistosomiasis in selected settings (Knopp, S.*et al.*, 2013, Rollinson, D.*et al.*, 2013, Knopp, S.*et al.*, 2015, Utzinger, J.*et al.*, 2015). New research and funding efforts should target the well-known weaknesses of currently available diagnostic assays for urogenital schistosomiasis.

Competing interests

The authors declare that they have no conflict of interest concerning the work reported in this paper.

Authors' contributions

SJK, HG, KS, JTC, LYA, JZ, EKN and JU designed the study; SJK, HG, KS, JTC, SIT, BNRN and LYA implemented the study; SJK, HG and KS managed the data; SJK, HG and KS analysed and interpreted the data; SJK wrote the first draft of the paper; HG, KS, JTC, SIT, BNRN, LYA, JZ, EKN and JU revised the paper. All authors read and approved the final version of the manuscript before submission.

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11. The spatial and seasonal distribution of *Bulinus truncatus, Bulinus forskalii* and *Biomphalaria pfeifferi,* the intermediate host snails of schistosomiasis, in N'Djamena, Chad

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11.1. Abstract

There is a paucity of epidemiological and malacological data pertaining to schistosomiasis in Chad. In view of a recently articulated elimination agenda, a deeper understanding of the spatio-temporal distribution of schistosomiasis intermediate host snails is pivotal. We conducted cross-sectional malacological surveys during the dry season (April/May 2013) and after the short rainy season (October 2013) in N'Djamena, the capital of Chad. Snails were identified at the genus and species level using morphological keys and molecular DNA barcoding approaches. Those belonging to *Bulinus* and *Biomphalaria* were examined for cercarial shedding. Snail habitats were characterised and their predictive potential for the presence of schistosomiasis intermediate host snails explored. Seasonal patterns were studied using geographical information system and kriging in order to interpolate snail abundance data to make predictions at non-sampled locations across N'Djamena. Overall, 413 *Bulinus truncatus*, 369 *Bulinus forskalii* and 108 *Biomphalaria pfeifferi* snails were collected and subjected to cercarial shedding. During the dry season, one *Bu. truncatus* of 119 snails collected shed *Schistosoma* spp. cercariae (0.84%), while *S. mansoni* was shed by one of 108 *Bi. pfeifferi* snails (0.93%). None of the snails collected after the rainy season shed *Schistosoma* spp. cercariae. The abundance of *Bu. truncatus* and *Bu. forskalii* showed an inverse U-shape relationship with the square term of conductivity, i.e. low abundance at the lowest and highest levels of conductivity and high abundance at intermediate levels. *Bi. pfeifferi* showed a negative, linear association with pH in the dry seasons. It is planned to link these intermediate host snail data to infection data in human populations with the goal to draw a predictive risk map that can be utilised for control and elimination of schistosomiasis in N'Djamena.

Keywords: *Biomphalaria pfeifferi*, *Bulinus forskalii, Bulinus truncatus,* Chad, geographical information system, intermediate host snail*,* kriging, schistosomiasis, spatio-temporal distribution.

11.2. Introduction

Schistosomiasis represents the most important snail-transmitted disease with an estimated global burden of 3.3 million disability-adjusted life years (Murray, C. J.*et al.*, 2012). According to recent calculations put forth by the World Health Organization (WHO), 243 million people in 52 countries require periodic treatment with the antischistosomal drug praziquantel (WHO, 2012a). WHO recently announced the goal of schistosomiasis elimination by 2025 (WHO, 2012a). Transmission of schistosomiasis is entrenched into social-ecological systems (Utzinger, J.*et al.*, 2011) with a characteristic, focal distribution of the disease due to its obligate snail intermediate host that requires human contact with contaminated freshwater bodies to close the parasite's life cycle. In

Africa, *Schistosoma haematobium* and *S. mansoni* are the two predominant species parasitising humans and the intermediate hosts are freshwater snails of the genus *Bulinus* and *Biomphalaria*, respectively (Colley, D. G.*et al.*, 2014). *S. bovis* poses a considerable veterinary public health problem, since it is the key species infecting livestock (Moné, H.*et al.*, 1999). To assess local transmission of these parasites, an appraisal of intermediate host snail distribution, climatic suitability and human water contact patterns are essential (Brooker, S., 2002, Pedersen, U. B.*et al.*, 2014).

To our knowledge, there are no published malacological survey data available for Chad and very little data on human prevalence in school-aged children. A national survey carried out in 2000 revealed a country-wide prevalence of 13.2% for *S. haematobium* and 1.0% for *S. mansoni* among school-aged children (Beasley, M.*et al.*, 2002). More recently, Alio et al. (2013) reported a somewhat higher prevalence of 3.4% for *S. mansoni*. For the peripheral neighbourhoods of the capital N'Djamena, a *S. haematobium* infection prevalence of 11.8% was reported in the mid-1990s (Massenet, D.*et al.*, 1995). For N'Djamena itself, a prevalence of 2.6% for *S. haematobium* (Beasley, M.*et al.*, 2002) and nil for *S. mansoni* (Alio, H. M.*et al.*, 2013) have been reported.

To deepen our understanding of the spatio-temporal distribution of snail-borne diseases, epidemiological and malacological investigations are important. Previous research has shown that microhabitat factors influence the presence and abundance of intermediate host snails (Utzinger, J.*et al.*, 1997a). Water temperature in lentic environments and current velocity in lotic environments are among the most important abiotic factors identified to date (Appleton, C. C., 1978, Utzinger, J.*et al.*, 1997b). Several abiotic water factors are affecting snail habitats, and thus influence snail presence and abundance. Conductivity, a measure of the total solids and dissolved ions in the water, and pH have been suggested to be of importance for the distribution of *Bulinus* and *Biomphalaria* (Kader, A. A., 2001, Kariuki, H. C.*et al.*, 2004, Kazibwe, F.*et al.*, 2006), while turbidity and oxygen are of lesser relevance (Abdel-Malek, E., 1958, Appleton, C. C., 1978). Additionally, biotic factors are associated with the presence or absence of intermediate host snails, most importantly different species of aquatic vegetation (Boelee, E. and Laamrani, H., 2004, Chingwena, G.*et al.*, 2004, Owojori, O. J.*et al.*, 2006). Yet, there is a paucity of knowledge about limiting factors for snail habitat preferences, and hence, malacological studies are required to fill this gap (Adema, C. M.*et al.*, 2012).

For predictive risk mapping of schistosomiasis and intermediate host snail distribution, geographical information system (GIS) and remotely sensed satellite data have been shown as useful tools at different spatial and temporal scales (Brooker, S., 2002, Simoonga, C.*et al.*, 2009, Schur, N.*et al.*, 2011, Standley, C. J.*et al.*, 2012, Stensgaard, A. S.*et al.*, 2013). However, the relatively coarse spatial resolutions (e.g. 1 x 1 km) of freely available data do not allow for small-scale prediction with covariates.

The purpose of this study was to determine the spatio-temporal distribution of schistosomiasis intermediate host snails in N'Djamena, Chad and to assess for *Schistosoma* infection in snails. We pursued an integrated eco-geographical approach, consisting of malacological surveys, freshwater habitat characterisation, GIS and kriging. Surveys were conducted both in the dry season and after the short rainy season to generate spatially explicit risk maps for schistosomiasis transmissions. Our findings are of contemporary relevance, particularly in view of defining a schistosomiasis elimination agenda, as expressed by Rollinson et al.(2013).

11.3. Material and methods

Study area and water-contact activities

N'Djamena (geographical coordinates 12° 6' 47" N latitude and 15° 2' 57" E longitude) is the capital of Chad. It is administratively divided into 10 districts with a total population estimated at 1 million in 2009 (GeoHive, 2014). In the south-west, the Chari River and the Logone River form a natural border to Cameroon. The climate of N'Djamena is semi-arid with a short rainy season from June to September and a long dry season with peak temperatures recorded in April, when the monthly average is above 40 °C (measured at the N'Djamena airport weather station).

The water levels of rivers and ponds within the city are highly variable. During the rainy season, depending on the amount of rainfall, rivers might flood adjacent dwellings. After the rainy season in 2012, the Walia district, situated between the Chari and Logone rivers in the south-eastern part of the city, experienced serious flooding. During the dry season, the Chari River bed consists of hundreds of puddles and ponds of varying sizes. There is extensive vegetation (grass, bushes, trees and aquatic plants), which is intensively used by humans and animals. Common activities include cloth washing and bathing, mud brick production, grazing of herds of cattle, sheep and goats, and agricultural activities (e.g. urban farming). Within the city limits, the ponds nearly disappear towards the end of the dry season. The lack of sanitation, keeps the river bed contaminated with human and animal excreta. Only the Ndjaré canal, which divides N'Djamena from east to west, still contains surface water during that time.

Design

The sampling was conducted during the dry season, from mid-April to mid-May 2013 and after the rainy season in October 2013. The surveyed area in N'Djamena extends over a surface of 11 x 16 km (Fig. 11.1). A systematic sampling approach was employed to define, in advance, the sampling points of three different water systems in N'Djamena: the Chari River, the Ndjaré canal and selected city

ponds. Pre-defined sampling points were determined using Google Earth version 7.1.2.2041 (Google Inc.; Mountain View, USA) after which coordinates (in WGS 1984, latitude and longitude) were transferred onto a global positioning system (GPS) device (Garmin eTrex 10; Olathe, USA) to facilitate geolocation in the field. Restricted areas and private dwellings were subsequently excluded.

For the sampling along the river, a random starting point was chosen on the northern river shore. After every km, a transect line (see Fig. 11.1, red line) was placed perpendicular to the previous and next point across the river. All water points within 100 m to the left and right from this transect line were used for sampling and defined as a transect (see Fig. 11.1, orange band). The canal sampling points were chosen every 500 m and collections were carried out either at the right or at the left bank within a buffer of 50 m of the pre-defined point. The larger ponds, which contained water during the dry and rainy seasons, were sampled once on the western and once on the eastern bank. During the second sampling shortly after the rainy season (October), the sampling sites were chosen as close as possible to the previous sampling sites. Due to the different water levels between seasons, the second sampling sites were not always at exactly the same locations, but as close as possible to the sampling sites during the dry season (April/May).

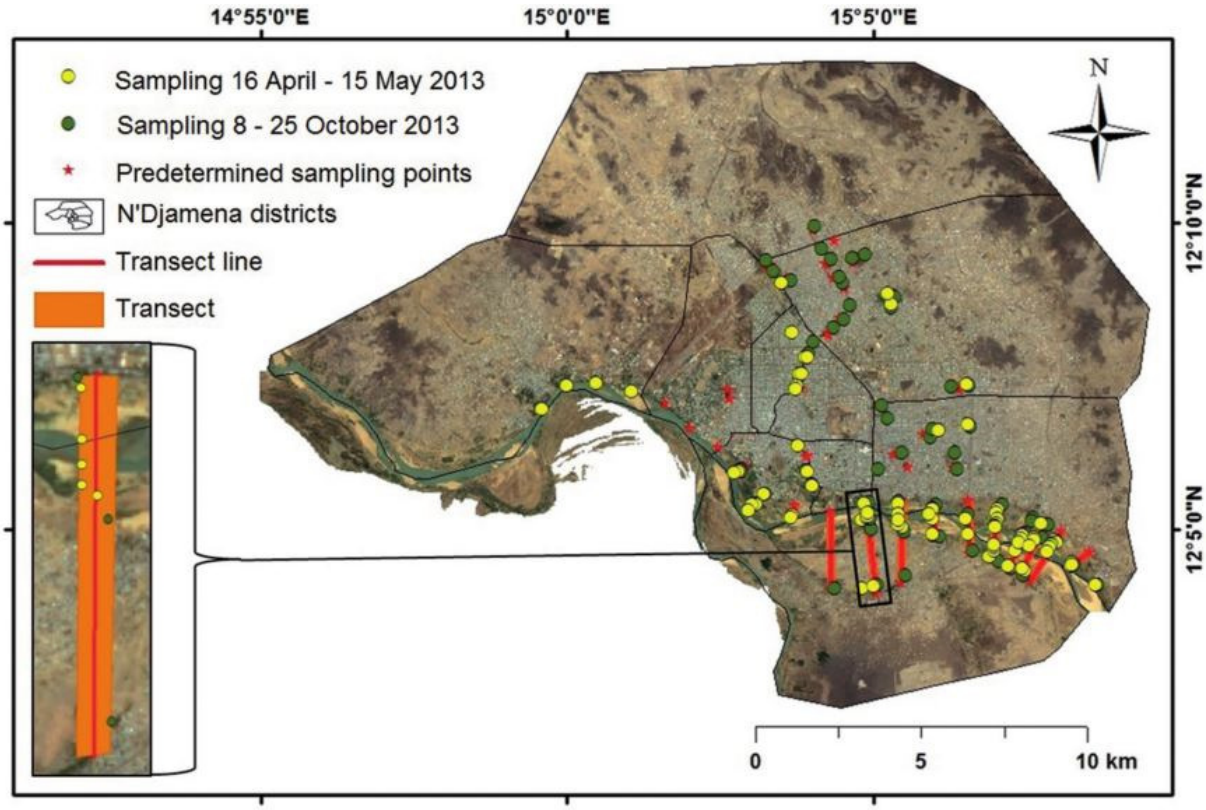

Figure 11.1. Study design and sampling sites at the end of the dry season (April/May 2013) and after the rainy season (October 2013) in N'Djamena, Chad.

Sampling

Snail surveys were carried out by the first author, adhering to standard protocols. In brief, snails were collected for 15 min at each site either with a scoop (Mandahl-Barth, G., 1962) or with forceps to remove them from aquatic plants. All collected snails were transferred to the laboratory of the "Institut de Recherches en Élevage pour le Développement" in N'Djamena, in labelled Petri-dishes on wet cotton. In the laboratory snail were identified a genus or species level, subjected to cercarial shedding and shell sizes were measured (in mm) using calipers. Snails of the genera Bulinus and Biomphalaria were identified using a morphological key (Mandahl-Barth, G., 1962). All snails were fixed in 70% ethanol and transferred to the Swiss Tropical and Public Health Institute (Swiss TPH) in Basel, Switzerland. Representative specimens of the genera Bulinus and Biomphalaria were sent to the Natural History Museum (NHM) in London, UK for species identification by molecular characterisation. Total genomic DNA was isolated using the "DNeasy Blood and Tissue kit" (http://www.qiagen.com/) and eluted into 200 µl purified water. A polymerase chain reaction (PCR) amplification of a partial cytochrome oxidase 1 (cox1) sequence was performed using primers LCO1490 (5'GGTCAACAAATCATAAAGATATTGG3' forward) and HCO2198 (5'TAAACTTCAGGGTGACCAAAAAATCA3' reverse) (Folmer, O.*et al.*, 1994). PCR investigations and sequencing conditions were chosen as outlined by Kane et al. (2008). The electropherograms produced were checked and *cox1* sequences edited using Geneious, version 5.6 (http://www.geneious.com/). Sequences were checked by performing BLAST searches via the National Center for Biotechnology Information against GenBank and EMBL sequence databases; and aligned with reference material (Kane, R. A.*et al.*, 2008) using Geneious version 5.6.

At around 11 a.m., living, sampled snails were placed individually in 24-well plates filled with tap water and placed under artificial light to induce shedding. After 3-4 hours (Steinauer, M. L.*et al.*, 2008), each well was examined for the presence of cercariae under a stereo microscope (magnification: 12-25 x). All cercariae found were transferred onto a slide, inspected with a compound microscope (magnification: 4x /0.10 and 10x /0.25) and identified by the morphological characteristics using the identification key of Frandsen and Christensen (1984).

Environmental data

The following water parameters were recorded using a portable multimeter (Hach®, HQ40D, Loveland, USA) for temperature (°C), pH, conductivity (µS/cm) and oxygen (mg/l; data only available for the end of the rainy season). The turbidity (FNU) was measured with a portable turbidimeter (Hach®, 2100P ISo). Several different habitat characteristics were noted, including the type of habitat

(flowing river, river bed puddle, canal and city pond), vegetation (aquatic, subaquatic and detritus) and surrounding growth (trees, bushes and grass).

Spatial and statistical analysis

Two satellite images, one taken during the dry season (23 May 2013) and the other taken after the short rainy season (30 October 2013), were acquired from the Landsat 8 for the Universal Transverse Mercator (UTM) zone 33 N (path 184 row 52), which includes N'Djamena. The eight bands of the satellite images were processed and pan sharpened to a resolution of 15 x 15 m. Shapefiles of the extent of water surface were created using ArcGIS version 10.1 (ESRI; Redlands, USA). As no images from the Landsat 7 and 8 were available for the maximum water surface extent, available Google Earth images spanning a 3-year period ending in 2013 were compared. The image obtained on 23 October 2012 was chosen. Indicator kriging using ArcGIS was performed for three snail species (*Bu. truncatus*, *Bu. forskalii* and *Bi. pfeifferi*), using punctual sample data (Guimaraes, R. J.*et al.*, 2009) to interpolate and estimate snail abundance at non-sampled areas of N'Djamena.

Comparisons were stratified by type of habitat (river versus city). The Wilcoxon rank-sum test was used to compare the mean snail shell sizes and additionally the mean of the water parameters at sites with presence of Bu. truncatus between seasons. The snail species compositions of the habitats were assessed by Fisher's exact test.

Whether the presence of snails showed a habitat shift between seasons was assessed using exact logistic regression with habitat (city versus river), season (dry versus end of rainy season) and interaction between habitat and season taken as a factor. Ordinary logistic regression was used to assess the association of water parameters with the presence and absence of snail species. The models were adjusted for habitat types (city versus river) and season. For each quantitative predictor variable, we assessed whether linearity could be assumed for its influence on the logit of snail abundance. This was done by categorising the variable or by adding a square term. Among the different parameterisations, the one with the lowest Akaike information criterion (AIC) was chosen. Due to the small number of observations, a maximum of two parameters were kept and parameters which did not improve the model in terms of AIC were removed. All statistical analyses were done with STATA version 12.1 (Stata Corporation; College Station, USA).

Ethical considerations

The study protocol was approved by the ethics committees of Basel (EKBB; reference no. 64/13) and Chad (reference no. 343/MSP/SE/DGAS/2013). For the transfer of snails from Chad to Switzerland, a

material transfer agreement was obtained from the "Institut de Recherches en Élevage pour le Développement" (reference no. 626/PR/PM/MDPPA/SG/IRED/2013).

11.4. Results

Snail presence, abundance and infection

The following snail species were identified morphologically and confirmed by molecular characterisation *Bu. truncatus* (Audoin, 1827), *Bu. Forskalii* (Ehrenberg, 1831) and *Bi. pfeifferi* (Krauss, 1848). Additionally another snail of medical importance - *Lymnaea natalensis* (Krauss, 1848) - was identified morphologically only. While the presence and abundance of Bulinus and Biomphalaria were estimated, with regard to *L. natalensis*, only the presence/absence at each sample site was noted. Overall, 144 sites (Fig. 11.1) were sampled for snails and characterised physico-morphologically and chemically; 83 during the dry season and the remaining 61 after the rainy season. In 51 of the sampled sites, a total of 892 snails were collected: 413 (46.3%) *Bu. truncatus*, 371 (41.6%) *Bu. forskalii* and 108 (12.1%) *Bi. pfeifferi* (Table 11.1).

Table 11.1. Abundance and size (mm) of *Bulinus truncatus*, *Bulinus forskalii* and *Biomphalaria pfeifferi*, collected in the dry season (April/May 2013) and at the end of the rainy season (October 2013) in N'Djamena, Chad.

Season	Species	Abundance		Size		
		No (%)	Infected snails (%)	Mean (mm)	Standard deviation	Range (mm)
Dry	*Bu. truncatus* [1,2]	119 (52.0)	1 (0.84)	7.4[3]	2.5	3.0-12.5
	Bu. forskalii	2 (0.9)	0			
	Bi. pfeifferi [2,4]	108 (47.1)	1 (0.93)	3.3	0.5	2.0-4.5
Rainy	*Bu. truncatus* [1]	294 (44.3)	0	6.4 [3]	1.3	3.0-11.5
	Bu. forskalii [4]	369 (55.7)	0	6.7	1.9	3.5-15.0

[1]Significant seasonal difference in the spatial distribution city *versus* river (P = 0.01 for interaction term between habitat and season in an exact logistic regression model);
[2] Significant positive association with abundance of *L. natalensis* (Fishers exact test P <0.001);
[3] Significant difference in mean between the dry and wet season (Wilcoxon sum-rank test, P <0.001);
[4] Significant positive association with abundance of *Bu. truncatus* (Fishers exact test P <0.01)

The species composition was dependent on the season. Whilst *Bu. truncatus* was present in high numbers during both rainy and dry seasons, *Bu. forskalii* and *Bi. pfeifferi* showed seasonal preferences. During the dry season, *Bu. truncatus* snails were found only in ponds at the riverbed at 14 collection sites with a mean number of 8.3 snails (n = 119, standard deviation (SD) 10.9, range 1-

40). At the end of the rainy season this species was found at 10 sites in the city (n = 282) and three at the river (n = 12) with a mean number of 22.6 snails (SD 23.8, range 1-61). We found a high seasonal variation of mean snail numbers (P = 0.074). In April-May, *Bu. forskalii* was lowest in number with only two specimens, collected at different sites along the river. However, after the rains ceased in October, *Bu. forskalii* was the predominant species with an average of 18.9 snails (SD 16.8, range 1-52) collected per site within the city. The opposite was observed for *Bi. pfeifferi*, as this species was only collected in the dry season with a mean of 10.7 snails (SD 16.6, range 1-59) at 11 sampling sites along the river.

All *Bu. truncates* were examined for cercarial shedding (Table 11.1). One of the 119 snails collected during the dry season shed *Schistosoma* spp. cercariae (infection rate 0.84%; 95% confidence interval (CI) 0.02-4.6%), whereas *S. mansoni* was present in one of the 108 *Bi. pfeifferi* specimens analysed (infection rate 0.93%; 95% CI 0.02-5.0%). No Schistosoma infections were found in snails during the collection at the end of the rainy season. However, a larger number of snails in both seasons were infected with several other cercariae species, the majority amphistome cercariae.

Structure and composition of snail populations

The recorded shell sizes of the intermediate host snails showed seasonal differences. As shown in Table 1, the average shell size of *Bu. truncatus* collected in April/May was highly significantly larger than that of the snails collected in October (7.4 mm versus 6.4 mm, P <0.001).

Most of the snail populations at the sample sites during the dry season at the Chari River and at the end of the rainy season within the city were composed of several species. The results of the Fisher's exact test, including all sampled sites of the dry season, showed that the presence of *Bu. truncatus* was positively associated with the presence *Bi. pfeifferi* (P <0.001) and *L. natalensis* (P <0.001) at the river. The presence of *Bi. pfeifferi* showed a significant positive association with *L. natalensis* (P <0.001) in the dry season. At the end of the rainy season, the abundance of *Bu. Forskalii* was significantly associated with *Bu. Truncates* (P <0.001).

Water data

Specific water parameters for the presence of each snail species were recorded (Table 11.2). For habitats containing *Bu. truncatus*, the pH changed from April/May (mean = 6.3) to October (mean = 8.3) and this increase showed a highly statistically significant difference (P <0.001). We found a borderline significant difference in turbidity (218.8 versus 55.9 FNU) according to season (Wilcoxon rank-sum, P = 0.065). The pH and turbidity showed no significant difference between seasons after

stratification by habitat (city *versus* river), however the conductivity was significantly different between the seasons (P = 0.020 after Bonferroni correction).

Table 11.2. Water parameters for habitats where schistosomiasis intermediate host snails were present.

Season	Species	Temperature (°C)			pH			Conductivity (µS/cm)			Turbidity (FNU)		
		Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Dry	*Bu. Truncates*	31.8	3.2	28.5-37.9	6.3[1]	0.6	5.6-7.6	217[2]	96	86-361	219[3]	273	21-1.000
	Bi. pfeifferi	30.7	1.7	28.1-34.1	6.1	0.5	5.7-7.6	228	138	86-525	170	289	21-1.000
Rainy	*Bu. truncatus*	29.2	3.0	25.4-33.6	8.3[1]	0.9	7.1-9.8	271[3]	139	52-489	56[2]	43	8-152
	Bu. forskalii	28.2	2.7	24.7-33.6	8.3	0.7	6.9-9.8	453	207	52-832	80	60	8-250

Observations: *Bu. truncatus* dry (n=14), *Bi. pfeifferi* dry (n=11), *Bu. truncatus* rainy (n=13), *Bu. forskalii* rainy (n=20); [1] Significant difference in mean between dry and rainy season (Wilcoxon rank-sum test, P <0.001) but not significant after stratification by habitat (city *versus* river); [2] Significant difference in mean between dry and rainy season after stratification by habitat (city *versus* river) (p = 0.02, after Bonferroni correction); [3] Marginally significant difference in mean between dry and rainy season (Wilcoxon rank-sum test P = 0.065) but not significant after stratification by habitat (city *vs.* river).

Logistic regression models

None of the models displayed a cluster effect (habitat: puddles, river, canal and city ponds). The interaction term of the exact logistic regression showed a significant difference of habitats (city versus river) between seasons (P = 0.01). In the model for both seasons, we found an inverse U-shape between the presence of *Bu. truncatus* and conductivity (P = 0.001). The model for *Bu. forskalii* included a quadratic term of conductivity (P = 0.030) and the respective functional form showed an inverse U-shape. Regarding the abundance of *Bi. pfeifferi* in its exclusive habitat (ponds and puddles) along the river, conductivity was not a significant predictor, while pH showed a significant negative association (P <0.001).

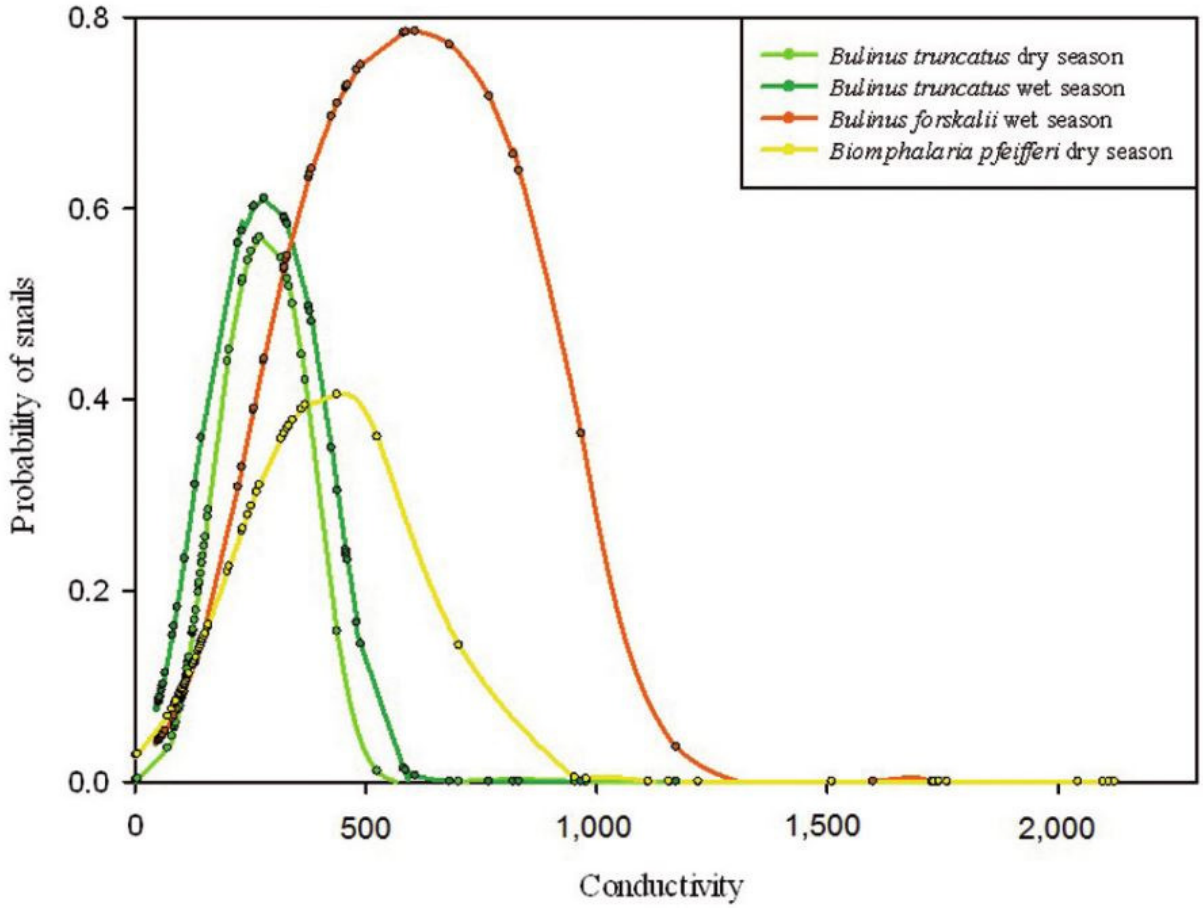

Figure 11.2. Logistic regression models for conductivity and the presence of snails.

Spatial distribution of intermediate host snails

The spatial distribution and spread of the aquatic snails in N'Djamena depends on the presence of water and therefore on the surface extent of the water bodies. During the dry season, the Chari River carries little water, and hence the sandy river bed partly dries up. The remaining puddles and ponds contain aquatic and subaquatic vegetation, creating suitable habitats for aquatic snails. Bu. truncatus snails were present at 12 out of 43 sites and *Bi. pfeifferi* snails were found in 10 out of 43 sites, in contrast to the flowing river where these snail species were found only at two sites and one site, respectively.

The water bodies in the city (ponds and canal) were either almost or fully dried out during the dry season and no snails were found. After the rainy season, the ponds and canal carried more water and the total water surface increased, thus providing potentially suitable habitats for aquatic snails. At five out of 14 sampling sites along the canal, Bu. forskalii snails were found. Bu. forskalii and Bu. truncatus were also detected at 14 out of 18 sampling sites at city ponds. There was a significant shift of Bu. truncatus from the river (puddles and flowing river habitats) into the city (canal and pond habitat) according to season (P = 0.011).

The results of the interpolated abundance of the snails, obtained from indicator kriging (Figure 11.3), showed a possible shift of the snail "hotspots" from the river to the city depending on the season. The kriged abundance maps displayed a high abundance for Bu. truncatus at the river during the dry season and in city ponds and the canal at the end of the rainy season. *Bi. pfeifferi* was only present along the river. Bu. forskalii was present in city water bodies after the rainy season, but this species was rarely found at the river sites. The boundary of the highest water extent in the 3 years preceding this study (23 October 2012) showed the potential habitat extent for aquatic snails. Because snail microhabitat preferences are restricted to shallow water with a depth less than 50 cm and a distance to the shoreline of 40 cm (Utzinger, J. and Tanner, M., 2000, Boelee, E. and Laamrani, H., 2004), the area between the boundaries of the highest water extent and the actual water surface extent shows the potential maximum spread of the snails within N'Djamena.

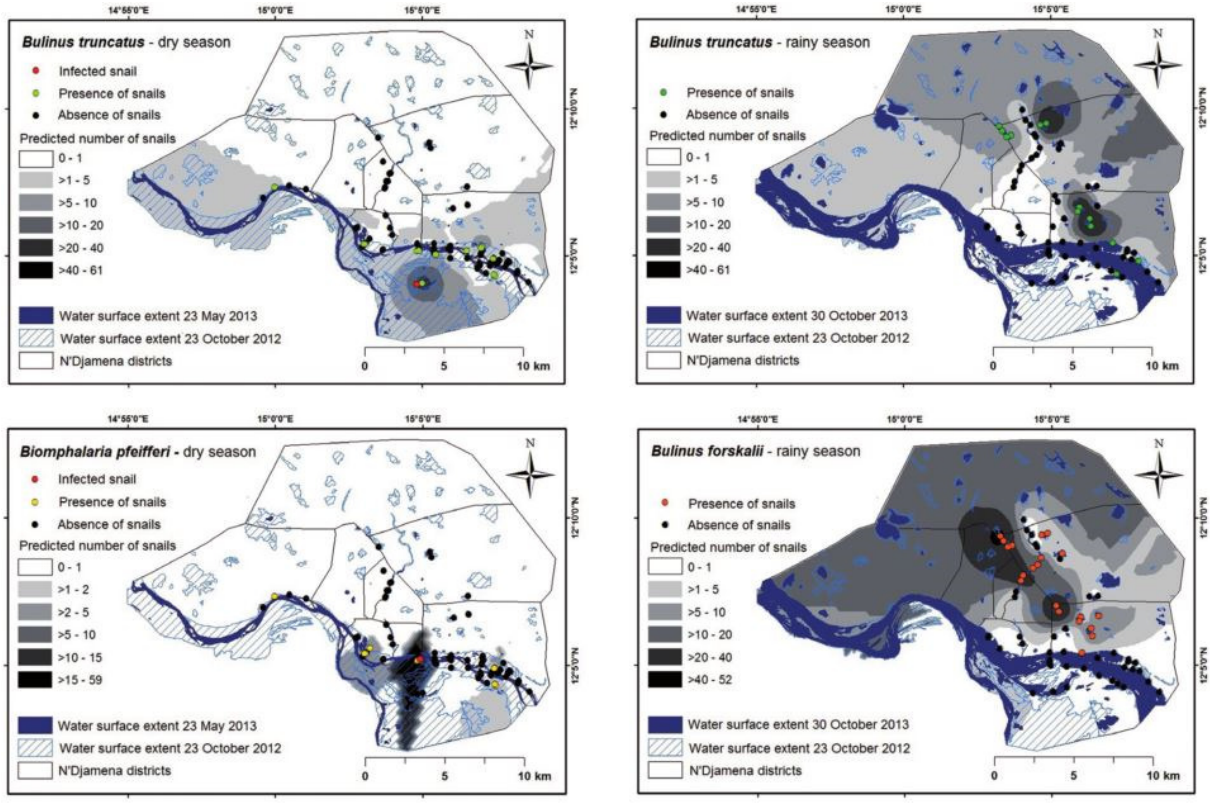

Figure 11.3. The estimated snail abundance for N'Djamena, Chad.

11.5. Discussion

During the dry season the residual puddles and ponds along the Chari River in N'Djamena provide suitable habitats for *Bi. pfeifferi* (Utzinger, J. and Tanner, M., 2000), as well as for the two Bulinus species. However, most of the ponds in the city were dry in the April/May survey, and hence no living snails were found. Importantly though, snails are able to aestivate at the bottom of dried ponds at a depth up to 3 cm in the soil (Betterton, C.*et al.*, 1988), and can rapidly repopulate when the rains

refill the ponds. Hence, when conditions become suitable, snail populations can rapidly restore, as has been observed for *Bu. truncates* and *Bu. forskalii* in the current study. After the short rainy season the surfaces of the Chari River reaches the highest level with fastest water velocity, which is a limiting factor for Bi. pfeifferi abundance (Utzinger, J.*et al.*, 1997b).

Snail population composition varied by seasons. For instance, Bu. truncatus was found in both seasons, with a higher abundance at the end of the rainy season within the city. Our results are in line with findings from Nigeria (Owojori, O. J.*et al.*, 2006), but are in contrast with observations made by Ngonseu et al. (1992) in Cameroon, where peak numbers of *Bu. truncatus* snails were reported at the end of the dry season. Studies conducted in Nigeria (Owojori, O. J.*et al.*, 2006, Ayanda, O. I., 2009) showed the same pattern for *Bi. pfeifferi* but a different pattern for Bu. forskalii, where the peak was observed in the dry season.

Only single specimens of *Bu. truncatus* and *Bi. pfeifferi* were found to shed *Schistosoma* cercariae, thus demonstrating low active transmission of schistosomiasis in N'Djamena. However, given the relatively small numbers of snails collected (slightly more than 100 specimens in the dry season), the confidence interval around the point estimates are quite large. Additional studies are needed and efforts should be made to collect larger numbers of snails to obtain more precise infection information with smaller accompanying confidence intervals. Despite this shortcoming, it is interesting to compare our results with findings by other researchers. Ngonseu et al. (1992) in the Sudano-Sahelian zone of Cameroon, reported low infection rates of *S. haematobium* in *Bu. truncatus* snails (1.2%), whereas Labbo et al. (2003) in Niamey, Niger found a several-fold higher infection rate (5%). In our study, the infection rate of *Bi. pfeifferi* was more than a magnitude lower compared to previous studies in urban (9.7%) (Njiokou, E.*et al.*, 2004) and rural parts of Cameroon (21.3%) (Ayanda, O. I., 2009). In our study, none of the 371 collected *Bu. forskalii* snails shed *Schistosoma* spp., whilst previous investigations carried out in Cameroon and Niger revealed, very low infection rates of 0.08% and 0.05%, respectively (Ngonseu, E.*et al.*, 1992, Labbo, R.*et al.*, 2007).

The number of *Bu. truncatus* snails correlated with *Bi. pfeifferi* and *L. natalensis* during the dry season, and with *Bu. forskalii* after the end of the rainy season. In a previous study conducted in Nigeria, Owojori et al. (2006) found an association between *Bu. truncatus* and *Bi. pfeifferi*. *L. natalensis* acts as intermediate hosts of the livestock (and human) trematodes Fasciola (Brown, D. S., 1994) and was found to be associated with the presence of *Bi. pfeifferi* and *Bu. truncatus* in N'Djamena.

The models across the seasons for *Bu. truncatus* and for *Bu. forskalii* with the lowest AIC included a quadratic term of conductivity with a negative coefficient, describing an inverse U-shape relation. Prior studies also suggest an association of the presence of *Bu. truncatus* and *Bu. forskalii* with

conductivity (Kader, A. A., 2001, Kariuki, H. C.*et al.*, 2004). The model with the lowest AIC for *Bi. pfeifferi* included a significant negative effect of pH, confirming previous observations by Kazibwe et al. (2006). Our results are based on relatively small sample sizes. Hence, only two parameters were included in each model, which leaves room for confounding effects. Conductivity might have a specific influence on the presence of the snails and should be assessed in future studies to specify the effect of con- ductivity. The abundance of snails is not depending on a single environmental factor, but is rather the result of complex interactions of multiple habitat factors (Utzinger, J.*et al.*, 1997a). Moreover, the air temperature has an impact on water temperature and therefore on the water parameters and depends on season, size of water body and daily change of the air temperature. The measurement of the water temperature was performed in parallel with the snail collection in the morning (between 7 a.m. and 11 a.m.). Given our limited financial and human resources, we were unable to measure daily variation. Further investigations studying diurnal changes of water temperature are warranted to adjust for water temperature and water parameters according to the exact sampling time.

GIS-based modelling to determine environmental requirements of intermediate host snails, coupled with parasitological data and advanced Bayesian geostatistical modelling have been utilised for predictive risk profiling of schistosomiasis (Kristensen, T. K.*et al.*, 2001, Brooker, S., 2002, Standley, C. J.*et al.*, 2012, Stensgaard, A. S.*et al.*, 2013). Such prediction maps can likely be enhanced by incorporating significant environmental covariates and aquatic factors using Bayesian inference. Since environmental data at a suitably high spatial resolution were not freely available, the snail abundance data were instead kriged to create a predictive map of the abundance of the different schistosomiasis intermediate host snails in N'Djamena. To further improve modelling attempts, environmental and aquatic factors (e.g. habitat types, ground substrate, turbulence, water velocity and water chemistry) should be considered as covariates. Our preliminary kriging maps, however, highlight the abundance of *Bu. truncates* and *Bi. pfeifferi* along the Chari River, the main abundance and transmission "hotspots" during the dry season and showed reasonable agreement with a previous report (Labbo, R.*et al.*, 2003). At the end of the rainy season, the main abundance for Bu. truncatus and Bu. forskalii shifted towards the city, where no infected snails were found.

The kriged maps provide an estimate of the real seasonal distribution and could provide an "auxiliary" tool (Guimaraes, R. J.*et al.*, 2009) for the planning of prevention, control, surveillance and elimination of schistosomiasis. The distribution of the intermediate hosts snail should be reflected by infection within the human population. Future studies should investigate the prevalence of Schistosoma spp. in school-aged children and other high risk groups in N'Djamena. Finally, predictive risk maps of N'Djamena, using data of human schistosomiasis and snail abundance, should be drawn

up for planning and implementing strategies to eliminate schistosomiasis in this major town in the Sahelian belt of Central Africa.

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Conflict of interest

None declared.

12. Discussion and conclusions

12.1. Overall Significance of the Research

This research was initiated through transdisciplinary processes which allowed to identify human and animal health priorities in the population under concern and which were translated into research questions. Thereby mobile pastoralists, local authorities and scientists engaged in a dialogue that yielded a list of local priorities. For example, liver fluke were stated as one of the most important prevailing animal health problem. In return, our results have direct practical applications for the mobile pastoralists and their livestock at Lake Chad. They also add much needed fundamental data for the development of mathematical transmission models and risk modelling that will guide the development of future public and veterinary public health interventions and policy. The spectrum of the work presented in this thesis is broad, incorporating conceptual and methodological theory and practice of One Health, local human and cattle trematode epidemiology, malacology, validation of diagnostic tools, investigations of access to health care and a test of anthelmintic treatment for humans and livestock. An array of methods were employed to address these questions, including transdisciplinary stakeholder processes, field epidemiology (see photos in Appendix 2), classical parasitological methods, new diagnostic tools adapted to a field laboratory, and statistical and geospatial analyses. Capacity building was an integral part of this work. Besides two Master of Science in Epidemiology thesis, one at Swiss TPH and one at IRED, the training of laboratory technicians at IRED and field workers in Chad supported research capacity in Chad. The training of local staff lead to good quality data and improved the institutional and human resource capacity for future related projects.

12.2. Innovation, Validation, Application

This dissertation pertains to the epidemiology, diagnosis and control of human and livestock schistosomiasis and fascioliasis in a mobile pastoralist setting on the eastern shores of Lake Chad. It was embedded in the concept of “innovation, validation, application”, which forms the foundation of Swiss TPH’s research and development activities. Innovation refers to the translation of ideas, concepts and methods into research findings, new tools and knowledge. Through validation, innovations are evaluated in the field to reveal their potential for application in the real world. Application refers to a validated innovation that can then be transformed into interventions and integrated into health systems and policies (Swiss-TPH, 2014). The contributions of this research to all three areas are listed in Table 12.1.

Chapters 3 and 4 are reporting on experiences in health research among mobile populations. They show how a One Health approach translates into benefits for human and animal health and adds value at the research, implementation and health economic level. Moreover, on the local scale, the epidemiology and the mutual predictive potential of human and animal trematode infections in mobile pastoralists and their cattle of four different ethnic groups is presented (chapters 5 and 6). We tested the performance of diagnostic tools for schistosomiasis in a field laboratory under extreme climatic conditions of the Sahel (chapters 9 and 10). Knowledge, attitude and practice regarding access to health care and use of treatment for human helminth infections and livestock fascioliasis were investigated, including a testing of anthelmintic drug active component and quantity as a proxy for drug quality (chapter 7). To our knowledge, ours was the first application of triclabendazole against fascioliasis caused by *F. gigantica* in naturally infected cattle in Chad (chapter 8). Finally, a malacological survey gave insight into the species composition of intermediate host snails and their seasonal abundance and distribution in N'Djamena (chapter 11).

As a further innovation, this work shows that the One Health approach cannot only contribute to generating added value in research and control of zoonotic diseases, but also in research and control of human and animal infecting pathogens that share similar ecological life traits.

Table 12.1. Stratification of research to the strategic axis of innovation, validation and application, the principles of Swiss TPH research activities

Chapter	INNOVATION	VALIDATION	APPLICATION
3 + 4 *The benefits of One Health for pastoralists in Africa* and *Health surveys among mobile populations*		Health research among mobile pastoralists is possible and highly effective when using a One Health approach. Here, validated results and applications and their benefits for pastoralists in Africa are are presented, which had evolved from research projects of the Swiss TPH and its partners.	
5 *Prevalence of Fasciola gigantica infection in slaughtered animals in south-eastern Lake Chad area in relation to husbandry practice*	Initiated by the mobile pastoralists concern, a first abattoir based survey on fascioliasis revealed its high veterinary importance in the Lake Chad area. Fascioliasis risk seems to be associated to husbandry practice.		
6 *The mutal predictive potential of human and animal trematode infections in a mobile pastoralists setting*	The first comprehensive investigation of intestinal parasitism among mobile pastoralists and their cattle, revealing culturally defined human and animal exposure patterns.		
7 *Treatment of human and animal helminth infections: access, common practice and active ingredient in drugs*		Despite active health seeking and treatment of livestock, outcome satisfaction was low. The most effective drugs against trematodes are not available in the study area.	Recommendations on the use of Praziquantel for human schistosomiasis treatment Role of informal market for drug dispensing for mobile pastoralists
8 *Rapid re-infection with Fasciola gigantica after triclabendazole treatment in cattle*		The first triclabendazole treatment applied against *F. gigantica* infections in cattle in a mobile pastoralist husbandry system	Recommendation for the use of Triclabendazole for fascioliasis treatment in cattle. Recommendation to initiate a regulatory trial in Chad
9 + 10 *Validation of a POC-CCA urine test for S. mansoni diagnosis in the Sahel* and *experiences with reagent strip testing for urogenital schistosomiasis*		Validation of the performance of a POC-CCA test for *S. mansoni* under Sahelian conditions. The validity of reagent strip testing for microhematuria as a proxy for *S. haematobium* infections was explored, specifically focusing on low prevalence settings.	
11 *The spatial and seasonal distribution intermediate host snails of schistosomiasis, in N'Djamena, Chad*	Survey on the species composition of intermediate host snails of *Schistosoma* and *Fasciola* in N'Djamena, Chad, and modelling of seasonal distribution and abundance.		

12.3. Research Outputs versus Objectives

This discussion section started with presenting the achievements of this PhD research. However, we could not achieve all objectives that were presented in Chapter 1. In the following section, the contribution of this research to each objective is reflected.

Objective 1: Human and animal trematode epidemiology in a mobile pastoralist setting

Objective 1 addressed trematode epidemiology in mobile pastoralists and their cattle by translating the One Health concept into a practical approach to human and animal schistosomiasis and fascioliasis in the field. In doing so, it provided the first high quality data on human and animal trematode infections in Chad. In humans, the survey comprised urine and stool sample analysis and was expanded to include an investigation on intestinal protozoa. It revealed that mobile pastoralists suffered most of schistosomiasis and intestinal protozoa infections. Urogenital schistosomiasis has previously been shown to be highly prevalent in other Sahelian countries (Garba, A.*et al.*, 2006). In this study, soil-transmitted helminths (STH) were found at much lower prevalence than in other studies from the area (Bechir, M.*et al.*, 2012a, Alio, H. M.*et al.*, 2013). This could partly be explained by the low sensitivity of the single Kato-Katz analysis applied in our field laboratory. However, 199 of the total 284 stool samples (70%) were analysed by two techniques, the single Kato-Katz and the ether-concentration method, and their combined results were used in the final analyses. The contrasting findings may thus rather be explained by the fact that the other studies included also sedentary populations. These populations live in a slightly different social-ecological system, where different parasites and infection pressures may persist.

Schistosomiasis, the most prevalent helminth infection in our samples, is intrinsically linked to water related social-ecological systems and a multitude of interconnected factors (Utzinger, J.*et al.*, 2011). Our findings show that this is also true for fascioliasis and schistosomiasis in livestock. By using a systems epidemiology approach, we identified cultural and environmental factors influencing not only the prevalence of human schistosomiasis but also of livestock schistosomiasis and fascioliasis. Hence, socio-culturally husbandry practices determine human and livestock trematode transmission. For example the Buduma and Fulani pastoralists grazing their animals inside Lake Chad flooding area expose themselves and their animals to a higher risk of infection with schistosomiasis and fascioliasis than Arab and Gorane pastoralists who remain largely in the drylands surrounding the lake.

The high fascioliasis prevalence in cattle shown in our abattoir study was confirmed in live animals and revealed a similar distribution among the different husbandry systems practiced at Lake Chad (Jean-Richard, V.*et al.*, 2014a). *Schistosoma bovis* infection in cattle was found at high levels, equally in live and slaughtered animals. Besides a report from the 1970s, to the best of our knowledge, our

study provides the first recent data on bovine schistosomiasis from Lake Chad (Quéval, R.*et al.*, 1971). Not surprisingly therefore, there was limited awareness of the disease among Chadian livestock breeders and veterinarians. Its clinical manifestations in naturally infected animals are not well documented and are assumed to be subclinical and chronic (Calavas, D. and Martin, P. M., 2014). It therewith receives no veterinary attention and no treatment is administered (Webster, J. P.*et al.*, 2016). This leads to the conclusion, that pastoralists erroneously attribute all morbidity caused by bovine schistosomiasis to fascioliasis. Given its high prevalence shown in this study, the epidemiology of livestock schistosomiasis seems largely understudied in Africa today. Hardly any prevalence data could be found in the published literature and most publications only report on occurrence (Christensen, N. O.*et al.*, 1983, Moné, H.*et al.*, 1999, Stothard, J. R.*et al.*, 2004). More data on its molecular biology can be found than on its epidemiology (Webster, B. L.*et al.*, 2010). However, *S. bovis* is gaining attention since several researchers have shown hybridisation with the human infective species *S. haematobium* (Webster, B. L.*et al.*, 2013). The most prominent example was the 2013 schistosomiasis outbreak on the French island of Corsica that brought schistosomiasis back into Europe (Holtfreter, M. C.*et al.*, 2014). As Calvatas et al (2014) reminded in a letter, bovine schistosomiasis had been reported from Corsica until the 1960s which shows, that the intermediate host snails were present and the parasite's life cycle could be completed on the island. The findings of our One Health study revealed the geospatial co-occurrence of *S. haematobium, S. mansoni* and *S. bovis* in the Lake Chad area. Such co-occurrence could potentially promote hybridization of *S. haematobium* and *S. bovis*, leading to novel phenotypical properties in the hybrid parasites. This finding would not have been possible without the simoultaneous study of humans and cattle and hence demonstrates an added value in terms of incremental knowledge of an integrated human and animal health research approach. Research on existing or on-going hybridisation is of greath interest, specifically in the light of the elimination goal for schistosomiasis. A potential host switch could change the known epidemiology of human schistosomiasis. Elimination and control programs would then need to be expanded to include the animal hosts (Webster, J. P.*et al.*, 2016). More molecular biology work on these parasites is therefore encouraged. New valuable insight into parasite-host interactions can be obtained. Understanding the genetic information will also contribute to the identification of vaccine candidates and development of specific diagnostic tools and novel drugs (Alvarez Rojas, C. A.*et al.*, 2014, Lv, Z.*et al.*, 2015).

This epidemiological study provides baseline parasitological prevalence data, however, there are limitations. We could not establish the seasonality of infection as initially planned. Due to security issues connected to the activities of the terroristic group Boko Haram in the study zone, certain adaptations were necessary (Box 1). The initially planned cohort study with 3 follow-ups was transformed into a repeated cross-sectional study, combined with an open cohort study. During

three field visits, enrolled participants were followed-up and new groups were enrolled. This methodological change allowed us to collect sufficient data for the epidemiological study, but did not allow for an analysis of seasonal dynamics. However, it did show that a cohort study among mobile pastoralists is feasible thanks to the fast growing mobile communication network in the area and the ubiquitous use of mobile phones (Jean-Richard, V.*et al.*, 2014c). During follow-up, mobile communication allowed us to contact group leaders in advance to announce our planned visit and to coordinate field trips. Additionally, the development of the mobile communication network in the area will now contribute to future studies by providing a statistically appropriate sampling frame. In co-operation with the representatives of the mobile pastoralists of all ethnic groups we can now develop a list with the mobile phone numbers of all group leaders of the different ethnic groups in the zone. This list fulfils the theoretical assumption of a simple random sample, and will allow us to randomly select and enrol groups in future studies (Schelling, E. and Hattendorf, J., 2015).

Box 1: Research in a conflict zone

Our research had to adapt to local conflicts caused by the periodic insurgence of Boko Haram terrorists who indiscriminantly targeted civilians and authorities in the Chadian capital N'Djaména and rural areas in the Lake Chad area by threats including attacks on villages and suicide bombings. The field work had to become conflict sensitive by permanent information and communication on the security status and reducing activities during time periods of highest risks. The longstanding partnership between Chadian institutions (CSSI and IRED) and Swiss TPH allowed to sustain activities throughout risk periods thanks to a permanent communication and flexibility of operations. Hence our work confirms similar experiences and practices adopted during the civil war in Côte d'Ivoire (Bonfoh, B.*et al.*, 2011b).

Objective 2: Disease Knowledge, Treatment and Control

The findings of our studies on treatment of human schistosomiasis and livestock fascioliasis (chapters 7 and 8) support hypothesis number 2 (page 22), stating that the current control measures for the two trematode infections are not effective and adequate treatment is absent in the study area.

Initiatives to fight NTDs have increased in recent years and considerable progress towards control and elimination has been made (WHO, 2015a). A key element to disease control and prevention are safe and efficacious drugs such as praziquantel (Tambo, E.*et al.*, 2015). The control programmes of human schistosomiasis have delivered remarkable results by reducing prevalence and incidence in highly affected areas, specifically in Asia. In Africa, lacking access to praziquantel and resources for implementation of treatment have been identified as the major factors hindering the launch of

schistosomiasis control programs in the past (Fenwick, A.*et al.*, 2009, Hotez, P. J.*et al.*, 2010). In Niger, the neighbouring country of Chad, a national schistosomiasis control program was launched by the schistosomiasis control initiative (SCI) in 2004. The program started at the same time in two other Sahelian countries, namely in Burkina Faso and Mali, as well as in Uganda (Garba, A.*et al.*, 2009). A first evaluation three years into these initiatives supported the success and feasibility of such programs in West Africa (Garba, A.*et al.*, 2006). Today, mass drug administration (MDA) without individual diagnosis is widely practiced and shows impressive results in reducing schistosomiasis prevalence, also in sub-Saharan Africa (Webster, J. P.*et al.*, 2014). Donors support planning and intervention of control programs and the pharma industry (Merck KGaA) is donating praziquantel for preventive chemotherapy programs until schistosomiasis is eliminated. Elimination is now the declared goal of WHO (WHO, 2012a, 2015b).

In contrast, no schistosomiasis control programme is in place in Chad to date (Figure 1.5). Our study revealed that praziquantel is not accessible for the population at Lake Chad and is not even available at rural health centres (chapter 7). Interestingly, albendazole and mebendazole was available at health centres, at village markets and with doctor Choukou. This indicates that drug distribution systems, both authorized and informal, are in place. Future schistosomiasis control program at Lake Chad could thus be developed using existing distribution systems. Combined MDAs of praziquantel and albendazole are widely supported and are also recommended for the Lake Chad area (WHO, 2015a). The introduction of praziquantel to the area would target several parasitic diseases our research has revealed to be endemic in the area. It is highly effective against schistosomiasis, but also *Hymenolepis nana* can be treated (Panic, G.*et al.*, 2014).

Similar, triclabendazole, is a highly effective treatment for livestock fascioliasis and can be administered as a single-dose treatment, which is ideal for a mobile population (Tambo, E.*et al.*, 2015). This research showed that triclabendazole is not available in the study area and beyond. The same appears to be the case for East Africa, Nigeria and Sudan (pers. Comm. N. Elumu) (Keyyu, J. D.*et al.*, 2009). Our triclabendazole treatment in cattle at Lake Chad revealed a rapid re-infection during the rainy season. To identify seasonal occurrence of fasciolisis transmission, a field trial covering all seasons will be needed to better understand treatment efficacy and re-infection dynamics in mobile pastoralist husbandry systems at Lake Chad.

Schistosomiasis patients regularly reported that treatment received at health centres lead to a short improvement period of 2 to 3 days before the symptoms appeared again. This suggests that at health centres or with doctor Choukou, the symptoms are attributed to bacterial urinary tract infection and antibiotic treatment is administered. An appraisal at the health centres in the study area and interviews with the health workers was not done within this study, but would add important

information on current diagnostic possibilities, disease knowledge and treatment strategies at health centres. One health centre chief referred a patient who had presented with haematuria to our field laboratory. He had taken this decision due to the lack of diagnostic possibilities and praziquantel at his health centre. This underlines the importance of new diagnostic tools such as RDTs and POC tests(Utzinger, J.*et al.*, 2015). Our findings underline the persisting importance of the informal sector and doctor Choukou in health service providing in remote areas at Lake Chad (Wiese, M., 2000, Hampshire, K., 2002b, Schelling, E., 2002, Gauthier, B. and Wane, W., 2011, Djimouko, S. and Mbairo, P., 2014). Interviews with doctor Choukou should be considered in future research. Despite them being untrained in medical procedures and certainly causing harm with inappropriate treatments, they represent a potential for health delivery to mobile populations that should not be ignored (Schelling, E., 2002). Finally, the assessment of disease knowledge, access to and use of anthelmintic treatment at patient and health provider level would have strongly benefited from the input of social scientists, specifically to refine the methodology. Nevertheless, our results do provide useful background information about knowledge, attitude and practice regarding human schistosomiasis and livestock fascioliasis, on which further research questions can be built.

Objective 3: Diagnostics

In view of the elimination goal for schistosomiasis and the limited capacities of the health system in areas where most NTDs are endemic, we cannot emphasize enough the need and importance of point-of-care, easy-to-use, cheap and accurate diagnostic tools (Utzinger, J.*et al.*, 2011). Such tools are also of high importance in low prevalence settings and settings with advanced intervention programs, when MDA will be replaced by a test-and-treat strategy (Utzinger, J.*et al.*, 2015). The point-of-care circulating cathodic antigen (POC-CCA) urine cassette test for the detection of *S. mansoni* infections shows all these qualities and has been validated in resource-constrained settings (Colley, D. G.*et al.*, 2013, Bergquist, R.*et al.*, 2015). During three field work phases, our study team has performed 795 POC-CCA tests in a mobile field laboratory under trees, often by temperatures above 40°C. Its simple procedure and clear interpretation could be validated in the harsh environmental conditions found in the Sahel. However, due to the very low prevalence of *S. mansoni* infections revealed by the coprology survey, our results obtained from POC-CCA testing could not be used to systematically test its performance under the Sahelian conditions. As became clear during later work, a more promissing validation test could be planned in N'Djamena. During the training of the laboratory technicians we had identified several cases of *S. mansoni* infection among voluntary providers of stool samples, which demonstrates that *S. mansoni* infection is present in N'Djamena. Since the climate in N'Djamena is comparable to that at Lake Chad, parallel testing could be done

under a tree and in an air-conditioned laboratory at recommended temperatures. However, among the few participants whose sample produced a positive POC-CCA test results we had observed several pregnant women and could reproduce a positive POC-CCA test result twice in one of two pregnant women with no history of exposure to schistosomiasis. A potential cross-reactivity with non-schistosome related metabolites in pregnant women's urine cannot be excluded. Systematic research on this aspect will further strengthen the usefulness and safe application of the POC-CCA urine cassette test.

In contrast, no comparable rapid diagnostic test is available for the diagnosis of *S. haematobium* infection today. Instead, the detection of blood in urine, a typical symptom of urinary schistosomiasis, is widely used for rapid detection of the disease. A simple reagent strip test can be performed, which is feasible without any laboratory equipment and its interpretation is straight-forward. The usefulness of this method for the diagnosis of urinary schistosomiasis has been repeatedly assessed and confirmed in comprehensive reviews (King, C. H. and Bertsch, D., 2015, Ochodo, E. A.*et al.*, 2015). Yet, microhaematuria can also result from several other diseases such as urinary tract infections, tumours or, important for endemic settings, sickle cell anaemia (Tomson, C. and Porter, T., 2002, McDonald, M. M.*et al.*, 2006). Most published surveys using the methodology showed on average 13% of positive reagent strip results that could not be related to urine filtration-confirmed *S. haematobium* infection. This finding remained also in studies reporting results from post-treatment surveys. In view of the elimination goal of schistosomiasis, our findings have major implications and underline the urgent need for new, additional tools for the direct diagnosis of schistosomiasis infection with a higher sensitivity than today's urine filtration method and a higher specificity than the reagent strip test.

Objective 4: Mutual predictive potential of human and animal trematode infection

Our findings reveal a pattern of similar levels of trematode infection in humans and cattle within ethnic groups. This pattern is not visible at group level. Only at a certain aggregation level a pattern can be identified. This has been shown before in human and livestock studies on zoonotic brucellosis in Kyrgyzstan zoonotic diseases, in studies about brucellosis (Bonfoh, B.*et al.*, 2012). The identified disease distribution points to a close interrelation of mobile pastoralists and their cattle and underlines the multitude of factors associated to these diseases in the specific social-ecological system. Exposure to environmental risks of disease are shared by humans and animals, since humans and animals move together and have one basic need in common: the need for water. This exposes both humans and animals to the, from a public health perspective, most important water-based disease, namely schistosomiasis and fascioliasis (Steinmann, P.*et al.*, 2006). In the study setting, the

Gorane pastoralists were least affected. This can be explained by their strict use of traditional wells for their own water consumption as well as for watering their livestock. At a recent stakeholder meeting that took place in January 2016, we could present and discuss these results with the mobile pastoralists and representatives of the ministry of public health and ministry of livestock production.

12.4. Applications of Research

The direct practical applications of the research can be summarized by the following statements.

- In the frame of our research we have set up a mobile field laboratory using solar powered microscopes that has been shown to be highly functional. This mobile field laboratory is now managed by IRED and can be deployed for further parasitological research in rural areas of Chad.
- The simple, safe, and sensitive POC-CCA urine cassette test can be used in Sahelian conditions and should be further promoted for rural health centres should.
- Although the majority of anthelmintic drugs tested contained more than the labelled amount of active ingredients, the use of available products at Lake Chad is safe and effective against albendazole and mebendazole sensitive helminths. No falsified anthelmintic drugs were identified.
- Praziquantel should be used for the treatment of human schistosomiasis and triclabendazole for the treatment of livestock fascioliasis. Both drugs should be introduced to the area.
- Community health education activities and control programs at Lake Chad should be more appropriately targeted by taking into account de distinct husbandry practices and other behavioural differences between ethnicities.

12.5. Outlook

A deeper understanding of the transmission dynamics of snail-borne trematodes in humans and animals will allow for optimizing intervention strategies with the goal to interrupt transmission (Keiser, J. and Utzinger, J., 2010, Utzinger, J., 2012, Bergquist, R.*et al.*, 2015, Zinsstag, J.*et al.*, 2015b). Targeted interventions can only be developed if the ecology of the disease is well understood (Webster, J. P.*et al.*, 2016). In the specific case of human and animal trematode infections, which additionally have a zoonotic potential, an ecological understanding of the disease is crucial to identify the level at which transmission interruption could be initiated (Zinsstag, J.*et al.*, 2015b). The transmission of trematode infections in the social-ecological system of mobile pastoralist husbandry

systems, livestock fascioliasis and schistosomiasis elimination could potentially be reached through constant transmission reduction over a long term (Gray, D. J.*et al.*, 2010). Potential leverage points can include decreasing egg contamination of the environment through strategic temporal treatments. But also behavioural adaptations, adapting water contact patterns and husbandry system could be efficient. Molluscicides have been applied as a further instrument in transmission interruption strategies. The multitude of factors involved in trematode transmission open a large number of possibilities for potential future intervention strategies. Williams et al (2002) showed a pictorial model of the transmission cycle and possible interventions for the zoonotic *S. japonicum,* which illustrated the facette of potential intervention points (Figure 12.1).

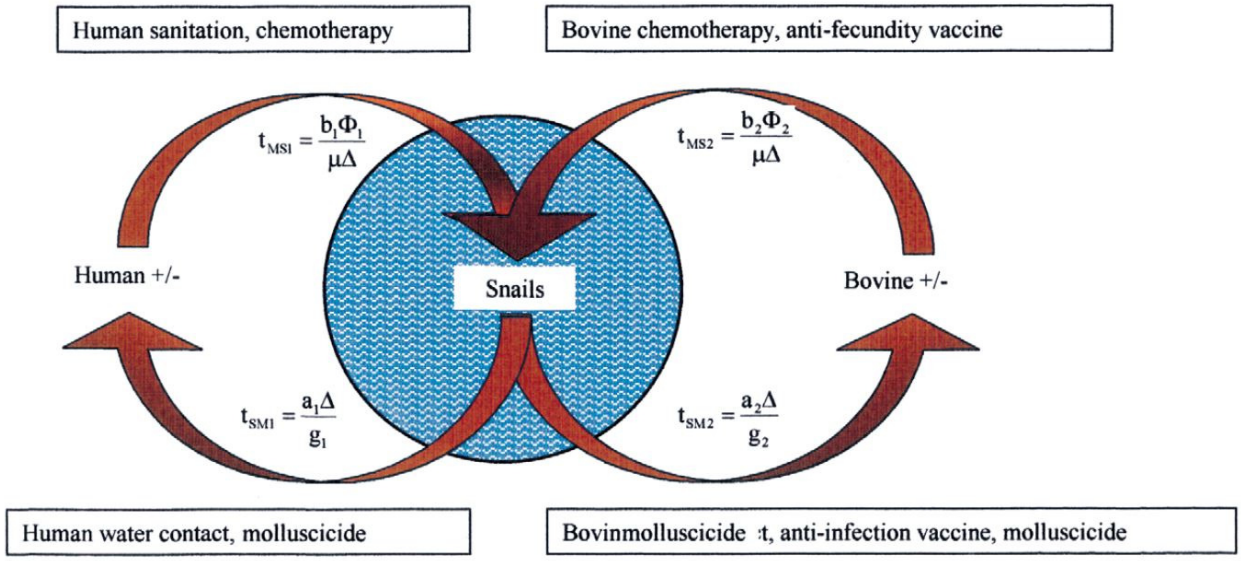

Figure 12.1 Pictorial transmission model of *Schistosoma japonicum* transmission and control (Williams et al. 2002)

The development of a mathematical transmission model allows for identifying most effective intervention strategies. Effective in terms of reduction of burden of disease, but also in regard to increase cost effectiveness of interventions. These may include different levels, be it the final host, intermediate host and the environment (Zinsstag, J.*et al.*, 2015b). A first transmission model for *F. gigantica* has been developed and is presented here in Figure 12.1. The epidemiological data collected during this research will contribute to estimate parameters of the model. Further data will be partly available from literature review and partly needs to be generated in forthcoming research projects. The mathematical transmission model will allow innovating interventions that can then be validated in the real world and hopefully lead to the identification of highly effective, sustainable interventions. In this way, we can work towards a systemic approach to parasite control considering con-linear dynamics in hosts, vectors, and parasites within a highly dynamic social-ecological system. Such examples contribute to a systems understanding at the biology, population and community level.

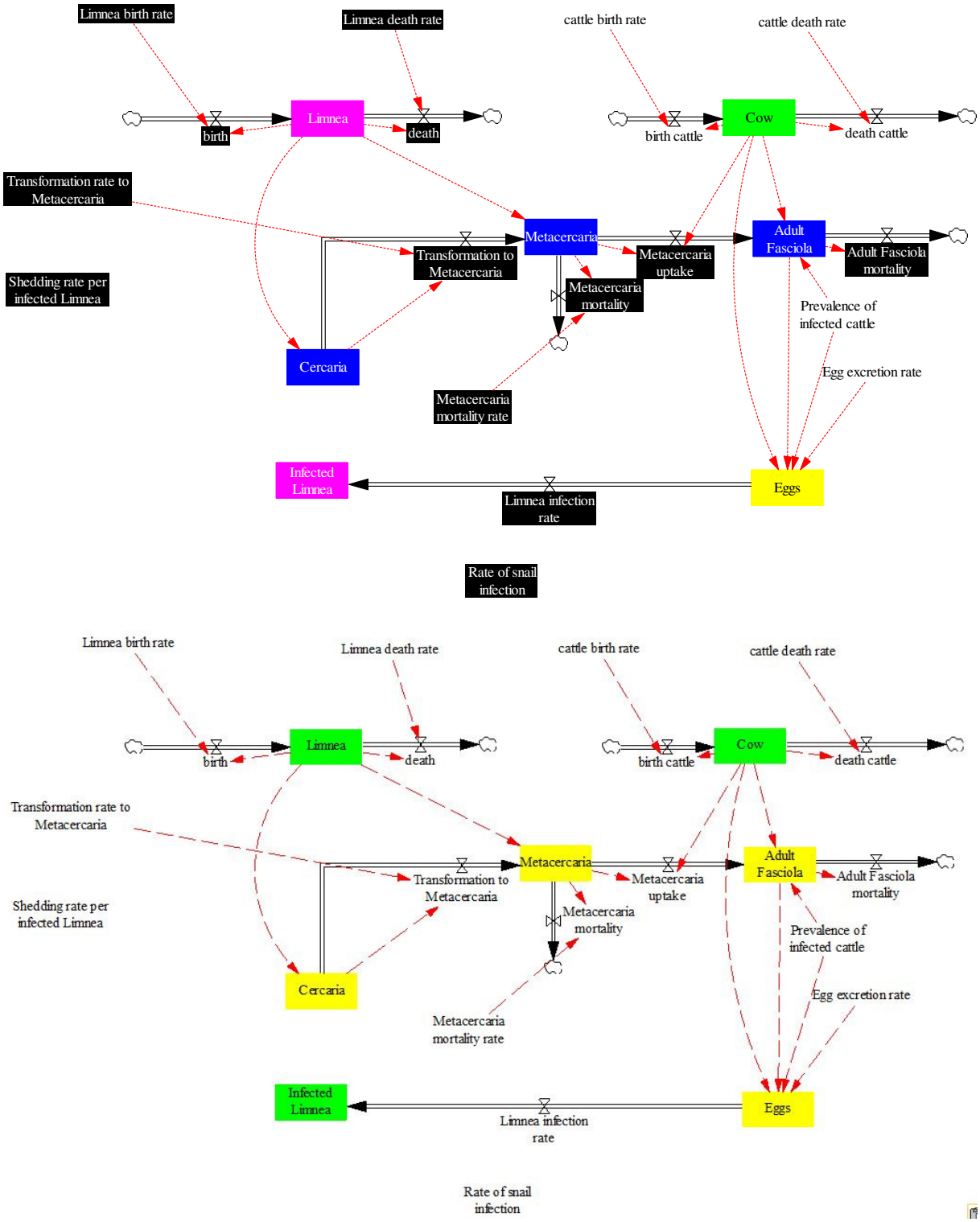

Figure 12.2. First layout of a transmission model for *Fasciola gigantica* (by Jakob Zinsstag)

12.6. Identified research needs

During the field, bench and desk work of this doctoral thesis, further research questions kept arising. An array of these is suggested here:

- A comprehensive abattoir study on livestock schistosomiasis and fascioliasis in the eastern Lake Chad area has been conducted (MSc Annour A. Batil). The analysis of these data will provide additional epidemiological data on the two trematode infections in cattle, sheep and goats from different husbandry systems
- At abattoirs, condemnation of fascioliasis damaged livers was recorded. Analysis of these data will allow for the estimation of the economic losses caused by fascioliasis at Lake Chad
- Water quality persists as a fundamental problem in the mobile pastoralist population at Lake Chad. Research on simple, save and practicable water cleaning techniques for mobile populations is encouraged
- The shores of Lake Chad are densely populated by mobile pastoralists and a sedentary population. An epidemiological survey including the sedentary population in the area is needed to complete our knowledge on schistosomiasis prevalence and transmission cycles
- A malacological survey at Lake Chad will provide data on species composition, seasonal abundance and infection intensity in intermediate host snails. The assessment of *Fasciola* and *Schistosoma* infection levels in snails throughout the seasons will provide fundamental data for mathematical transmission modelling.
- Human and animal infective *Schistosoma* spp occurrence is overlapping at Lake Chad. Molecular analysis will reveal potential hybridization between *Schistosoma* species.
- A deeper insight into the molecular biology of *Schistosoma* spp and *Fasciola* spp composition in all hosts involved, be it human, veterebrate or snail, will add fundamental information to a holistic view of trematode disease ecology and epidemiology at Lake Chad
- A molecular determination of the *Schistosoma* spp and *Fasciola* spp will also contribute to the research and development of new, targeted diagnostic tools
- The complex cultural setting of mobile pastoralism and different ethnicities can be best addressed when researchers from social sciences, ethnologist and health researches work together. Continuing and intensifying interdisciplinary and transdisciplinary approaches for future health research is strongly encouraged.

12.7. Conclusions

The epidemiology of snail born trematode infections in humans and livestock at Lake Chad is influenced by environmental and cultural factors. The findings of this dissertation contribute to human and veterinary public health and are of value at the regional level and beyond. Besides the direct, practical application of this research, it provides an evidence base for further transdisciplinary collaborations and broader, integrated systems thinking approaches. Research is encouraged that addresses complex interactions between people, animals and their environment. This will provide the fundament for the development of targeted research questions and, eventually, for the design and implementation of human and animal health interventions and policy.

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Appendix 1: The mobile pastoralists of Lake Chad

Housing styles of the Buduma (top) and the Fulani (bottom) pastoralists.

Housing styles of the Gorane (top) and the Arab (bottom) pastoralists.

Fulani pastoralists prepare for travelling to a new camp site. Mbororo cattle carry housing material.

Arab pastoralists use also Camels for their travels.

A herd of Kouri cattle, passing by our field laboratory in the late afternoon.

Appendix 2: Impressions from the bench and the field

Parasitology training at IRED: The course participants (from left to right:) Annour Adoum Batil, Ferdinand Mbainaissem, Mahamat Hissein Mahamat, Raison Djimassingar and Lucie Ndilassoum Meloum group around their “doyenne”, Madame Michelle Dobler from Swiss TPH.

The field team is ready for a 10 day field work visit at Lake Chad. From left to right: Ferdinand Mbainaissem (lab technician), Annour Adoum Batil (veterinarian), Fayiz Mahamat Abakar (friend), the driver, the author, and Moussa Issa, the guide, nurse and translater.

At the Gredaya abattoire site, the master student Annour Adoum Batil (left) learns from Prof. Jakob Zinsstag how to identify *Schistosoma bovis* flukes in the mesenteric venules of a slaughtered goat.

The author together with the nurse conducting a interview with the mother of a schistosomiasis infected child in a Buduma camp at Lake Chad

Appendix 3: IOHC Poster Award

At the 3rd International One Health Congress that took place in March 2015 in Amsterdam, NL, the project was one of two winners of the poster award. It was a big honor to receive the prize handed over by Nobel Prize winner Prof. Peter Dogherty, who stated in his speech that the project had a "truly fascinating title".

"*The jury was impressed with the high quality of both posters and presentations by young scientists at IOHC2015. This scientific work forms the basis of a sustainable scientific One Health community. The jury highly valued the collaboration between scientists and public health workers of both developed and developing countries and has awarded in particular posters and presentations that deal with all three basic aspects of One Health: the health of man, animal and the environment.*

The poster prize winners both described studies carried ou in remote areas with the locally available technology and had a firm impact on health management in the region. Helena Greter and Stephanie Burniston got their prize handed over by Prof. Dr. Peter Doherty, Nobel Prize Laureate. The poster awards were sponsored by MSD Animal Health represented by Dr. Ruud Segers."

(http://iohc2015.com/nieuws/lezen/i-21/award_winners_iohc2015)

IOHC Poster Award ceremony (from left to right: Dr. Ruud Segers, Prof. Dr. Peter Doherty, Stephanie Burniston, Helena Greter. Photo: Marcel de Cnock)

Appendix 4: Report in the SNSF Magazine HORIZONS, 2014, No 101, p. 29-31

With the text and pictures from Dr. Christian Heuss, communications officer, Swiss TPH

Blood, sweat and stool

In Chad, Swiss researchers are investigating parasitic diseases in humans and animals. The aim is to improve the health of vulnerable nomadic communities.
By Christian Heuss

It's with a handshake and a black goat that a research deal is sealed with the clan chief of the Foulbé nomads. "Only through long-lasting relationships and mutual respect can we research their health", says Jakob Zinsstag from the Swiss Tropical and Public Health Institute in Basel, who, over the last twenty years, has often been here on the southern banks of Lake Chad.

So with the goat safely stowed in the luggage area, ready for handover when we reach the nomads, our 4x4 vehicle drives out of the town of Gredaya, heading further and further into the green bush of the Sahel. The going is bumpy, and the puddles in the mud road testify to the recent rains. From the trees and bushes, there's the twittering of the first migratory birds to arrive from Europe. For outsiders, every fork in the road adds to the sense of disorientation. The only person who seems to know exactly where we're going is Ali Baye Abba Abakar. "My brain is like sat-nav", he laughs. Along with the nurse Hadjé Falmata and the driver, Abba Abakar is a crucial member of the team led by Zinsstag's doctoral student Helena Greter. Abba Abakar speaks the local languages and has stored on his mobile the telephone numbers of many nomadic families.

The Foulbés, the Goranes and the Kuris are very different in their nomadic lifestyles, their social structures and the routes they take, but they all belong to the most vulnerable population groups in one of the world's poorest countries. Because they have no fixed place of residence and move from one pasture to the next along with their several hundred cattle, donkeys and carts, they fall through the gaps in the state's net. Nomadic children have no schools, have poor access to medical services and are prone to a high rate of child mortality.

Zinsstag pursues a systematic approach. "If we want to improve the health of these people, then we have to understand how they live". Health is not just a medical problem, it's just one aspect of the overall socio-economic picture. That is why Zinsstag, a veterinarian and epidemiologist, does not just work together with doctors of human medicine but also with geographers, ethnologists and public health experts.

Runaway cattle

Despite Abba Abakar's sense of direction, it's only after a difficult search that Greter and her team find the nomads. Their cattle had broken out that afternoon, which meant that they'd had to relocate their overnight camp. Now, dotted across a space

Open-air health care: Helena Greter taking a dung sample (top right); the nomads with cattle-branding tongs (top left), milking a cow (opposite page) and preparing to catch cows (previous page). Photos: Christian Heuss

measuring roughly one hectare, ten families camp with their children at the base of a large tree. They have their cooking areas here, their colourful canvas tents and all their meagre belongings. Behind them, the cattle are grazing. As daylight fades, the smoke from little fires serves to ward off the malaria-carrying anopheles mosquito.

Shortly after the research team arrives, the men of the group sit with them in a circle. Over cups of bitter-sweet black tea, Greter explains her research plans. She and her team would like to visit the group three times in the coming months to examine both people and animals for parasitic worms. Her work is geared to solving a health problem that the nomads themselves have identified. Their cattle are often affected by a worm – the large liver fluke – which reduces their ability to give milk and consequently also their market price. The people also host a similar flatworm, the *schistosoma haematobia*. This parasite leads to schistosomiasis, an often chronic illness that weakens those who suffer from it, and that can lead to blood in the urine and even to death. Earlier examinations showed at least one in ten children to be infected.

Here, humans and animals share the same living space. So are there similarities in how they are all infected by these parasites? This is the question occupying Greter. At their watering holes, she collects water snails, which serve as intermediate hosts during the life-cycle of the flatworm. Greter determines how many of the snails carry worm larvae. Using this data and a mathematical model, she and Zinsstag want to determine the best point in time for a medical treatment to be applied simultaneously to both men and animals. Zinsstag is convinced that "this way, we hope to eliminate the parasitic infestation completely in the long term".

Greter speaks French, Abba Abakar translates. The nomads ask a lot of questions, and there is much laughter. Greter wants to examine fifteen animals and fifteen people in the next 24 hours. She keeps to a strict protocol that was agreed by ethics commissions in both Switzerland and Chad. She has medicines with her, in case she discovers any illness.

Caught with a lasso

With a clear, starry night spent under only mosquito nets, the hard manual work begins shortly after daybreak. Greter has to examine dung samples in order to find out which cattle are infected with worms. The Foulbé men catch these impressive beasts with a lasso and hold them fast by the horns. Greter plunges her arm – clad in a green plastic glove – deep into the rectum to draw out fresh dung.

In the early morning, things are already busy at the campsite. While the men are helping Greter, the children are standing around and enjoying the unusual sight of a researcher at work. Meanwhile, the women are seeing to the food. Some of them are milking cows, others are churning butter and grinding corn into a kind of polenta pulp. After a short break, Greter sits with the men to discuss the examinations she needs to carry out among their families. She picks a random sample of men and women from the group and supplies each with two white plastic cups – one for a stool sample, the other for urine. Falmata, the nurse, will then use a detailed questionnaire to ask them about their general state of health.

By ten in the morning, the sun is burning mercilessly in the sky, and time is pressing. Greter sets up her field laboratory in the shade of a bush. Her solar-powered microscope stands on a folding table. She had practised every step of the process for analysing urine and faeces back home in Basel – but now, out here in the field, everything is different. Blowflies flock to the dung samples, and Greter has to adjust both the staining method and her own timing. But with her second dung sample she immediately recognises the worm eggs with their typical, spindle-shaped silhouettes.

In the coming months, Greter will conduct hundreds of examinations, all of which will be very carefully documented and statistically evaluated. This will not just provide a more precise picture of the health situation of the nomads. Greter will also improve her overall knowledge of nomadic life in Chad. "It is an extraordinary opportunity", she says, while putting the next specimen slide under her microscope.

Christian Heuss runs the Communication Department of the Swiss Tropical and Public Health Institute in Basel.