Epidemiology of brucellosis and Q fever in Togo and the risk of disease spread through cattle trade in West Africa

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Summary

Background

Zoonotic diseases account for more than half of infectious diseases of humans and three quarters of all emerging infectious diseases. Most of this burden lies with poor, rural communities who rely on livestock for nutrition and income. In order to advocate on behalf of these communities and reduce the disease burden, there is a need for high quality epidemiological data acquired through transdisciplinary approaches involving the natural, health and social science fields.

Brucellosis is one of the most common zoonoses in the world. This bacterial disease has a major impact on human health, livestock production and the economy. In recent years, Q Fever has become a bacterial zoonosis of increasing interest, in part due to an unprecedented, large-scale outbreak in the Netherlands in 2007-2010. However, there is an absence of high quality epidemiological data from West Africa for both brucellois and Q Fever.

Member countries of the Economic Community of West African States (ECOWAS) are required to permit free movement of people and goods across borders. This mobility is important culturally, socially and economically. However, mobile populations in sub-Saharan Africa suffer from the least access to health services. Cross-border trade and seasonal transhumance are central to livestock production to West Africa, but these movements can facilitate the rapid spread of pathogens across large distances. The Savannah Region of northern Togo is the country's main livestock raising area. This is a dynamic zone, with frequent movement of both people and livestock across its borders with Ghana, Burkina Faso and Benin.

Aims

- To assess the global burden of human brucellosis and identify data gaps
- To provide the first data on the epidemiology of brucellosis and Q Fever in people and animals in Togo
- To conduct the first quantitative assessment of cattle trade in Togo, including the potential impact on disease spread in West Africa

- To assess the mobility of people and livestock and the access to health care and other social services in northern Togo
- To foster intersectoral collaboration and transdisciplinarity in Togo

Methods

A systematic review and meta-analysis of the burden of human brucellosis was undertaken in the framework of the Global Burden of Disease Study 2010. Additionally, primary data collection was conducted in the Savannah Region of northern Togo. A brucellosis and Q Fever serosurvey was conducted in cattle, sheep, goats and people in 25 randomly selected villages in 2011 and 18 transhumant herds from Burkina Faso in 2012. The serological testing methods included the Rose Bengal Test (RBT), Enzyme-linked Immunosorbent Assay (ELISA), Complement Fixation Test (CFT) and Immunofluoresence Assay (IFA). Multiple Loci Variable Number of Tandem Repeats Analysis (MLVA) was used to genotype *Brucella* strains isolated from bovine hygroma fluid. Semi-structured interviews were conducted in 2011 in parallel with the serosurvey to assess the mobility of the local population and their access to health care and other social services. In 2012, semi-structured interviews were conducted with cattle traders in the 9 principal cattle markets in the study zone. The data collected was used to simulate cross-border livestock flows and assess the potential risk of disease spread in West Africa through cattle trade.

Principal Findings

The systematic review quantified the severe and chronic impact of brucellosis on its sufferers and provided the first informed estimates of acute and chronic disability weights for brucellosis. Major knowledge gaps regarding the incidence of brucellosis are evident for Sub-Saharan Africa, Eastern Europe, the Asia-Pacific, and Central and South America.

In Togo, the association between cattle seropositivity and a history of abortion in cows suggests that brucellosis and Q Fever have an important impact on livestock production. Given the absence of small ruminants seropositive for brucellosis, it is likely that only *B. abortus*, not *B. melitensis,* is circulating in the study zone. People of Fulani ethnicity were shown to have greater exposure to zoonoses than the rest of the population, suggesting that cultural factors may influence the disease epidemiology. These results

represent the first epidemiological data for zoonoses in linked human and animal populations in Togo. The impact of these diseases on human health and the economy warrants further investigation.

The *B. abortus* strains isolated from cattle hygromas are among the first *Brucella* genotypic data available from West Africa. The identification of three distinct strains from only a small geographical area highlights the genetic diversity of circulating strains in the study zone. The two deletions detected in the BruAb2_0186 gene have important diagnostic and, possibly, epidemiological consequences. More molecular and epidemiological data are needed from the region, in order to better understand transmission patterns and develop more suitable diagnostic assays. The use of a chaotrophic buffer to preserve and inactivate DNA was both a safer and more sensitive diagnostic method than culture, and is of particular relevance to countries with poor laboratory biosafety.

The first quantitive assessment of the risk of disease spread in West Africa through cross-border cattle trade demonstrated that surveillance for emerging infectious diseases as well as control activities targeting endemic diseases are likely to be ineffective if only conducted at a national level. The trade network in northern Togo extended into Burkina Faso, Ghana, Benin and Nigeria, with their epidemiological systems likely to be intimately linked to one another. There is a need for greater collaboration between countries at the regional level in order to strengthen disease surveillance and control efforts.

In addition to the large number of foreign herdsmen undertaking transhumance in northern Togo every dry season, one third of local herds were reported to be seasonally transhumant. Women were also shown to be a more mobile sub-group of the local population, and further investigation of the needs of these mobile groups is warranted. Although the level of satisfaction with the local health centres was generally high, the cost of services was an exception and may reflect a financial barrier. Increased distance to the local health centre reduced the utilisation of skilled birth attendants by women, and may be an important barrier to accessing health care. Livestock herding duties of Fulani children resulted in poorer school attendance, and the impact on physical, intellectual and social development should be explored.

Conclusions

This PhD thesis addresses the global disease burden of brucellosis, the regional mobility of livestock and people, and the local epidemiology of zoonoses and access to essential services. The research was conduced across the continuum of innovation-validation-application that forms the basis of the activities of the Swiss Tropical and Public Health Institute. The findings provide a public health contribution that is valuable from the global to regional to national level. In addition to the direct, pratical applications of this research, it also provides a much needed evidence base on which to advocate on behalf of communities, develop more targeted research questions and, eventually, design public health interventions and policy.

Résumé

Les maladies zoonotiques sont responsables de plus de la moitié des maladies infectieuses affectant l'homme, et de trois-quarts des maladies infectieuses émergentes. Le poids de ces maladies est le plus élevé parmi les communautés pauvres et rurales, pour lesquelles l'élevage est une source importante de protéines et de revenue. Afin de promouvoir des investissements plus importants pour le contrôle et la prévention des maladies affectant ces communautés, il est essentiel de collecter des données épidémiologiques de qualité à travers une approche transdisciplinaire impliquant les sciences naturelles, les sciences de la santé et les sciences sociales.

La brucellose est l'une des zoonoses les plus répandues au monde. Cette maladie bactérienne a un impact majeur sur la santé humaine, la production animale et l'économie. Depuis quelques années, la fièvre Q, une zoonose bactérienne, a soulevé un intérêt croissant en partie dû à l'épidémie sans précèdent, de par son échelle, qui a touché les Pays-Bas entre 2007 et 2010. Néanmoins, il n'existe pas de données de qualité sur l'épidémiologie de ces deux maladies en Afrique de l'Ouest.

Les pays membres de la Communauté Economique des Etats de l'Afrique de l'Ouest (CEDEAO) se sont engagés à permettre la libre circulation des biens et des personnes à travers leurs frontières communes. Cependant, les populations mobiles d'Afrique sub-saharienne souffrent d'un accès réduit aux services de santé. Le commerce transfrontalier et la transhumance saisonnière sont des piliers de la production animale en Afrique de l'Ouest, mais de tels mouvements peuvent faciliter la dissémination rapide de pathogènes sur de longues distances. La région des Savanes, au nord du Togo, est la principale zone d'élevage de bétail du pays. C'est une zone avec de fréquents mouvements de personnes et de bétail au travers des frontières partagées avec le Ghana, le Burkina Faso et le Benin.

Objectifs

- Evaluer le poids global de la brucellose humaine et identifier les manques en matière de données.

- Fournir la première base de données épidémiologique sur la brucellose et la fièvre Q affectant les populations humaines et animales au Togo.
- Effectuer la première évaluation quantitative des mouvements commerciaux de bovins au Togo, et de son impact sur la dissémination de maladies en Afrique de l'Ouest.
- Evaluer la mobilité et l'accès aux services de santé et autres services sociaux des personnes et de leur bétail au nord du Togo.
- Promouvoir la collaboration intersectorielle et transdisciplinaire au Togo.

Méthodes

Une revue systématique de la littérature et une méta-analyse du poids global de la brucellose humaines ont été réalisées dans le cadre du rapport sur la Charge Mondiale de Morbidité en 2010. De plus, une étude de terrain a été réalisée dans la région des Savanes au nord du Togo. Une étude de la séroprévalence de la brucellose et de la fièvre Q a été réalisée au sein des populations humaines, bovines, ovines et caprines dans 25 villages sélectionnés aléatoirement en 2011, et dans 18 élevages transhumants originaires du Burkina Faso en 2012. Les tests sérologiques employés comprennent le test au Rose Bengale (TRB), la méthode immuno-enzymatique ELISA, le test de fixation du complément (TFC) et l'analyse par immunofluorescence (AIF). L'analyse de plusieurs locus en répétition en tandem polymorphe (MLVA) a été utilisée pour caractériser le génotype des souches de *Brucella* isolées à partir du fluide d'hygromas de bovins. Des entretiens semi-structurées ont été effectuées en 2011 en parallèle de l'étude de séroprévalence afin d'évaluer la mobilité des populations locales et leur accès aux services de santé et aux autres services sociaux. En 2012, des entretiens semi-structurées ont été réalisées avec les commerçants de bovins dans les cinq principaux marchés de la zone d'étude. Les données collectées ont été utilisées pour simuler les mouvements commerciaux transfrontaliers de bovins et évaluer le risque que ces mouvements représentent pour la dissémination de maladies en Afrique de l'Ouest.

Principaux résultats

La revue systématique de la littérature a permis de mettre en lumière le sévère et chronique impact de la brucellose pour les populations humaines infectées, et a permis de calculer les premières estimations du poids de l'incapacité aiguë et chronique de la brucellose. Les lacunes de connaissances sur l'incidence de la brucellose sont majeures en Afrique sub-saharienne, en Europe de l'Est, dans la zone Asie-Pacifique, en Amérique Centrale et en Amérique du Sud.

Au Togo, l'association entre la séropositivité du bétail et la survenue d'avortements parmi les animaux échantillonnés suggère que la brucellose et la fièvre Q ont un impact important sur la production animale. Etant donne l'absence de séropositivité des petits ruminants pour la brucellose, il est vraisemblable que *B. abortus*, et non pas *B. melitensis*, circule dans la zone d'étude. Les individus de l'ethnie Peul avaient des niveaux d'exposition aux zoonoses étudiées plus élevés que le reste de la population, suggérant que des facteurs culturels pourraient influencer l'épidémiologie de ces maladies infectieuses. Ces résultats représentent les premières données sur les zoonoses au sein de populations humaines et animales en interaction au Togo. L'évaluation de l'impact de ces maladies sur la santé humaine et l'économie requiert de plus amples investigations.

Les trois souches de *B abortus* isolées à partir d'hygromas de bovins sont parmi les premières données génotypiques disponibles sur les souches de *Brucella* circulant en Afrique de l'Ouest. L'identification de trois souches distinctes dans une zone géographique de faible dimension met en lumière la diversité génétique des souches circulant dans la zone d'étude. Les deux délétions détectées dans le gène BruAb2_0186 ont d'importantes conséquences en matière de diagnostic et, potentiellement, d'épidémiologie. Des données épidémiologiques et moléculaires supplémentaires sont nécessaires afin de mieux comprendre la transmission de la brucellose, et de développer des tests diagnostiques appropriés. L'utilisation d'une solution tampon chaotrophique pour préserver et inactiver l'ADN s'est avérée une méthode diagnostique plus sure et plus sensible que la mise en culture, et est pertinente pour des pays dont les laboratoires ont un faible niveau de biosécurité.

La première évaluation quantitative du risque de dissémination de maladies en Afrique de l'Ouest par le commerce transfrontalier de bovins a démontré que la surveillance des maladies infectieuses émergentes, ainsi que les activités de contrôle ciblant les maladies endémiques sont vraisemblablement inefficaces si elles sont conduites au niveau national. Le réseau formé par les mouvements commerciaux de bovins au nord du Togo s'étendait également au Burkina Faso, au Ghana, au Bénin et au Nigéria, vraisemblablement connectant les systèmes épidémiologiques de ces pays entre eux. Une plus grande collaboration entre pays au niveau régional est nécessaire afin de renforcer la surveillance et le contrôle des maladies.

En plus du nombre important de bouviers étrangers transhumant dans le nord du Togo à chaque saison sèche, un tiers des troupeaux locaux ont été identifiés comme étant des troupeaux transhumants saisonniers. L'étude a identifié les femmes comme étant un sous-groupe plus mobile au sein de la population locale. De plus amples investigations ciblant ces sous-groupes sont nécessaires. Bien que le niveau de satisfaction des populations au sujet des centres locaux de santé était généralement élevé, ces populations trouvaient le coût des services élevé, ce qui pourrait constituer une barrière à l'accès aux soins. Le recours à un assistant d'accouchement était plus faible parmi les femmes habitant à une distance importante des centres de santé, suggérant que cette distance constitue une barrière à l'accès aux soins. Les tâches liées à l'élevage dont s'acquittent les enfants Peuls se traduisent par un absentéisme scolaire élevé. L'impact de cet absentéisme sur le développement physique, intellectuel et social de ces enfants doit être exploré.

Conclusions

Cette thèse de doctorat a traité du poids global de la brucellose pour la santé humaine, la mobilité régionale des personnes et du bétail, l'épidémiologie locale des zoonoses et l'accès aux services essentiels. Cette recherche a été menée à travers le continuum innovation-validation-application qui forme la base des activités de l'Institut Tropical et de Santé Publique Suisse. La contribution de ces résultats à la sante publique est précieuse tant à l'échelle globale, que régionale et locale. En plus des applications directes et pratiques de cette recherche, elle fournit également une base d'évidences pour promouvoir des investissements plus importants pour le contrôle et la prévention des maladies affectant ces communautés, développer des questions de recherche plus ciblées, et développer des mesures et des politiques de santé publique.

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List of Abbreviations

CDC	Centers for Disease Control and Prevention
CFT	Complement Fixation Test
CI	Confidence Interval
CSRS	Centre Suisse de Recherches Scientifiques en Côte d'Ivoire
DALYs	Disability-Adjusted Life Years
DNA	Deoxyribonucleic Acid
ECOWAS	Economic Community of West African States
ELISA	Enyme-linked Immunosorbent Assay
ESA	Ecole Supérieure d'Agronomie
FAO	Food and Agricultural Organization of the United Nations
FERG	Foodborne Disease Burden Epidemiology Reference Group
FMD	Foot and Mouth Disease
GBD	Global Burden of Disease
GNI	Gross National Income
GSCC	Giant Strongly Connected Component
GWCC	Giant Weakly Connected Component
IFA	Immunofluorescent Assay
IgG	Immunoglobulin G
IgM	Immunglobulin M
IQR	Interquartile Range
MAT	Microscopic Agglutination Test
MLVA	Multiple Loci Variable Number of Tandem Repeats Analysis
NCCR	National Centres of Competence in Research
OR	Odds Ratio
OIE	World Organisation for Animal Health
PCR	Polymerase Chain Reaction
PPP	Purchasing Power Parity
RBT	Rose Bengal Test
R_0	Basic Reproduction Number
Swiss TPH	Swiss Tropical and Public Health Institute

STAT	Standard Tube Agglutination Test
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
VNTR	Variable Number of Tandem Repeats
WAT	Wright Agglutination Test
WHO	World Health Organization
2ME	2-Mercaptoethylamine Test

1 Introduction

1.1 Impact and Burden of Zoonotic Diseases

Zoonotic diseases account for more than half (61%) of pathogens known to infect humans and 75% of all emerging infectious diseases (*Taylor et al. 2001*; *Jones et al. 2008*). In recent years, interest in zoonoses has increased due to high profile disease emergent events with an impact on global travel and trade, including Bovine Spongiform Encephalopathy, Highly Pathogenic Avian Influenza A H5N1 and Sudden Acute Respiratory Syndrome (SARS). However, it is the endemic diseases, rather than newly emerging pathogens, that threaten livelihoods and prevent communities from rising out of poverty (*WHO 2006*). The major burden of endemic zoonoses lies with poor, rural communities, who rely on livestock for their nutrition (milk, meat), agricultural activities (traction, fertiliser), cash income, and cultural and ceremonial purposes (*McDermott & Arimi 2002*).

These communities generally have poor access to health care and are overlooked by the global research and public health agenda. Only 0.6% of international global health assistance is dedicated to neglected tropical diseases, of which less than 10% is estimated to be allocated to neglected zoonotic diseases. Neglected zoonotic diseases constitute an open-ended list of underreported, often severe endemic diseases which have been largely eliminated from high income countries, and for which cost-effective control options often already exist (Figure 1). They have been very aptly described as the “poor cousins of the poor cousins” (*WHO 2010*).

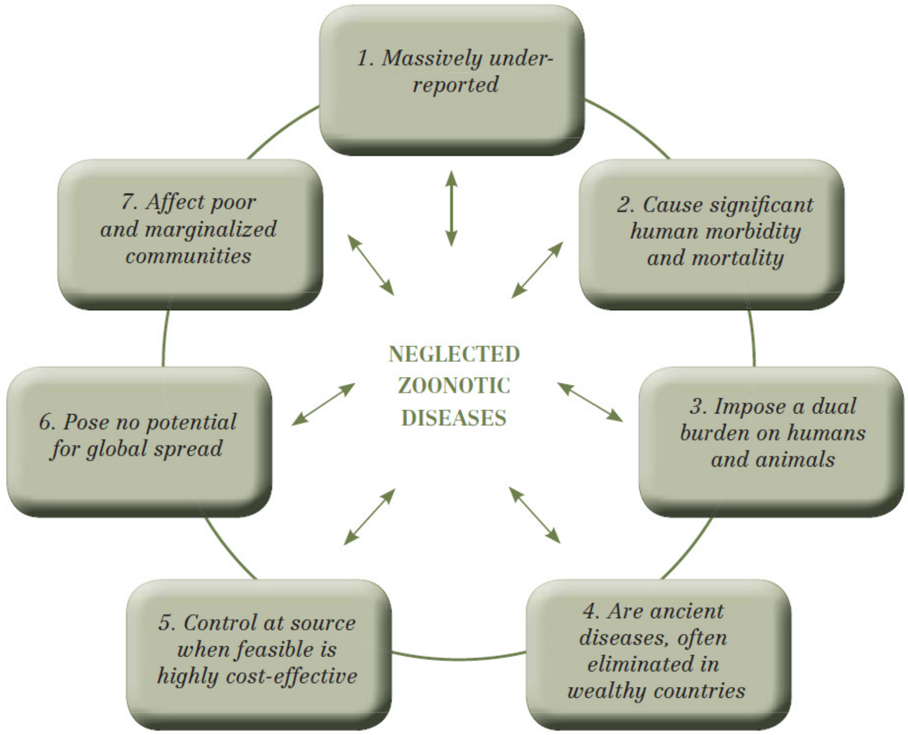

Figure 1: Characteristics of neglected zoonotic diseases

Source: (*WHO 2010*)

In malaria-endemic countries, accurately diagnosing and treating zoonotic infections is particularly challenging. Fever cases are often treated with anti-malarial medicines without a definitive diagnosis. In sub-Saharan Africa, the policy of presumptive malaria treatment for childhood fever cases has been brought into question, given declining rates of malaria transmission (*D'Acremont et al. 2009*; *D'Acremont et al. 2010*). It has been estimated that more than half (57%) of children presenting with fever to government health services in Africa do not actually have malaria. In some countries, this proportion is greater than 90% (*Gething et al. 2010*). Poor people are less likely to seek medical care for the treatment of fevers, resulting in a delay in diagnosis and treatment. This is then compounded by misdiagnosis when/if assistance is eventually sought at a health facility (*Amexo et al. 2004*). Thus, many zoonotic infections remain undiagnosed and untreated, and the disease burden grossly under-estimated.

There is a need for a high quality, comprehensive evidence base in order to advocate for greater investment in neglected zoonoses. The Disability-Adjusted Life Year (DALY) is defined as a "time-based measure that combines years of life lost due to premature mortality and years of life lost due to time lived in states of less than full health" (*WHO 2013b*). Although not without its criticisms (*Mont 2007*), the DALY provides policy-makers, funding bodies and researchers with a comparative view of population health, guiding the decision-making process about where global health efforts should be directed (*Horton 2012*). Estimates for DALYs are given for 291 diseases in the recently released results of the Global Burden of Disease Study 2010, a consortium of seven partners: Harvard University, Institute for Health Metrics and Evaluation (IHME) at the University of Washington, Johns Hopkins University, University of Queensland, Imperial College London, University of Tokyo, and the World Health Organization (WHO) (*Horton 2012*). Fifteen of these 291 are neglected tropical diseases, of which only several are neglected zoonoses; namely, rabies, echinococcosis, cysticercosis, leishmaniasis, and African trypanosomiasis (*Murray et al. 2012*).

1.2 Tackling Zoonotic Diseases: Ecohealth and One Health Approaches

The Millennium Ecoystem Assessment of the United Nations called for the preservation of the benefits provided by the natural environment to human health and well-being. Improved ecosystem management was identified as central to the strategy of achieving the Millennium Development Goals (*WHO 2005*). The concept of Ecohealth considers the health of an ecosystem as a whole, through transdisciplinary approaches involving the natural, health and social science fields. Local communities are engaged in the process of developing sustainable solutions to health problems, whilst preserving the natural, social and cultural capital required for the health of future generations (*International Association for Ecology & Health 2012*).

Embedded within Ecohealth is One Health, which extends the previous concept of One Medicine from a physiological and clinical focus to a broader public health approach including zoonotic disease epidemiology, food safety and health service delivery (*Zinsstag et al. 2011*). Mainstreaming One Health into the global health agenda would bring benefits not only to health, but also to conservation and development (*Zinsstag et al. 2012*). From an economic perspective, the World Bank's cost-benefit study of a One Health approach to zoonotic disease prevention and control at a global level showed that benefits far exceeded costs in all plausible scenarios of disease prevalence and outbreak severity. These benefits are the direct result of joint planning and communication as well as the sharing of staff, facilities and equipment (*The World Bank 2012*).

In order to safeguard human health, any zoonotic disease control strategy must address disease in the animal reservoir. Thinking should be shifted away from unisectoral benefits of disease control activities towards the overall benefits to society as a whole (*Zinsstag et al. 2007a*). These include economic benefits, such as in Mongolia where a mass vaccination campaign of livestock against brucellosis was shown to be a highly cost-effective disease control intervention only when all beneficiaries were considered. These benefits included not only improved livestock productivity but also a reduction in public and private health spending, which in turn protected household incomes (Figure 2) (*Roth et al. 2003*).

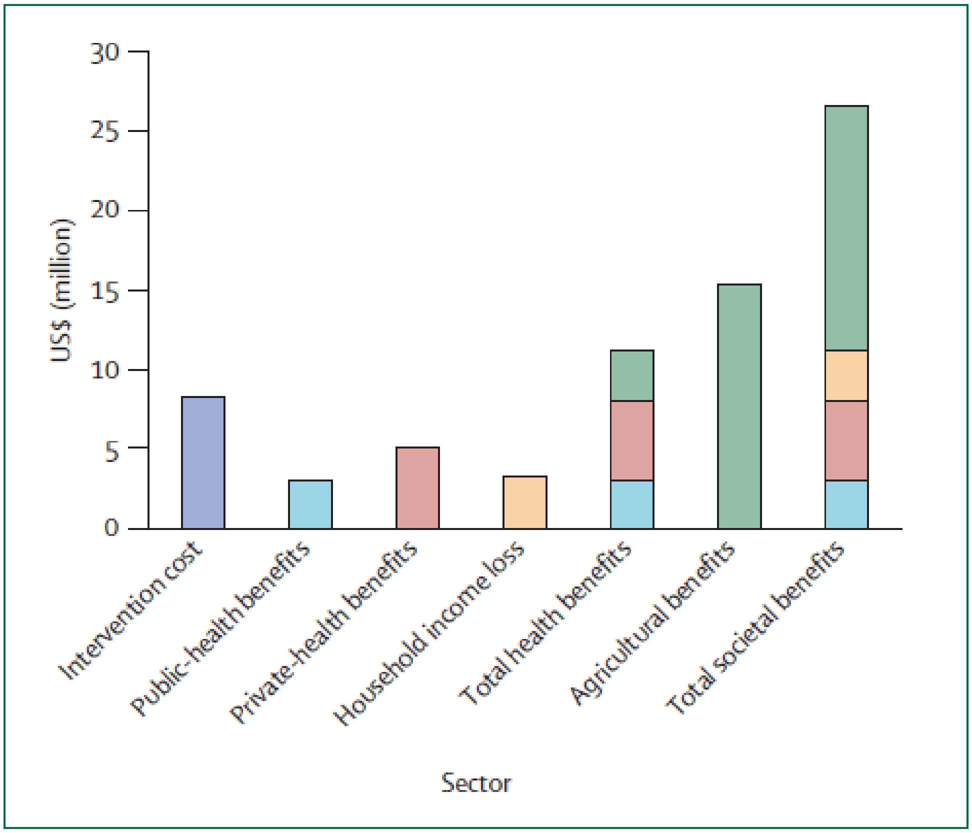

Figure 2: Distribution of benefits of mass vaccination of livestock against brucellosis in Mongolia

Source: (***Zinsstag et al. 2005***)

A combined approach to zoonotic disease epidemiology increases the efficiency and capability of disease surveillance and response activities. For example, in Mauritania, presumed outbreaks of Yellow Fever were only correctly diagnosed as Rift Valley Fever when contact was made with livestock authorities who reported abortion storms in livestock due to Rift Valley Fever (*Zinsstag et al. 2005*; *Zinsstag et al. 2007a*). Similarly, integrated human health and veterinary systems for preventive interventions may be more successful at achieving the desired health outcomes, as well as being more cost-effective. The sharing of transport and equipment between health and veterinary sectors in Chad in a joint vaccination achieved higher vaccination coverage of nomadic children than via a conventional, unisectoral approach, as well as a 15% reduction in operational costs (*Zinsstag et al. 2005*; *Schelling et al. 2007*). Closer cooperation between the two sectors not only strengthened each health system, but resulted in a synergy (*Schelling et al. 2005*).

1.3 Epidemiology, Impact and Burden of Brucellosis

1.3.1 Animal Brucellosis

The *Brucella* genus includes both zoonotic and non-zoonotic species. These intracellular, Gram negative bacteria can infect a range of land and marine mammals, but do exhibit some host-specificity. In terms of livestock production, the most important species are *B. abortus* adapted to cattle, *B. melitensis* in sheep and goats and *B. suis* in pigs, all of which are zoonotic. In livestock, the most common outcomes of brucellosis infection are abortions, usually in the second half of gestation, reduced milk yield, and arthritis, often manifested by joint hygromas (*WHO et al. 2006*; *Seleem et al. 2010*). The disease has important implications for animal welfare, the economy and public health. The Food and Agricultural Organization of the United Nations (FAO) estimated in 2002 that bovine milk and meat production in West Africa could increase by US$56-168 million/year if brucellosis were eliminated (*Mangen et al. 2002*). Additionally, it should be remembered that the economic impact of brucellosis is not solely due to a reduction in animal productivity, but also the financial consequences of human infection (*Roth et al. 2003*) and livestock export bans.

Despite being one of the most common zoonotic diseases in the world, very little high quality data have been published regarding animal brucellosis in West Africa. Brucellosis antibodies have been detected in livestock sera and/or milk samples in Burkina Faso (*Gidel et al. 1974*), the Gambia (*Unger et al. 2003*), Ghana (*Kubuafor et al. 2000*; *Mensah et al. 2011*), Guinea (*Sylla et al. 1982*; *Unger et al. 2003*), Guinea-Bissau (*Unger et al. 2003*), Ivory Coast (*Pilo-Moron et al. 1979*; *Thys et al. 2005*; *Sanogo et al. 2008*), Mali (*Tounkara et al. 1994*; *Bonfoh et al. 2002*), Niger (*Gidel et al. 1974*), Nigeria (*Cadmus et al. 2006*), Senegal (*Unger et al. 2003*; *Tialla 2012*), and Togo (*Sonhaye 1980*; *Kponmassi 1991*). However, most of these studies are limited by questionable quality or a lack of generalisability. Some molecular data are available regarding the species and biovar of *Brucella* strains isolated in West Africa, but much of these data were collected more than 30 years ago (*Sanogo et al. 2013*).

A major difficulty in interpreting cross-sectional serosurvey results is that serological testing methods are imperfect. Serological methods used in disease surveillance activities include the Rose Bengal Test (RBT) enzyme-linked immunosorbent assay (ELISA), complement fixation test (CFT), and fluorescence polarisation assy. The ELISA and milk ring test can also be used for bulk milk samples from cows. A positive test result should be confirmed using a complementary strategy (*OIE 2009a*; *OIE 2009b*). *Brucella* organisms can be cultured from aborted foetal tissues (*Leyla et al. 2003*; *Muendo et al. 2012*), vaginal fluid (*Muendo et al. 2012*), hygroma fluid (*Bankole et al. 2010*) and milk (*Schelling et al. 2003*; *Muendo et al. 2012*). However, given the high risk of laboratory-acquired infection, with *Brucella* being classified by the US Centers for Disease Control and Prevention (CDC) in Atlanta as a potential Category B bioweapon (*CDC 2013*), laboratories in developing countries often do not have an adequate level of biosafety for safely performing microbiological culture. Loci with Variable Number of Tandem Repeats (VNTR) can be used as markers to discriminate *Brucella* strains. In this way, molecular typing by Multiple Locus VNTR Analysis (MLVA) can be used as a molecular epidemiology tool to understand transmission patterns and monitor surveillance and control programs (*Le Flèche et al. 2006*; *Al Dahouk et al. 2007a*). In Africa, MLVA has been performed on *Brucella* field strains from cattle in the Gambia (*Bankole et al. 2010*), Kenya (*Muendo et al. 2012*), Zimbabwe and Chad (*Le Flèche et al. 2006*), and from a camel in Sudan (*Le Flèche et al. 2006*).

Some industrialised countries have successfully eliminated brucellosis. Vaccination, either implemented on mass or targeting only replacement stock, is a key component of a brucellosis control strategy. In certain circumstances, vaccination should be combined with test-and-slaughter of adult animals. When disease prevalence is very low and there is no mixing between herds, an exclusive test-and-slaughter or stamping-out policy will lead to disease elimination if rigorously implemented (*European Commission 2009*; *Minas 2006*). Deciding whether disease control or disease elimination should be the goal in a given setting depends on the disease prevalence, structure of the livestock production system, mixing and mobility of the animal population, availability of economic and other resources, potential ramifications on international trade, and economic and public health impact (*Benkirane 2006*).

1.3.2 Human Brucellosis

Brucella can infect a human host through ingestion, particularly of unpasteurised dairy products, from inhalation, or via direct contact through skin abrasions (*Franco et al. 2007*). Assisting the delivery of an aborted animal foetus poses a particular risk (*Schelling et al. 2003*; *John et al. 2010*). Infection with *Brucella* can cause a severely debilitating, disabling, chronic and relapsing illness in people. Brucellosis is a “great imitator”, with a wide spectrum of clinical presentations including fever, fatigue, weight loss, arthralgia and arthritis, myalgia, endocarditis, and neurological and respiratory symptoms (*Franco et al. 2007*). The chronic nature of the illness has important economic ramifications, through reduced household income and increased out-of-pocket spending on health care (*Roth et al. 2003*). Diagnosis can be challenging not only due to the broad clinical spectrum, but also because of imperfect serological tests (*Franco et al. 2007*). If a regimen of combination therapy of doxycycline with rifampicin or streptomycin for uncomplicated brucellosis is adhered to for at least 6 weeks, relapse may still occur (*Ariza et al. 2007*).

The RBT is rapid and simple to perform in the field but indicates only previous exposure to *Brucella*, not necessarily active infection. Although it can be a very useful test in resource-poor settings, it can give false positive and false negative results (*Diaz et al. 2011*). Additional confirmatory tests should be used and the results interpreted in light of the clinical status of the patient. These include the serum agglutination test, Coomb’s test, ELISA, immunochromatographic lateral flow assay, and immunocapture (*Orduña et al. 2000*; *Serra & Viñas 2004*; *Araj et al. 2005*; *Gómez et al. 2008*; *Casanova et al. 2009*;

2010). The sensitivity and specificity of these tests for diagnosing active infection vary according to the background level of exposure and antibodies in a given population. The cut-off thresholds of these tests may not, therefore, be appropriate for every epidemiological setting (*Franco et al. 2007*; *Araj 2010*). A novel indirect immunoassay using glycol-engineered antigen coupled with magnetic beads shows promise (*Ciocchini et al. 2013*).

The gold standard for diagnosis of active brucellosis is culture and isolation of the organism from blood, joint fluid, cerebrospinal fluid, or other bodily fluids and tissues (*Franco et al. 2007*). However, laboratories in developing countries often do not have the capacity to safely perform microbiological culture. MLVA of human-isolated strains can assist trace-back analysis for identification of the origin of an infection (*Le Flèche et al. 2006*; *Al Dahouk et al. 2007a*; *Marianelli et al. 2007*; *Kattar et al. 2008*; *Nöckler et al. 2009*; *Tiller et al. 2009*; *Valdezate et al. 2010*; *Kiliç et al. 2011*; *Ferreira et al. 2012*).

Despite being one of the most common zoonotic diseases in the world, brucellosis remains underdiagnosed and underreported (*Franco et al. 2007*) and may be an important cause of fevers of unknown origin in sub-Saharan. A study from rural Tanzania revealed that 1 in 5 patients diagnosed with brucellosis did not present to a health centre for assessment until more than one year after the onset of illness. Once at the health centre, nearly half (45%) were not diagnosed with brucellosis at their first visit (*Kunda et al. 2007*). Thus, diagnostic and therapeutic delays pose a major challenge to minimising the health and economic impact of this disease. This is particularly true in Sub-Saharan Africa, where the almost total absence of high quality data regarding the burden of human brucellosis (as shown in Figure 3) means that communities, health care workers and policy-makers are not aware of the disease.

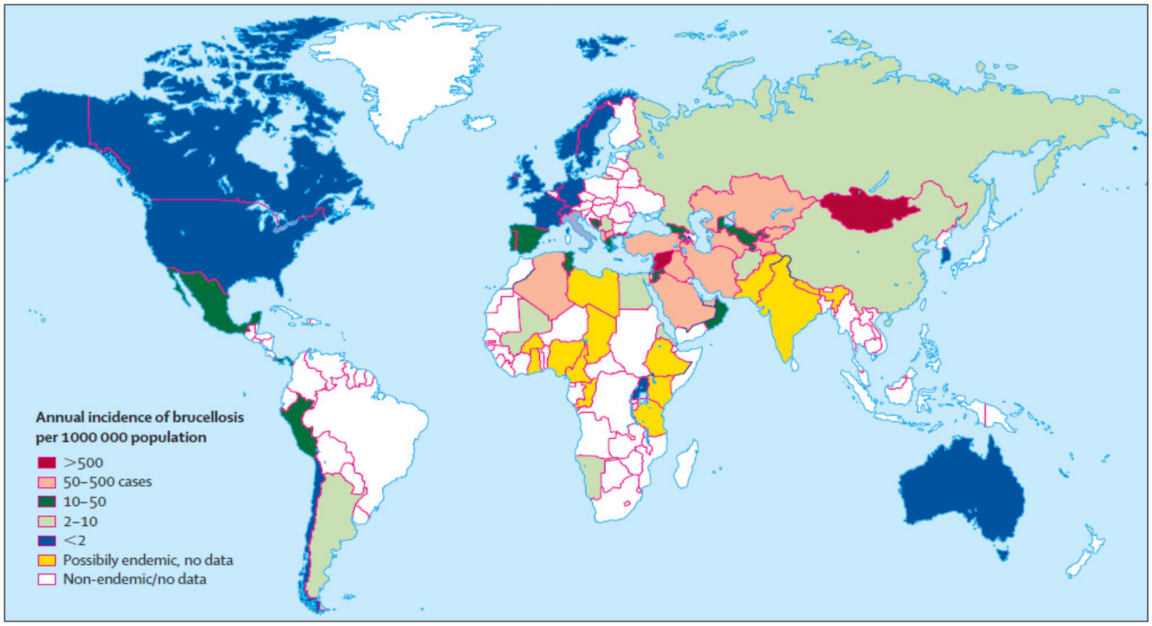

Figure 3: Worldwide incidence of human brucellosis

Source: (*Pappas et al. 2006*)

In West Africa, no high quality, recent data are available regarding the incidence or prevalence of human brucellosis in the general population. Although some seroprevalence data are available from Senegal (*Tialla 2012*), Ghana (*Kubuafor et al. 2000*), Benin (*Audurier et al. 1987*), Nigeria (*Baba et al. 2001*) and Mali (*Tasei et al. 1982*), their usefulness is limited by questionable quality or a lack of generalisability. However, studies conducted in central and eastern Africa suggest that the disease could pose a public health problem in pastoral communities (*Schelling et al. 2003*; *Regassa et al. 2009*) and in high risk occupational groups, such as abattoir workers (*Chantal et al. 1996*; *Swai & Schoonman 2009*). In Tanzania, 3.5% of febrile patients presenting to hospitals were determined to be unwell due to active brucellosis infection (*Bouley et al. 2012*), suggesting that the disease should be considered as a differential diagnosis for fevers of unknown origin in sub-Saharan Africa.

1.4 Epidemiology, Impact and Burden of Q Fever in People and Animals

More than 75 years after being first identified, Q Fever, so named to reflect the “query” surrounding the organism, still poses many unanswered questions (*de Valk 2012*). Q Fever was predominantly considered an occupational disease, affecting abattoir workers or farmers (*Parker et al. 2006*). However, a large-scale outbreak in the Netherlands which resulted in approximately 4,000 notified human cases from 2007-2010, revealed that the pathogenicity and transmissibility of the organism had been under-estimated (*de Valk 2012*). The inhalation of contaminated aerosols played an important role in disease transmission, with people living within a 2km radius of an affected goat farm having a much high risk of disease than people living 5km away or further (*Schimmer et al. 2010*). The underlying conditions which led to this unprecedented outbreak are not understood, but hypothesised factors include the high density of people and domesticated animals, particularly goat herds (*Dijkstra et al. 2012*), and the possible dissemination via infected rats (*Reusken et al. 2011*).

Q Fever is caused by *Coxiella burnetii*, a Gram negative intracellular bacteria with a worldwide distribution. Reservoirs include a range of domestic and wild animals, as well as ticks. In livestock, the disease is often sub-clinical but can cause reproductive problems, including aborting and infertility. Infected animals shed the organism in milk, birthing products, urine, and faeces. The most common route of infection to people is through the inhalation of dust contaminated by infected animal fluids, followed by ingestion, particularly of unpasteurised dairy products (*Maurin & Raoult 1999*; *Porter et al. 2011*). However, the role of dairy product consumption in *C. burnetii* transmission is not fully understood, and asymptomatic seroconversion without clinical illness has been reported (*Benson et al. 1963*; *Cerf & Condron 2006*). The organism is very environmentally resistant and, like *Brucella*, is classified by the Centers for Disease Control and Prevention (CDC) in Atlanta as a potential Category B bioweapon (*CDC 2013*).

In people, acute Q Fever usually presents as an influenza-like illness with non-specific symptoms similar to brucellosis, including fever, fatigue, weight loss, myalgia and arthralgia. Additionally, hepatitis, pneumonia, and abortions can also result from infection. Chronic disease can occur months or years after exposure and may manifest as endocarditis or chronic fatigue syndrome. However, exposure to the organism does not necessarily result in clinical disease, with as many as 50% of exposures estimated to be sub-clinical (*Parker et al. 2006*).

The reference method for diagnosis is the immunofluorescent assay (IFA), but other methods include ELISA and the CFT. The diagnosis of acute fever is based on elevated IgM and IgG antibodies against the Phase II organism, whilst chronic disease is characterised by high titres of IgG antibodies against Phase I. The presence of only high Phase II IgG antibodies indicates previous exposure or infection (*Blaauw et al. 2012*). Acute disease can be successfully treated with 2 weeks of doxycycline, but chronic disease may need 18 months of combination treatment (*Parker et al. 2006*).

The limited serology data available for Sub-Saharan Africa have demonstrated that exposure is common in livestock (*Vanek & Thimm 1976*; *Kponmassi 1991*; *Schelling et al. 2003*; *Scolamacchia et al. 2010*) and people (*Vanek & Thimm 1976*; *Dupont et al. 1995*; *Julvez et al. 1997*; *Steinmann et al. 2005*; *Kobbe et al. 2008*). However, given that many exposures are asymptomatic, understanding the clinical impact of the disease requires education of health workers and an active surveillance system. In fever patients presenting to hospitals in Tanzania, 5% of cases were determined to be due to acute Q Fever (*Prabhu et al. 2011*), suggesting that the disease should be considered as a differential diagnosis for fevers of unknown origin in sub-Saharan Africa.

Vaccines against *C. burnetti* exist for both livestock and people. During the aforementioned outbreak in the Netherlands, the vaccination of dairy goats and sheep reduced the disease prevalence and the bacterial load in bodily secretions in these species. This suggests that vaccination may reduce environmental contamination and human exposure to *C. burnetti* (*Hogerwerf et al. 2011*). In people, vaccination may be recommended for high-risk occupations such as veterinarians or abattoir workers. This is generally only practised in high income countries, as careful screening for previous exposure to the organism is essential in order to avoid adverse vaccine reactions in people with pre-existing immunity (*Zhang & Samuel 2004*).

1.5 Livestock Production in Sub-Saharan Africa

Livestock contribute to the livelihoods of at least 70% of the world's poor (*Ashley et al. 1999*). It is estimated that 50 million pastoralists and 200 million agro-pastoralists live within the drylands of Africa which cover 43% of Africa's inhabited surface. Many of these pastoral populations rely on mobility to maximise livestock production, access trading opportunities, or avoid drought, conflict, and even disease (*IIED and SOS Sahel UK 2010a*). Although newly arrived herds can place additional strain on already limited natural resources, resulting in conflict with the local population (*Kohlhagen 2002*; *Fokou et al. 2004*; *Tezike & Dewa-Kassa 2008*), potential benefits to these settled communities include meat and milk products and fertilisation of agricultural land (*Macpherson 1995*; *Tezike & Dewa-Kassa 2008*).

Seasonal, semi-nomadic livestock movements, known as transhumance, are central to livestock production in West Africa. The Economic Community of West African States (ECOWAS) recognised the importance of transhumance in the Decision passed in 1998. This Decision legally protects cross-border transhumance between all member countries, provided that only predetermined routes are followed and the required documentation is shown at check-points (*Ly 2007*). However, implementation of this legislation at the national level is not necessarily straightforward. In Togo, a national law in place since 2008 has placed additional restrictions on transhumance activities, with economic and social repercussions for transhumant herdsmen (*Grolimund 2010*).

Long-distance, cross-border cattle trade is culturally and economically important to West Africa, estimated to be worth US$150 million in 2000 (*Williams et al. 2006*). Traditionally, livestock have been raised in the semi-arid Sahel zone and traded with countries in the southerly forested zones (*Williams et al. 2006*). The spread of *Trypanosoma brucei rhodesiense*, one of the pathogens causing Human African Trypanosomiasis, through cattle trade in Uganda (*Hutchinson et al. 2003*) clearly demonstrated that livestock movements can facilitate the spread of pathogens across large distances. This is particularly a concern in resource-poor settings where veterinary services and surveillance systems are weak (*Fèvre et al. 2006*).

1.6 Access to Health Care

1.6.1 The Health Access Livelihood Framework

Improving access to health care is central to the achievement of the Millennium Development Goals (*United Nations 2000*). Despite the recognised need for vast improvements in equity of access to health care, there are no formal international standards. A novel framework for analysing and improving access to health care has been developed in the context of malaria treatment and care in Tanzania. The Health Access Livelihood Framework considers access within the broader context of livelihood insecurity (*Obrist et al. 2007*). The following five dimensions of access are considered:

- Availability – the existing services and goods meet the client's needs, with adequate supplies and appropriately skilled personnel
- Accessibility – the location of services is convenient
- Affordability – the prices of services meet the client's ability to pay both direct and indirect costs
- Adequacy – the organisation of health care meets client's expectations in terms of opening hours, structure and cleanliness
- Acceptability – the characteristics of health care providers match those expected by the client, in terms of social values and personality

Improvements in access across these five dimensions depend on the interplay between: (i) health care services and the governing policies, institutions, organisations, and processes, and (ii) the livelihood assets that can be mobilised by people in particular vulnerability contexts. Outcomes of interventions aiming to improve access can be measured in terms of health status, patient satisfaction, and equity (*Obrist et al. 2007*).

1.6.2 Health and Mobility in Sub-Saharan Africa

More than half of the world's nomadic and semi-nomadic populations live in Africa (*Sheik-Mahomed & Velema 1999*). Mobility can lead to the importation of diseases into communities and even across national boundaries, with the last documented case of smallpox infection transmitted by a nomadic Ethiopian child in Somalia. A mobile lifestyle can, however, also offer opportunities to avoid disease. A serosurvey in nomadic camps in Niger demonstrated a low transmission rate of measles with 15% of adults remaining unexposed (*Loutan & Paillard 1992*). Periodic movement away from accumulated waste as well as regular milk consumption may explain the lower burden of intestinal parasites in nomadic communities in Somalia compared to settled communities (*Sheik-Mahomed & Velema 1999*).

Lowering barriers to movement and improving the treatment of mobile people could reap substantial benefits to human development (*UNDP 2009*). Mobile populations in sub-Saharan Africa suffer from the least access to health services (*Sheik-Mahomed &*

Velema 1999; *Fokou et al. 2004*). Combining outreach veterinary and human health services has been shown to increase coverage amongst pastoral nomadic communities (*Schelling et al. 2005*). Novel strategies are being developed for baseline demographic and health-related surveillance (*Weibel et al. 2008*; *Weibel et al. 2011*), but there is a need for more practical interventions addressing the priorities of communities beyond the reach of conventional services.

The national borders marked out by the previous colonial powers transect communities which share common cultural, ethnic and linguistic heritages. In order to overcome these divisions and to promote regional economic development, member countries of ECOWAS are bound to permit free movement of people and goods across borders (*Adepoju 2005*; *Adepoju et al. 2007*). The Fulani are the most dispersed ethnic group in West Africa and are traditionally associated with livestock production activities in the region. The previous 30 years have seen significant migration of Fulani pastoral communities from the Sudano-Sahelian zone into the more humid Sudano-Guinean savannahs of West Africa, as a result of social and agroecological adjustments (*Oppong 2002*; *Bassett & Turner 2007*). This migration includes groups seeking permanent settlement as well as those following transhumance pathways (*Diallo 2001*).

2 Study Rationale and Design

2.1 Research Needs

One of the main barriers to reducing the impact of zoonotic diseases in developing countries is the absence of high quality epidemiological data. Not only is a sound evidence base needed for the design of appropriate disease control interventions, but also for advocating for greater commitment of funds and other resources by governments and international organisations.

Given the cultural and economic importance of smallholder livestock production in West Africa, it is hypothesised that the zoonotic disease burden is significant. In order to fully assess the impact on health and livelihoods, more empirical, population-based research is needed. This includes investigation into disease prevalence and incidence, risk factors, transmission dynamics, clinical sequelae and economic outcomes. Conducting the research using an Ecohealth approach may foster closer collaboration between different sectors at the provincial and national levels by providing a practical example of transdisciplinarity in action. Based on the research findings, practical interventions appropriate for the local context could be designed and trialled in collaboration with stakeholders, and validated within the targeted communities. These outcomes could then be used as an advocacy tool at the national, regional and international levels.

Given that most of West Africa is endemic for malaria, an issue of particular relevance is malaria misdiagnosis. Zoonotic diseases may contribute to a significant proportion of fevers of unknown origin. Training health care workers and strengthening laboratory capacity would be central to quantifying this. As most laboratory diagnostic tests have been developed and validated in industrialised countries, it may be necessary to modify to the protocols for different epidemiological settings.

One factor that must be considered in any epidemiological study in West Africa is the movement of people and livestock within and between countries. This mobility is important culturally, socially and economically, occurring frequently across somewhat “porous” borders. Mobile population health is a developing field in Africa, but very little data are available regarding the way in which mobility influences disease epidemiology, as well as implications for accessing health care and other social services. There is a need to develop practical disease control interventions and social infrastructure which explicitly account for mobile populations, whilst reaping mutual benefits for mobile and settled communities.

2.2 Goal, Aims and Objectives

2.2.1 Goal

The overarching goal of this research was to generate high quality data on the burden and epidemiology of zoonotic diseases in livestock and people in West Africa, including consideration of mobile populations, and to use this evidence base for policy development and as an advocacy tool for greater investment in prevention and control activities in the region.

2.2.2 Aims

The aims of this PhD thesis were:

- To assess the global burden of human brucellosis and calculate the DALYs for brucellosis
- To provide the first high quality data on the epidemiology of brucellosis and Q Fever in Togo, and to use this evidence to provide guidance to policy-makers
- To provide the first high quality data on cattle trade patterns in northern Togo, including the potential risk of disease spread in West Africa through trade, and to use this evidence to provide guidance to policy-makers
- To foster intersectoral collaboration and transdisciplinarity in zoonoses prevention and control activities in Togo
- To assess mobility and access to health care and other social services in northern Togo

2.2.3 Objectives

In order to address the objectives of this PhD research, a combination of desk-based review, cross-sectional field surveys, laboratory analyses, and statistical analyses and modelling were used. The objectives were:

Objective 1: Global burden of human brucellosis

- To perform a systematic review and meta-analysis of human brucellosis within the framework of the Global Burden of Disease Study 2010 in collaboration with the Foodborne Disease Burden Epidemiology Reference Group (FERG) of WHO
- To determine regional and global estimates of the incidence of human brucellosis
- To assess the morbidity due to human brucellosis and specify a disability weight which could be used for a future calculation of the DALYs due to brucellosis

Objective 2: Burden and epidemiology of brucellosis and Q Fever in northern Togo

- To determine the burden of brucellosis and Q Fever in people and livestock, including transhumant populations
- To assess the performance of different serological tests such as ELISA, RBT, CFT and IFA and to provide diagnostic training to laboratory staff
- To describe the epidemiology of brucellosis and Q Fever, including molecular aspects such as MLVA, and assess the risk posed to human health and livelihoods
- To communicate the findings to the medical and veterinary sectors and other stakeholders, and to develop practical recommendations together for reducing the impact of zoonotic diseases on human health and livelihoods

Objective 3: Livestock movements in northern Togo

- To quantitatively assess the scale of cross-border cattle trade through Togo
- To estimate the impact of this trade on the risk of disease spread in West Africa
- To communicate these findings to veterinary authorities and other stakeholders and to develop practical recommendations together for reducing the risk of regional disease spread through trade

Objective 4: One Health in Togo

- To put the concept of One Health into practice by providing practical training to nurses and animal health workers in the field
- To provide an environment for intersectoral dialogue regarding public health priorities in Togo, in the form of a stakeholder workshop

Objective 5: Mobile populations and access to health care

- To describe the mobile populations of northern Togo
- To assess their access to healthcare and other social services and identify barriers
- To identify priorities of mobile populations in relation to health care and other social services
- To develop practical recommendations and interventions addressing these priorities, in consultation with stakeholders

2.3 Study Design

2.3.1 Study Site

Primary data collection was originally planned for northern Côte d'Ivoire in the Savannah Region (Région des Savanes), bordering Mali and Burkina Faso. However, due to political instability, the field site was relocated during the second year of the PhD and all data collection took place in Togo.

Togo is a small, narrow country in West Africa, bordered by Ghana to the west, Burkina Faso to the north and Benin to the east. From its southern limit at the Gulf of Guinea, it extends northwards from a tropical coastal plain to a semi-arid savannah. A national census conducted in November 2010 estimated the population to be 5,753,324 people, of which more than half (62.6%) lived in rural areas (*Direction Générale de la Statistique et de la Comptabilité Nationale 2010*). The population includes 37 different ethnic groups (*Central Intelligence Agency, USA 2013*). Togo is classified by the World Bank as a low income country, its Gross National Income (GNI) at Purchasing Power Parity (PPP) per capita being one of the lowest in the world. In 2011, the GNI at PPP of Togo ranked 201st out of 214 countries (*The World Bank 2013*). Its Human Development Index ranked 162nd out of 187 countries, falling in the category of Low Human Development (*UNDP 2009*). The life expectancy at birth is low, at just 57 years for men at 61 years for women in 2009 (*WHO 2013a*).

All data were collected in the Savannah Region (Région des Savanes) of Togo, a semi-arid ecosystem which is the northernmost of five administrative regions of Togo (Figure 4). The population of the Region was estimated to be 776,710 in 2010, accounting for

13.5% of the total Togolese population (*Direction Générale de la Statistique et de la Comptabilité Nationale 2010*). The Savannah Region was selected because it is the country's main livestock-raising zone and is known to be an area of significant cross-border mobility (*Tezike & Dewa-Kassa 2008*; *Grolimund 2010*; *Kulo & Kada 2011*). Detailed livestock census data are not available, but the cattle population in the Savannah Region was estimated to be 138,000 in 2011 (personal communication, Direction de l'Elevage). Each dry season, the Region receives transhumant herds from the Sahel zone in search of watering sites and pasture, with the official period of transhumance being from January until May. In 2011, 47,000 cattle passed through official entry points in the Savannah Region, but it is likely that many more entered the country unofficially (personal communication, Direction de l'Elevage). Some of these remain in the Savannah Region whilst others descend further south. Additionally, internal transhumance within Togo is frequently undertaken by Togolese herds (*Tezike & Dewa-Kassa 2008*). The Savannah Region of northern Togo is, therefore, a highly dynamic zone. This mobility may have social, economic and health-related implications.

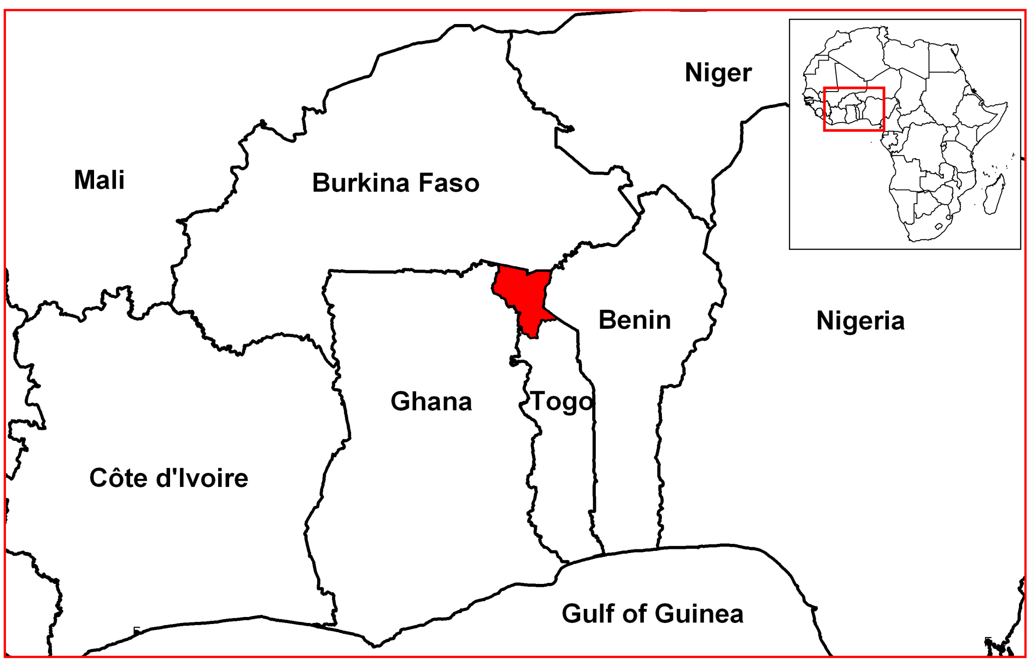

Figure 4: Map showing the study site

The Savannah Region in northern Togo is shaded red.

2.3.2 Field Activities

In Côte d'Ivoire, scoping and planning activities were undertaken from May-August 2010 and November 2010. This included conducting focus group discussions with stakeholders, developing and trialling data collection tools, and obtaining ethics approval.

Following the decision to relocate the field site to Togo in February 2011, the first field visit took place from March – July 2011. During this time, a cross-sectional study of people and livestock was conducted in 25 randomly selected villages in the Savannah Region, in order to investigate the burden and epidemiology and brucellosis and Q Fever, as well as access to health care and other social services. Questionnaire data and serum samples were collected. The serological testing and questionnaire data analysis were performed in Switzerland.

A second field visit took place from January – April 2012. The principal activity during this period was a cross-sectional survey of cattle traders in 9 cattle markets in the Savannah Region in order to investigate the cross-border trade network. Data were analysed in Switzerland. Additionally, a cross-sectional serosurvey of transhumant cattle from Burkina Faso was performed in order to determine the seroprevalence of brucellosis, complementing the survey conducted in the Togolese cattle population in 2011. The serological analyses were performed in Togo at the Laboratoire Central Vétérinaire. Additionally, cows with joint hygromas suggestive of brucellosis were identified and joint fluid collected for microbiological culture in Switzerland. In November 2012, a stakeholder workshop was held in Togo to share the research findings, discuss their application to policy and develop further research questions.

2.3.3 Collaborating Partners

This research project was undertaken within the framework of the National Centres of Competence in Research (NCCR) North-South, a research program focusing on global change and sustainable development, co-funded by the Swiss National Science Foundation and the Swiss Development Cooperation (*NCCR North-South 2012*).

Although headquartered in Bern, Switzerland, NCCR North-South encompasses partnerships with researchers from more than 40 countries. This PhD thesis contributed to the activities of Research Project (RP) 10, “Social services for mobile populations”, within Thematic Node 2, “Health, Services, Planning”.

The research was a collaborative project implemented by three academic institutions in Switzerland, Togo and Côte d’Ivoire: Swiss Tropical and Public Health Institute in Basel, Ecole Supérieure d’Agronomie at the Université of Lomé, and the Centre Suisse de Recherches Scientifiques en Côte d’Ivoire. The local representations of the Ministry of Health and the Department of Livestock Production (Direction de l’Elevage) were partners in the field during the data collection phase. The Institute for Veterinary Bacteriology at the University of Bern, the Laboratoire Central Vétérinaire at the Direction de l’Elevage in Togo, and Vircell S.L. in Spain were collaborating laboratories.

3 Global burden of human brucellosis: a systematic review of disease frequency

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3.1 Abstract

Background: This report presents a systematic review of scientific literature published between 1990-2010 relating to the frequency of human brucellosis, commissioned by WHO. The objectives were to identify high quality disease incidence data to complement existing knowledge of the global disease burden and, ultimately, to contribute towards the calculation of a Disability-Adjusted Life Years (DALY) estimate for brucellosis.

Methods/Principal Findings: Thirty three databases were searched, identifying 2,385 articles relating to human brucellosis. Based on strict screening criteria, 60 studies were selected for quality assessment, of which only 29 were of sufficient quality for data analysis. Data were only available from 15 countries in the regions of Northern Africa and Middle East, Western Europe, Central and South America, Sub-Saharan Africa, and Central Asia. Half of the studies presented incidence data, six of which were longitudinal prospective studies, and half presented seroprevalence data which were converted to incidence rates.

Brucellosis incidence varied widely between, and within, countries. Although study biases cannot be ruled out, demographic, occupational, and socioeconomic factors likely play a role. Aggregated data at national or regional levels do not capture these complexities of disease dynamics and, consequently, at-risk populations or areas may be overlooked. In many brucellosis-endemic countries, health systems are weak and passively-acquired official data underestimate the true disease burden.

Conclusions: High quality research is essential for an accurate assessment of disease burden, particularly in Eastern Europe, the Asia-Pacific, Central and South America and Africa where data are lacking. Providing formal epidemiological and statistical training to researchers is essential for improving study quality. An integrated approach to disease surveillance involving both human health and veterinary services would allow a better understanding of disease dynamics at the animal-human interface, as well as a more cost-effective utilisation of resources.

3.2 Author Summary

Brucellosis is a bacterial disease transmitted to humans by consumption of infected, unpasteurised animal milk or through direct contact with infected animals, particularly

aborted foetuses. The livestock production losses resulting from these abortions have a major economic impact on individuals and communities. Infected people often suffer from a chronic, debilitating illness. This systematic review of the incidence of human brucellosis is the first ever conducted. Using strict exclusion criteria, 28 scientific articles published between January 1990 – June 2010 which included high quality data were identified. Half of these studies presented incidence data and half presented seroprevalence data which were converted to incidence rates. Data were only available from 15 countries in the regions of Northern Africa and Middle East, Western Europe, Central and South America, Sub-Saharan Africa and Central Asia. Brucellosis incidence varied widely between and within countries. Demographic, occupational and socioeconomic factors may play a role in these differences. In many brucellosis-endemic countries, health systems are weak, and official data are likely to underestimate the true disease burden. High quality research is needed, particularly from Eastern Europe, the Asia-Pacific, Central and South America and Africa. An integrated approach to disease surveillance involving both human health and veterinary services would allow a better understanding of the disease, as well as a more cost-effective utilisation of resources.

3.3 Introduction

Brucellosis is one of the most common zoonotic infections globally (*Ariza et al. 2007*), transmitted to humans through consumption of unpasteurised dairy products or through direct contact with infected animals, placentas or aborted foetuses. This bacterial disease causes a severely debilitating and disabling illness, with fever, sweating, fatigue, weight loss, headache, and joint pain persisting for weeks to months. Neurological complications, endocarditis and testicular or bone abscess formation can also occur (*WHO et al. 2006*). Additionally, brucellosis has major economic ramifications due to time lost by patients from normal daily activities and losses in animal production (*Roth et al. 2003*). In a review of 76 diseases and syndromes of animals, brucellosis lies within the top 10 in terms of impact on impoverished people (*Perry 2002*)

In 1992, the World Bank commissioned the original Global Burden of Disease (GBD) study, providing a comprehensive assessment of 107 diseases and injuries and 10 risk factors in eight major regions (*WHO 2006*). This review did not include any neglected tropical zoonoses. Such diseases often do not attract the interest of health researchers or sufficient resources for adequate control, yet they continue to impact significantly on human health and wellbeing, livestock productivity and local and national economies. There is a need for more accurate data relating to the burden of neglected zoonoses to facilitate more effective implementation of disease control interventions. In 2009, the Foodborne Disease Burden Epidemiology Reference Group (FERG) of the World Health Organization (WHO) commissioned a series of systematic reviews on the burden of neglected zoonotic diseases, with the aim of incorporating the findings into the overall global burden of disease assessments.

This report presents a systematic review of scientific literature published between 1990-June 2010 relating to the frequency of human brucellosis. The objectives of this review were to identify high quality disease incidence data to complement existing knowledge (*Pappas et al. 2006*) of the global disease burden and, ultimately, to contribute towards the calculation of a Disability-Adjusted Life Years (DALY) estimate for brucellosis. A systematic review of scientific literature investigating the clinical manifestations of brucellosis is the subject of a companion paper (*Dean et al. 2012a*)

3.4 Methods

Searching

Thirty three databases were searched for relevant articles using the search strings of both (brucellosis OR malta fever) and (brucellosis OR malta fever OR brucella melitensis OR brucella abortus) AND (symptom* OR sequelae* OR morbidity OR mortality OR transmission mode OR foodborne), with a publication limitation of 1990 - 30 June, 2010. The search term was adapted to the predominate language of the database. If a database did not allow the combining of Boolean operators, (18 of 33 databases), 'brucellosis' was used as the sole term.

Reference Manager bibliographic software was used to manage citations. Duplicate entries were identified by considering the author, the year of publication, the title of the article, and the volume, issue and page numbers of the source. In questionable cases, the abstract texts were compared.

Selection

The articles were sorted by a team of four reviewers with a combined fluency in English, German, French, and Spanish. Articles in other languages were noted for future translation, pending resources.

All reports were classified into one of two categories, based on the abstracts:
Category 1: Relevant - articles related to human brucellosis infection in populations (i.e. disease frequency) or cases of human brucellosis (i.e. disease morbidity);
Category 2: Irrelevant - articles related to non-human brucellosis; articles addressing topics not related to the current review, such as genetics, laboratory diagnostic tests, experimental laboratory animal studies

The abstracts of studies belonging to Category 1 and meeting the following criteria for disease frequency were retained: published between 1990 and 30 June 2010, at least 100 study subjects drawn from the general population, prevalence or incidence data included and some information relating to diagnostic tests provided. The abstracts of studies meeting the following criteria for disease morbidity were also retained: published between 1990 and 30 June 2010, at least 10 study subjects, clinical symptoms/syndromes described and some information relating to diagnostic tests provided. The assessment and classification of morbidity articles will be the subject of a companion paper and will not be considered further here.

Articles for which the necessary data for classification could not be obtained were identified for possible future assessment, according to availability of resources. In general, non peer-reviewed or review articles, conference proceedings and book chapters were excluded.

Validity Assessment

After applying the aforementioned screening steps, the full text of each selected article was retrieved for detailed analysis. Each article was reviewed by two or three reviewers, and classification discrepancies were resolved by discussion.

Frequency studies were classified as prevalence studies if they stated a specified study population and area and an outcome expressed as the proportion of the study population identified as brucellosis seropositive (%); or as incidence studies if they presented the time period of observation, information about the study population size and area, and an outcome expressed as the number of new brucellosis cases per population at risk per time period.

Articles were coded based on the following parameters:

1) Study design

- Longitudinal - clear start/end date with a study period of several months to years
- Cross-sectional - a short study period of several weeks or, occasionally, several months
- Routine data – data officially reported by health services or routine data recorded by a health facility or local authority

2) Sampling methods

The sampling approaches were defined in order of decreasing quality as: cluster sampling proportional to size, simple random cluster sampling, simple random sampling without clustering or non-random sampling. The method of case acquisition (active, passive) was also evaluated. Studies not meeting any of these classifications were coded as “other”.

3) Study level

The study area was categorised in decreasing order of quality as: national, provincial, district or sub-district level. Studies not meeting any of these classifications were coded as “other”.

4) Diagnostic methods

Tests were categorised in decreasing order of quality as:

1. ELISA +/- Rose Bengal Test (RBT) or lateral flow assay only
2. RBT only
3. One of the following tests: microscopic agglutination test (MAT), complement fixation test (CFT), 2-Mercaptoethylamine test (2ME), standard tube agglutination test (STAT) of 1:160 or greater dilution, Wright agglutination test (WAT) or Huddleson test.

Studies diagnosing seropositives based on a STAT result of a dilution of less than 1:160 were excluded.

5) Study quality

Studies were given an overall quality grade of 1, 2, or 3, as shown in Table 1. Quality 1 studies had well described study design and methods. Their sampling approaches and study level were highly ranked, e.g. active sampling by cluster sampling proportional to size or simple random cluster sampling approaches at the national or provincial level. The diagnostic methods were also highly ranked, such as ELISA, lateral flow assay or RBT. Quality 2 studies contained some weaknesses in their sampling approach and/or diagnostic methods. Although data were extracted from Quality 3 studies, they were not included in the final analysis, due to either a lack of information about the methods and approaches preventing adequate assessment of the quality of the study or obvious biases in study design and implementation.

Data Extraction

The following information was extracted from each article, and they were grouped according to geographic region, as identified by the GBD consortium:

Seroprevalence studies: study period, size of study population, seroprevalence as a percentage

Incidence studies: study period, size of reference population, number of cases, incidence rate

Data Analysis

Seroprevalence data were multiplied by the duration of seropositivity, assumed to be 10.9 years (*Bonfoh et al. 2011a*), to determine the proportion of the general population seroconverting each year due to brucellosis exposure. Using a conservative estimate of 10% of seroconversions representing true clinical cases, these proportions were multiplied by 0.1 and converted to rates per 100,000 person-years for the general population.

Table 1: Grading of study quality based on study methodology criteria

Methodological Criteria	Overall Study Quality		
	Quality 1	Quality 2	Quality 3
Sampling approach			
Cluster sampling proportional to size	✓	✓	
Simple random cluster sampling	✓	✓	✓
Simple random sampling without clustering		✓	✓
Non-random sampling			✓
Case acquisition			
Active	✓	✓	
Passive			✓
Study level			
National	✓	✓	
Provincial	✓	✓	✓
District	✓	✓	✓
Sub-district		✓	✓
Diagnostic methods			
ELISA with/without additional method	✓	✓	
Lateral flow assay with/without additional method	✓	✓	
RBT with additional method	✓	✓	✓
RBT only		✓	✓
MAT, CFT, STAT, WAT, 2ME, Huddleson alone or in combination		✓	✓

Additional Targeted Searching

Given that high quality data were also likely to be available through routine reporting systems in developed countries with strong public health systems, additional data sources were identified through a non-systematic, targeted search.

3.5 Results

Searching

Table 2 lists the databases searched and the number of articles identified for each. A total of 28,824 articles were identified, of which 59% were duplicates, leaving 11,000 original reports.

Flow of Selected Studies

Figure 5 shows a detailed flow diagram of the selection of articles included in the systematic review. In total, 275 frequency and morbidity studies were selected, for which full text was available for 153. However, 14 of these were in languages in which the team was not competent (Croatian (6), Turkish (4), Korean (2), Persian (1), Mandarin(1)), leaving 61 frequency studies for quality assessment. Three were classified as Quality 1 and 26 as Quality 2. Thirty-two were excluded from further analysis as Quality 3, due to either a strong possibility of bias, a study population not representative of the general population, or a lack of adequate information to allow a proper assessment of study quality. Except for two articles in Spanish, all Quality 1 and Quality 2 studies were in English.

Table 2: Databases searched and number of hits

Database	Website	No. hits
Global databases		
Medline	http://www.ncbi.nlm.nih.gov/sites/pubmed	6176
ISI Web of Science	http://isiwebofknowledge.com	3458
EMBASE	http://www.embase.com	4980
Popline	http://www.popline.org	55
CAB	http://www.cabdirect.org	3424
ProMed	http://www.promedmail.org	666
The Cochrane Library	http://www.thecochranelibrary.com	100
BIOLINE	http://www.bioline.org.br	37
WHOLIS	http://www.bireme.br	76
Regional WHO databases		
African Index Medicus	http://indexmedicus.afro.who.int	14
Index Medicus for the Eastern Mediterranean Region	http://www.emro.who.int/whalecom0/Library/Databases/wxis.exe/Library/ Databases/iah/	526
Western Pacific Region Index Medicus	http://www.wprim.org/	96
Index Medicus for the South-East Asia Region	http://imsear.hellis.org/	247
Afro Library	http://afrolib.afro.who.int/	2
Other regional databases		
Health Information Locator	http://www.bireme.br	7
Institute of Tropical Medicine, Antwerp, Belgium	http://lib.itg.be:8000/webspirs/start.ws	122
King's Fund Information & Library Service	http://www.kingsfund.org.uk/library/	0
African Journals Online	http://ajol.info/	71
LILACS	http://www.bireme.br	538
MedCarib	http://www.bireme.br	9
REPIDISCA	http://www.bireme.br	29
PAHO	http://www.bireme.br	157
IBECS	http://www.bireme.br	148
CUIDEN	http://www.index-f.com/	17
Indian Medlars Center IndMed	http://indmed.nic.in/	84
KoreaMed	http://www.koreamed.org/SearchBasic.php	89

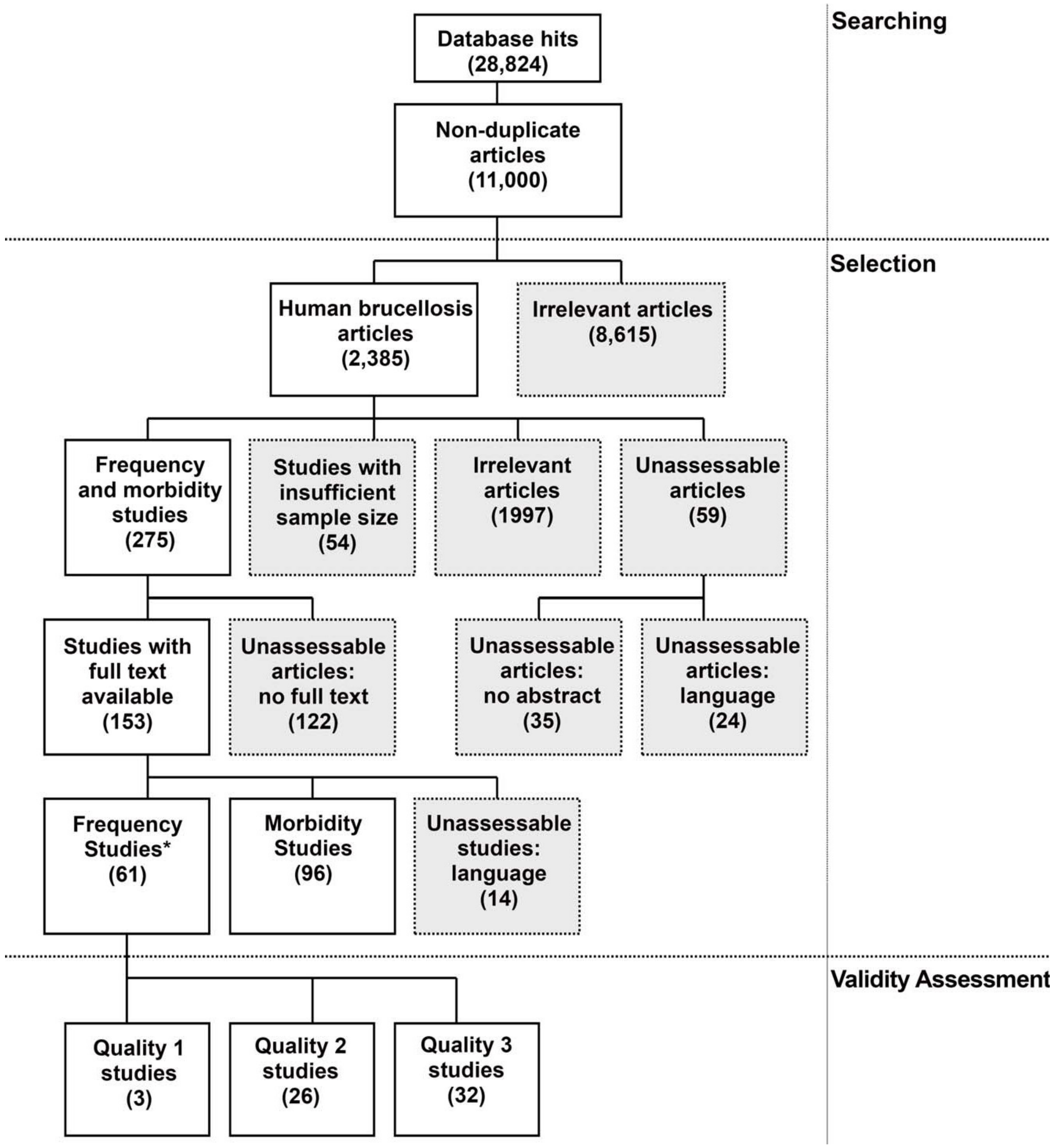

Figure 5: Flow of selected studies

* Some frequency studies were also classified as morbidity studies.

Study Characteristics

Fifteen articles presented incidence data. Six of these were longitudinal prospective studies (*Awad 1998*; *Crump et al. 2003*; *Avdikou et al. 2005*; *Jennings et al. 2007*; *Minas et al. 2007*; *Gargouri et al. 2009*), with the remainder retrospectively reviewing data collected mainly in health centres (*Doyle & Bryan 2000*; *Abu Shaqra 2000*; *Elbeltagy*

2001; *De Massis et al. 2005*; *Haghdoost et al. 2007*; *Al Dahouk et al. 2007b*; *Al Tawfiq & AbuKhamsin 2009*; *Jelastopulu et al. 2010*). Three incidence studies did not describe the diagnostic tests used but were included because they filled gaps in available data and had otherwise well-documented methods and results (*Doyle & Bryan 2000*; *De Massis et al. 2005*; *Jelastopulu et al. 2010*). Seroprevalence data were presented in fourteen articles, from surveys conducted in communities (*Alballa 1995*; *Al Sekait 1999*; *Karabay et al. 2004*; *Cetinkaya et al. 2005*; *Yacoub et al. 2006*; *El Sherbini et al. 2007*; *Vancelik et al. 2008*) or from blood donor screening (*Hernandez et al. 1999*; *Al-Ani et al. 2004*; *Marder et al. 2005*; *Khorasgani et al. 2008*). Due to a lack of data, several studies focusing on specific sub-groups of the general population where also included: two studies of nomadic communities (*Schelling et al. 2003*; *Alavi et al. 2007*) and one of school children (*Idris et al. 1993*).

Studies of Quality 1 and 2 were only available for 15 countries from the following GBD geographic regions: Northern African and Middle East (17 studies) (*Idris et al. 1993*; *Alballa 1995*; *Awad 1998*; *Al Sekait 1999*; *Abu Shaqra 2000*; *Elbeltagy 2001*; *Crump et al. 2003*; *Al-Ani et al. 2004*; *Yacoub et al. 2006*; *Alavi et al. 2007*; *El Sherbini et al. 2007*; *Haghdoost et al. 2007*; *Jennings et al. 2007*; *Hosseini et al. 2008*; *Khorasgani et al. 2008*; *Al Tawfiq & AbuKhamsin 2009*; *Gargouri et al. 2009*), Western Europe (8 studies) (*Karabay et al. 2004*; *Avdikou et al. 2005*; *Cetinkaya et al. 2005*; *De Massis et al. 2005*; *Al Dahouk et al. 2007b*; *Minas et al. 2007*; *Vancelik et al. 2008*; *Jelastopulu et al. 2010*), South and Central America (2 studies) (*Hernandez et al. 1999*; *Marder et al. 2005*), Sub-Saharan Africa (1 study) (*Schelling et al. 2003*) and North America (1 study) (*Doyle & Bryan 2000*), as shown in Figure 6. One additional Quality 1 seroprevalence study from Central Asia (*Bonfoh et al. 2011a*) was identified through targeted non-systematic searching which, although not fulfilling the publication date criteria of the systematic review, was included in data analysis because it provided otherwise missing data.

Study characteristics of Quality 1 and 2 seroprevalence and incidence articles are provided as Supplementary Information (Appendix 1, Tables 12 and 13), grouped by GBD geographic region. Confidence intervals for seroprevalence estimates were only provided in four articles (*Schelling et al. 2003*; *Yacoub et al. 2006*; *El Sherbini et al. 2007*; *Bonfoh et al. 2011a*). The normal approximation to the binomial was used to calculate confidence intervals for the prevalence estimates of the remaining articles.

Although some studies used a cluster-based sampling approach, they did not present adequate information for the calculation of adjusted confidence intervals.

Data Analysis

Table 3 shows the incidence of brucellosis in the general population per 100,000 person-years by country, including rates directly reported as well as those calculated from seroprevalences. The studies are classified as containing data from the national and/or sub-national level. Where incidence rates were reported for several years, only the most recent data are provided.

A wide variation in reported brucellosis incidence is evident regionally, as well as within countries. In the North Africa and Middle East region, for example, incidences calculated from a seroprevalence study in Iraq ranged from 52.3 cases per 100,000 person-years in a rural area to 268.8 cases per 100,000 person-years in a semi-rural area (*Yacoub et al. 2006*). In Egypt, two prospective incidence studies incorporating a surveillance system for acute febrile illness in different rural areas provided rates of 18 (*Crump et al. 2003*) and 70 (*Jennings et al. 2007*) cases per 1000,000 person-years. Only 5.7% of these cases were detected through passive hospital-based surveillance (*Jennings et al. 2007*).

Incidence rates in Western Europe and North America were generally much lower than in other regions, although some within-country variation was still evident. In Greece, for example, respective rates of 4 (*Jelastopulu et al. 2010*) and 32 cases (*Minas et al. 2007*) per 100,000 person-years were reported in western and central areas. The study in western Greece also identified that one quarter of these cases, although diagnosed in health facilities, were not officially reported to the provincial public health department. Although rates in the USA were very low, counties within 100km of the Mexican border had a higher disease incidence (0.18 compared to 0.02) than those in non-border states (*Doyle & Bryan 2000*).

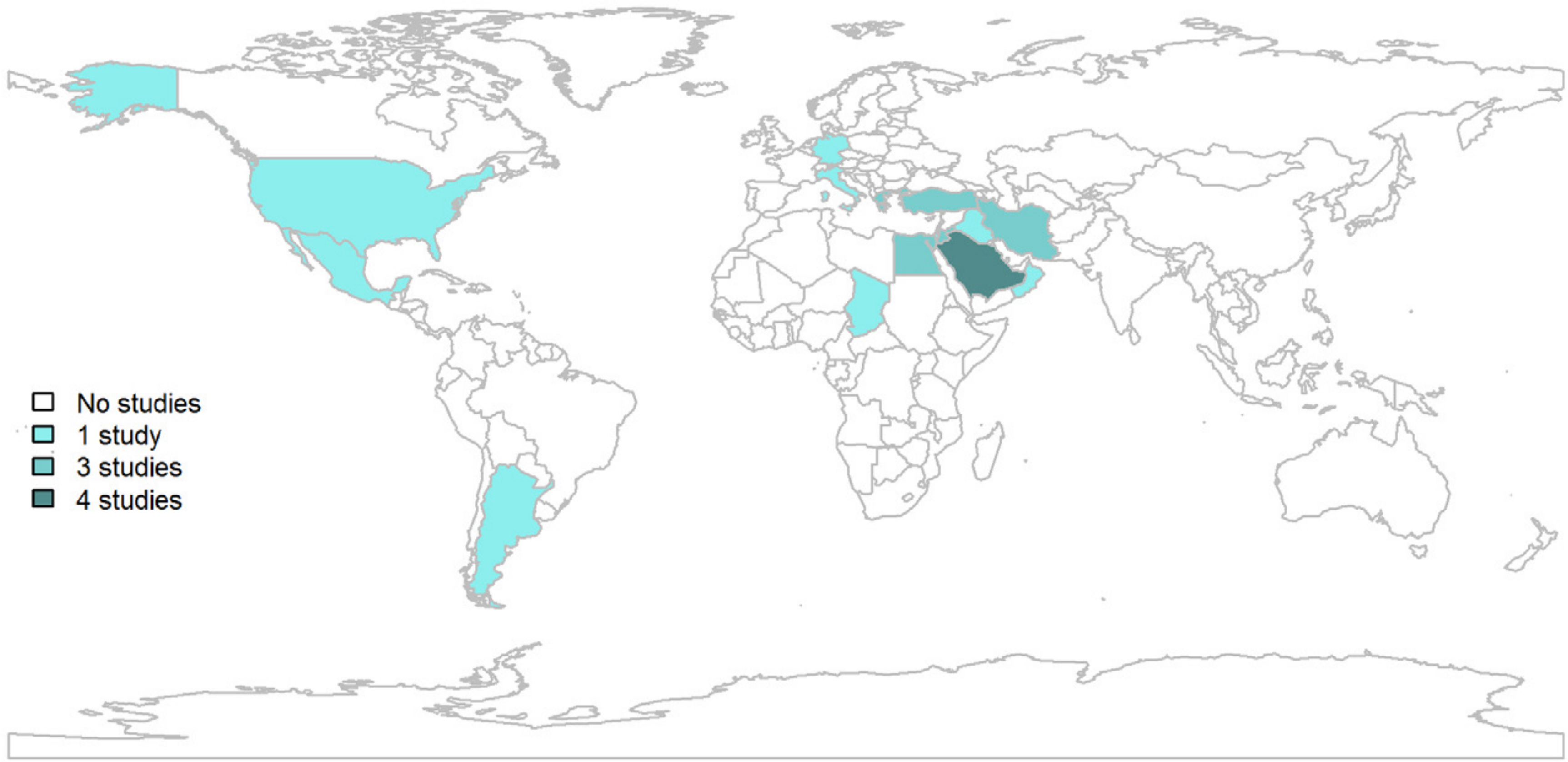

Figure 6: Geographical distribution of selected studies

Table 3: Brucellosis incidence by country (cases per 100,000 person-years)

Country	Study level	Incidence per 100,000 per year
	North Africa and Middle East	
Egypt	Sub-national	0.28-70.00
Iraq	Sub-national	52.29-268.81
Iran	Sub-nationalº	0.73-141.60
Jordan	National	25.70-130.00
Oman	Sub-national*	11.01
Palestine	Sub-national	8.00
Saudi Arabia	National	137.61
	Sub-national	6.00-149.54
Turkey	Sub-national	11.93-49.54
	Sub-Saharan Africa	
Chad	Sub-national+	34.86
	Western Europe	
Germany	National	0.03
Greece	Sub-national	4.00-32.49
Italy	National	1.40
	Central Asia	
Kyrgyzstan	National	88.00
	Central and Southern Latin America	
Argentina	Sub-national	12.84
Mexico	Sub-national	25.69
	North America	
USA	Sub-national	0.02-0.09

º includes one study of a nomadic community
* children only
+ nomadic community

Additional Targeted Searching

Surveillance data from the European Food Safety Authority were obtained, giving an overall incidence for the European Union of 0.08 cases per 100,000 person-years, three quarters of which were reported by Greece, Spain, and Portugal (*European Food Safety Authority & European Centre for Disease Prevention and Control 2009*). Global data obtained non-systematically from various sources including health ministries, international organisations and scientific articles has been previously summarised according to continent and country by Pappas, and a global map was produced (*Pappas et al. 2006*).

3.6 Discussion

The epidemiology of human brucellosis evolved over the previous 15 years, as a result of socioeconomic factors, improved surveillance systems, animal-based control programs and international tourism (*Pappas et al. 2006*). Additionally, political changes have influenced disease epidemiology, with brucellosis emerging as a major human health problem in countries of the former Soviet Union following its dissolution in 1991 (*Roth et al. 2003*).

The current review complements previous assessments of brucellosis disease burden (*Pappas et al. 2006*) by presenting epidemiological data from scientific studies published between 1990 – June 2010 which have been quality screened according to strict criteria. There is an obvious lack of high quality scientific data relating to brucellosis incidence globally, with the majority of data coming from the North Africa and Middle East region. Major gaps exist for Eastern Europe and the Asia-Pacific, both of which had no available data, as well as for Central and South America (only two studies) and Africa (excluding Egypt, only one study). One of the major factors limiting the usefulness of the identified studies was the lack of clearly described methods, particularly in relation to sampling approaches and case definitions. For many studies, it was not possible to assess whether the study had been conducted in such a way to minimise the risk of bias, resulting in the exclusion of data that may have been of acceptable quality.

Brucellosis incidence varies widely not only between countries but also within countries. Although it is not possible to rule out study biases as potential causes of these differences, rates differing by five times in one study in Iraq (*Yacoub et al. 2006*) or by four times in similarly designed studies in Egypt (*Crump et al. 2003*; *Jennings et al. 2007*) suggest that demographic, occupational, and socioeconomic factors may play a

role. Aggregated data at national or regional levels cannot capture these complexities of disease dynamics and, consequently, at-risk populations or areas may be overlooked.
A lower disease incidence is seen in developed countries when compared to low and middle income countries. However, brucellosis still targets specific sub-groups of these populations, such as Turkish immigrants in Germany (*Al Dahouk et al. 2007b*) or Hispanic communities of low socioeconomic status in the USA (*Doyle & Bryan 2000*). Brucellosis clearly remains a disease of public health importance even in developed countries.

Although grey literature can provide high quality data, data from well designed scientific studies are preferred. Passively acquired national data in many brucellosis-endemic countries are likely to underestimate the true disease burden. In an Egyptian study incorporating an active acute febrile illness surveillance system to identify and confirm suspected cases, brucellosis incidence in the study area was 70 cases per 100,000 person-years. Only 5.7% of these cases were identified through hospital-based surveillance, from which the incidence rate would be calculated as 3.8 cases per 100,000 person-years using a case definition based on laboratory confirmation or 6 cases per 100,000 person-years using a clinical definition. Reliance on routine hospital-based incidence data would have, therefore, underestimated incidence by 12-18 times (*Jennings et al. 2007*). Official data from the Ministry of Health provided an incidence rate of only 0.3 cases per 100,000 person-years (*Pappas et al. 2006*).

Such underestimations of disease incidence could relate to barriers to accessing health care or to case mismanagement and misdiagnosis. A retrospective review of hospital records in western Greece identified an additional source of error in official passively acquired data, with a brucellosis under-reporting rate from hospitals to the public health department of 26% (*Jelastopulu et al. 2010*). Consequently, one quarter of cases diagnosed through the hospital system were not included in the official government data. Indeed, incidence rates identified in studies conducted between 1999-2005 in different regions of Greece ranged from 4-32.5 cases per 100,000 person-years, whereas aggregated data published by the European Food Safety provided a national incidence of just 0.9 cases per 100,000 person-years in 2009 (*European Food Safety Authority & European Centre for Disease Prevention and Control 2009*).

Research Agenda

Strengthening public health systems would improve the quality of data captured through routine reporting. In many brucellosis endemic countries, however, health systems are weak and high quality research is needed. Brucellosis incidence and prevalence studies are notably lacking from Eastern Europe, the Asia-Pacific, Central and South America and Sub-Saharan Africa. Researchers must have an adequate foundation in the principles of epidemiology and biostatistics to ensure that their studies are designed, implemented, and analysed in a manner which minimises bias and maximises the usefulness of the data.

An integrated approach to disease surveillance involving both human health and veterinary services would allow a better understanding of disease dynamics at the animal-human interface, as well as a more cost-effective utilisation of resources (*Zinsstag et al. 2005*; *Zinsstag et al. 2009*). A checklist of requirements for representative seroprevalence and incidence studies is given in Table 4.

Limitations

Although test performance was considered in the initial ranking of each article and, thus, influenced study inclusion in the review, the individual results of each study were not adjusted for test performance. This is because test performance can vary significantly according to test manufacturer or laboratory protocol, and such detailed information was not available.

The calculation of incidence rates from seroprevalence studies is based on two assumptions. The assumed duration of seropositivity of 10.9 years has been determined mathematically using Vensim software (*Bonfoh et al. 2011a*). The assumption that 10% seroconversions represent true clinical cases is conservative and is likely to underestimate the true burden of disease.

Studies for which a title or abstract was not published in a language using the Latin alphabet, such as those published only in Chinese characters or Arabic script, may not have been identified during the original database search. Of the foreign language studies that were identified, those published in languages in which the team was not competent were excluded from the analysis. It is possible that some of these studies contained data that could have contributed to this global assessment of brucellosis frequency. Additionally, although studies in English were independently reviewed by three team members, this was not always possible for studies reviewed in other languages (German, French, Spanish).

3.7 Conclusion

This systematic review adds to the understanding of the global burden of brucellosis by identifying high quality data from scientific studies according to strict screening criteria. Disease incidence varied significantly within regions and within countries. Aggregated data do not capture the complexities of disease dynamics and at-risk populations may be overlooked. As many brucellosis-endemic countries do not have strong health systems, passively acquired official data likely underestimate the true burden. The brucellosis research agenda should focus on designing and implementing high quality studies to investigate disease seroprevalence and/or incidence, particularly in Eastern Europe, the Asia-Pacific, Central and South America and Africa.

3.8 Acknowledgements

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Table 4: Key considerations for representative brucellosis seroprevalence and incidence studies

	Seroprevalence	Incidence
Study population		
Defined study zone, geographically or in terms of an administrative unit	✓	✓
Clear inclusion and exclusion criteria	✓	✓
Study population representative of general population, not high risk groups alone	✓	✓
Ongoing community education campaigns to raise disease awareness	✓	✓
Sampling		
Sample size based on appropriate calculation, ideally including clustering of individuals	✓	
Random sampling strategy, ideally using probability proportional to size	✓	
Active surveillance system in health centres and/or in communities at household level		✓
Multidisciplinary study team to investigate disease dynamics at animal-human interface	✓	✓
Diagnostic testing		
Clearly described testing methods, including details of manufacturer or developer	✓	✓
Concise serological and clinical case definitions	✓	✓
ELISA, lateral flow or RBT preferred testing methods	✓	✓
Reporting		
STROBE checklist followed [40]	✓	✓
Consideration of test performance in the analysis of results	✓	✓
Consideration of sensitivity of the surveillance system in the analysis of results		✓

4 Clinical manifestations of human brucellosis: a systematic review and meta-analysis

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4.1 Abstract

Background: The objectives of this systematic review, commissioned by WHO, were to assess the frequency and severity of clinical manifestations of human brucellosis, in view of specifying a disability weight for a DALY calculation.

Methods/Principal Findings: Thirty three databases were searched, with 2,385 articles published between January 1990 – June 2010 identified as relating to human brucellosis. Fifty-seven studies were of sufficient quality for data extraction. Pooled proportions of cases with specific clinical manifestations were stratified by age category and sex and analysed using generalized linear mixed models. Data relating to duration of illness and risk factors were also extracted.
Severe complications of brucellosis infection were not rare, with 1 case of endocarditis and 4 neurological cases per 100 patients. One in 10 men suffered from epididymo-orchitis. Debilitating conditions such as arthralgia, myalgia and back pain affected around half of the patients (65%, 47% and 45%, respectively). Given that 78% patients had fever, brucellosis poses a diagnostic challenge in malaria-endemic areas. Significant delays in appropriate diagnosis and treatment were the result of health service inadequacies and socioeconomic factors. Based on disability weights from the 2004 Global Burden of Disease Study, a disability weight of 0.150 is proposed as the first informed estimate for chronic, localised brucellosis and 0.190 for acute brucellosis.

Conclusions: This systematic review adds to the understanding of the global burden of brucellosis, one of the most common zoonoses worldwide. The severe, debilitating, and chronic impact of brucellosis is highlighted. Well designed epidemiological studies from regions lacking in data would allow a more complete understanding of the clinical manifestations of disease and exposure risks, and provide further evidence for policy-makers. As this is the first informed estimate of a disability weight for brucellosis, there is a need for further debate amongst brucellosis experts and a consensus to be reached.

4.2 Author Summary

Brucellosis is a bacterial disease transmitted to humans by consumption of infected, unpasteurised animal milk or through direct contact with infected animals, particularly aborted foetuses. The livestock production losses resulting from these abortions have a

major economic impact on individuals and communities. Infected people often suffer from a chronic, debilitating illness. This systematic review on the symptoms of human brucellosis is the first ever conducted. Using strict exclusion criteria, 57 scientific articles published between January 1990 – June 2010 which included high quality data were identified. Severe complications of brucellosis infection were not rare, with 1 case of endocarditis and 4 neurological cases per 100 patients. One in 10 men suffered from testicular infection, which can case sterility. Debilitating conditions such as joint, muscle, and back pain affected around half of the patients. Given that most patients had fever, brucellosis poses a diagnostic challenge in malaria-endemic areas where fever is often assumed to be malaria. More high quality data is needed for a more complete understanding of the clinical manifestations of disease and exposure risks, and to provide further evidence for policy-makers.

4.3 Introduction

Brucellosis is one of the most common zoonotic infections globally (*Ariza et al. 2007*). This bacterial disease causes not only a severely debilitating and disabling illness, but it also has major economic ramifications due to time lost by patients from normal daily activities (*WHO et al. 2006*) and losses in animal production (*Roth et al. 2003*). In a review of 76 diseases and syndromes of animals, brucellosis lies within the top ten in terms of impact on impoverished people (*Perry 2002*). A brucellosis disability weighting of 0.2 has been previously proposed for Disability-Adjusted Life Years (DALY) calculation, based on the pain and impaired productivity known to result from infection (*Roth et al. 2003*). However, a more informed estimate is needed for an accurate assessment of disease burden.

In 1992, the World Bank commissioned the original Global Burden of Disease (GBD) study, providing a comprehensive assessment of 107 diseases and injuries and 10 risk factors in eight major regions (*Lopez AD et al. 2006*). This review did not include any neglected tropical zoonoses. Such diseases often do not attract the interest of health researchers or sufficient resources for adequate control, yet they continue to impact significantly on human health and wellbeing, livestock productivity, and local and national economies (*WHO 2006*). There is a need for more accurate data relating to the burden of neglected zoonoses to facilitate more effective implementation of disease control interventions. In 2009, the Foodborne Disease Burden Epidemiology Reference Group (FERG) of the World Health Organization (WHO) commissioned a series of systematic reviews on the burden of neglected zoonotic diseases, with the aim of incorporating the findings into the overall global burden of disease assessments.

This report presents a systematic review of scientific literature published between 1990-June 2010 relating to morbidity from human brucellosis infection. The objectives of this review were to assess the frequency and severity of the clinical manifestations of brucellosis, the duration of disease, the associated disabilities and important risk factors, with a view to estimating an appropriate disability weight for calculation of the brucellosis DALY. A systematic review of scientific literature investigating the incidence and prevalence of brucellosis globally is the subject of a companion paper (*Dean et al. 2012b*).

4.4 Methods

Searching

Thirty three databases were searched for relevant articles using the search terms of (brucellosis OR malta fever OR brucella melitensis OR brucella abortus) AND (symptom* OR sequelae* OR morbidity OR mortality OR transmission mode OR foodborne), with a publication limitation of 1990 - 30 June, 2010. The search term was adapted to the predominate language of the database. If a database did not allow the combining of Boolean operators, (18 of 33 databases), 'brucellosis' was used as the sole term.

Reference Manager bibliographic software was used to manage citations. Duplicate entries were identified by considering the author, the year of publication, the title of the article, and the volume, issue and page numbers of the source. In questionable cases, the abstract texts were compared.

Selection

The articles were sorted by a team of four reviewers with a combined fluency in English, German, French, and Spanish. Articles in other languages were noted for future translation, pending resources.

All reports were classified into one of two categories, based on their abstracts:
Category 1: Relevant – articles related to human brucellosis related to brucellosis infection in populations (i.e. disease frequency) or cases of human brucellosis (i.e. disease morbidity);
Category 2: Irrelevant - articles related to non-human brucellosis; articles addressing topics not related to the current review, such as genetics, laboratory diagnostic tests, experimental laboratory animal studies

The abstracts of studies belonging to Category 1 and meeting the following criteria for disease morbidity studies were retained: published between 1990 and 30 June 2010, at least 10 study subjects, clinical symptoms/syndromes described, and some information relating to diagnostic tests provided. Articles relating to disease frequency and meeting the following criteria were also retained: published between 1990 and 30 June 2010, at least 100 study subjects drawn from the general population, prevalence or incidence data included, and some information relating to diagnostic tests provided. The assessment and classification of frequency articles will be the subject of a companion paper and will not be considered further here.

Articles for which the necessary data for classification could not be obtained were identified for possible future assessment, according to availability of resources. In general, non peer-reviewed or review articles, conference proceedings and book chapters were excluded.

Validity Assessment

After applying the aforementioned screening steps, the full text of each selected article was retrieved for detailed analysis. Each article was reviewed by two or three reviewers, and classification discrepancies were resolved by discussion.

Using a pre-designed Access database, articles were coded according to the following parameters:

1) Study type

Studies were classified as a prospective case series, a retrospective case series, a case-control study, or of another type.

2) Study population

The populations studied were grouped according to age category – children only (<15 years), adults only (≥15 years), or including both children and adults. Additionally, they were coded according to whether the study population represented the general population of brucellosis cases in the age category, or only a specific sub-group.

3) Diagnostic methods

Studies were classified according to their use of microbial culture to diagnose brucellosis patients. In order for studies to be included in the review, they had to not only mention culture in their methods but to also present laboratory results.

4) Overall study quality

Studies were given an overall quality grade of 1, 2, or 3. Quality 1 studies provided data drawn from general brucellosis cases, of which 75% or more were diagnosed by culture, and had well described study design and methods. Quality 2 studies also presented data from general brucellosis cases, utilised culture as a method and presented relevant laboratory results. However, unlike for Quality 1 studies, the majority of cases did not have to be diagnosed by positive culture in order to be included as Quality 2. Quality 3 studies were either drawn from only a specific sub-group of brucellosis cases such that general conclusions could not be drawn, did not use culture as a diagnostic method or failed to present culture results, or had poorly described study design and methods such that the quality of the data could not be assured.

Data Extraction

Based on brucellosis literature (*Madkour 2001*) a comprehensive list of clinical manifestations associated with brucellosis cases was developed:

- General: documented fever, sweats, chills, fatigue, headache, malaise, weight loss, nausea/vomiting
- Abdominal: abdominal pain, splenomegaly, hepatomegaly, hepatitis
- Musculoskeletal: arthralgia, arthritis, myalgia, back pain, spondylitis, sacroiliitis
- Specific organ involvement: epididymo-orchitis, abortion, endocarditis, respiratory and neurological signs, cutaneous changes

Numbers of subjects with each symptom/syndrome were recorded for each study, as well as the number of male and female patients. For the sex-related outcomes of epididymo-orchitis and abortion, the study population was considered to be only the male and pregnant female sub-groups of the study population respectively. Information relating to duration of disease prior to treatment and exposure to potential risk factors were also recorded wherever provided.

Data Analysis

To calculate the proportion of patients by sex, numbers of male and female patients were aggregated across all studies as well as within each age category. 95% confidence intervals were calculated using the normal approximation to the binomial.

Where appropriate data were available from two or more studies, pooled proportions of patients with each clinical manifestation were estimated using generalized linear mixed models. Pooled estimates with 95% confidence intervals were calculated both within age categories and overall across all studies, using a Freeman-Tukey double arscine transformation. Homogeneity across studies was assessed using a Cochrane's Q test and total variability due to between-study variation was reflected in the I^2 index. The meta-analysis was performed with R statistical software (*R Development Core Team 2012*) using the meta package (*Schwarzer 2012*). Additionally, in order to assess the impact of study design, the same analysis was conducted according to study type category.

The pooled estimates for proportions of patients with each clinical manifestation were compared with the disability weights used in the GBD 2004 study (*WHO 2004*). A disability weight for brucellosis was then proposed.

Median proportions of patients with exposure to particular risk factors were calculated. Data relating to duration of illness and diagnostic delay were recorded. In order to assess the duration of untreated illness, an additional, non-systematic search for data prior to the availability of appropriate antibiotics was undertaken by manually searching library records.

4.5 Results

Searching

Table 2 lists the databases searched and the number of hits obtained for each. A total of 28,824 studies were identified, of which 59% were duplicates, leaving 11,000 original reports.

Flow of Included Studies

Figure 7 shows a flow diagram of the process for the selection of articles included in the review. In total, 289 frequency and morbidity studies were selected, for which full text was available for 153. However, 14 of these were in languages in which the team was not competent (Croatian (6), Turkish (4), Korean (2), Persian (1), Mandarin (1)), leaving 96 morbidity studies for quality assessment. Some articles contained both frequency and morbidity data and were thus counted in both categories.

Of the 96 morbidity studies for quality assessment, five were classified as Quality 1 and 52 as Quality 2. Thirty-nine were excluded from further analysis as Quality 3, one of which was due to duplication of data from another larger study. Two pairs of Quality 2 studies were based on the same data (*Colmenero et al. 1991*; *Colmenero et al. 1996*; *Pourbagher et al. 2006a*; *Pourbagher et al. 2006b*). These studies were included because each provided some unique information; however, the duplicated data were only included once in the meta-analysis. Except for two articles in Spanish and one in French, all Quality 1 and 2 studies were in English.

Study Characteristics

The median number of study subjects was 143 (IQR: 85-283), ranging from 20-1028. Studies from high income countries such as Germany, France, and USA were generally situated at the lower end of the range (less than 60 subjects), although larger studies were reported from Spain, including one study of over 900 subjects. Of the 57 studies selected, 24 were from Turkey. The next most represented country was Saudi Arabia, with 8 studies, followed by Spain with 4 and Greece with 4. One or two studies each came from Cuba, France, Germany, Israel, India, Iran, Jordan, Kuwait, Tunisia, USA, Uzbekistan and Yemen. The geographic distribution of the selected studies is shown in Figure 8.

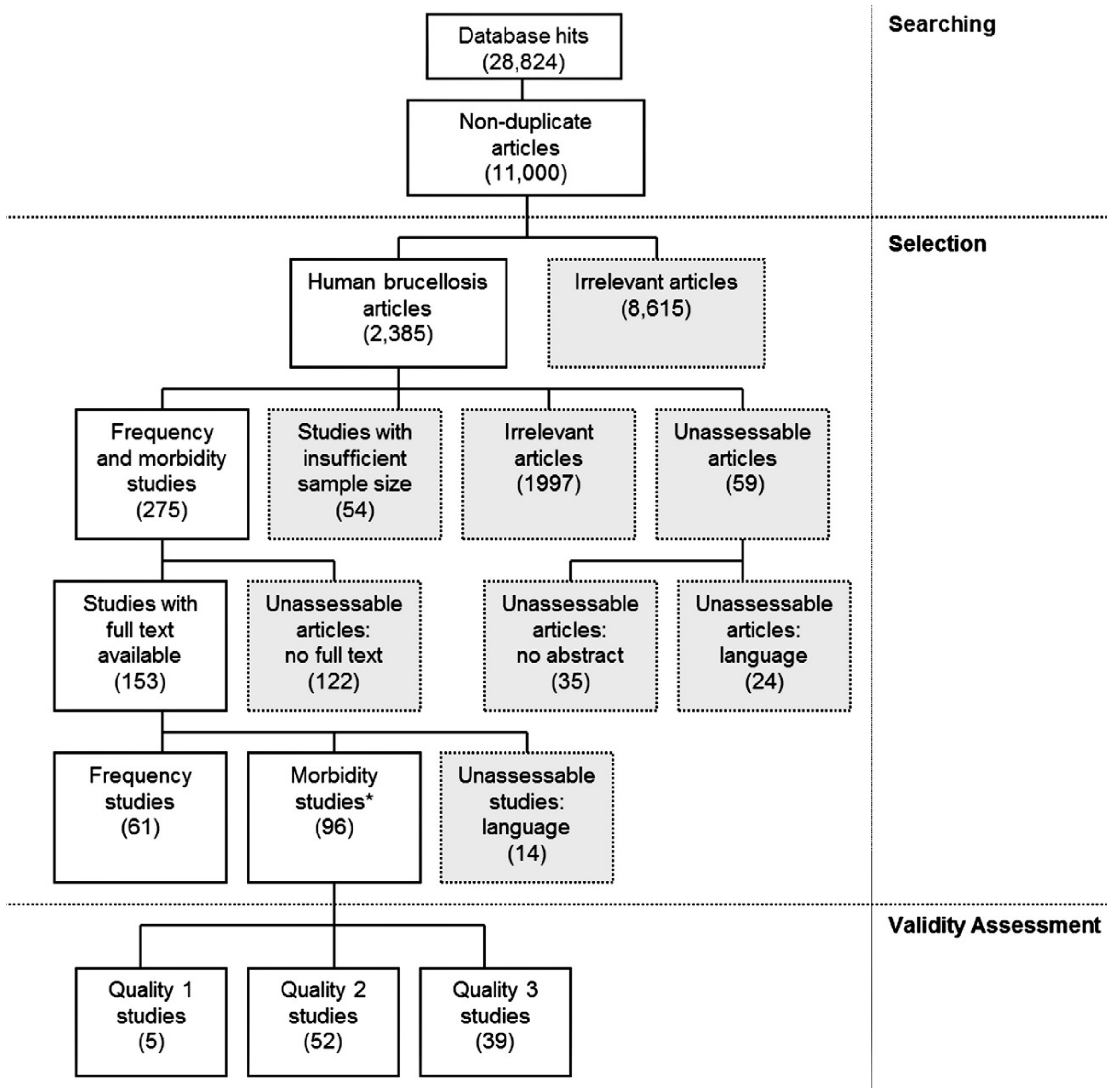

Figure 7: Flow of selected studies

* Some morbidity studies were also classified as frequency studies

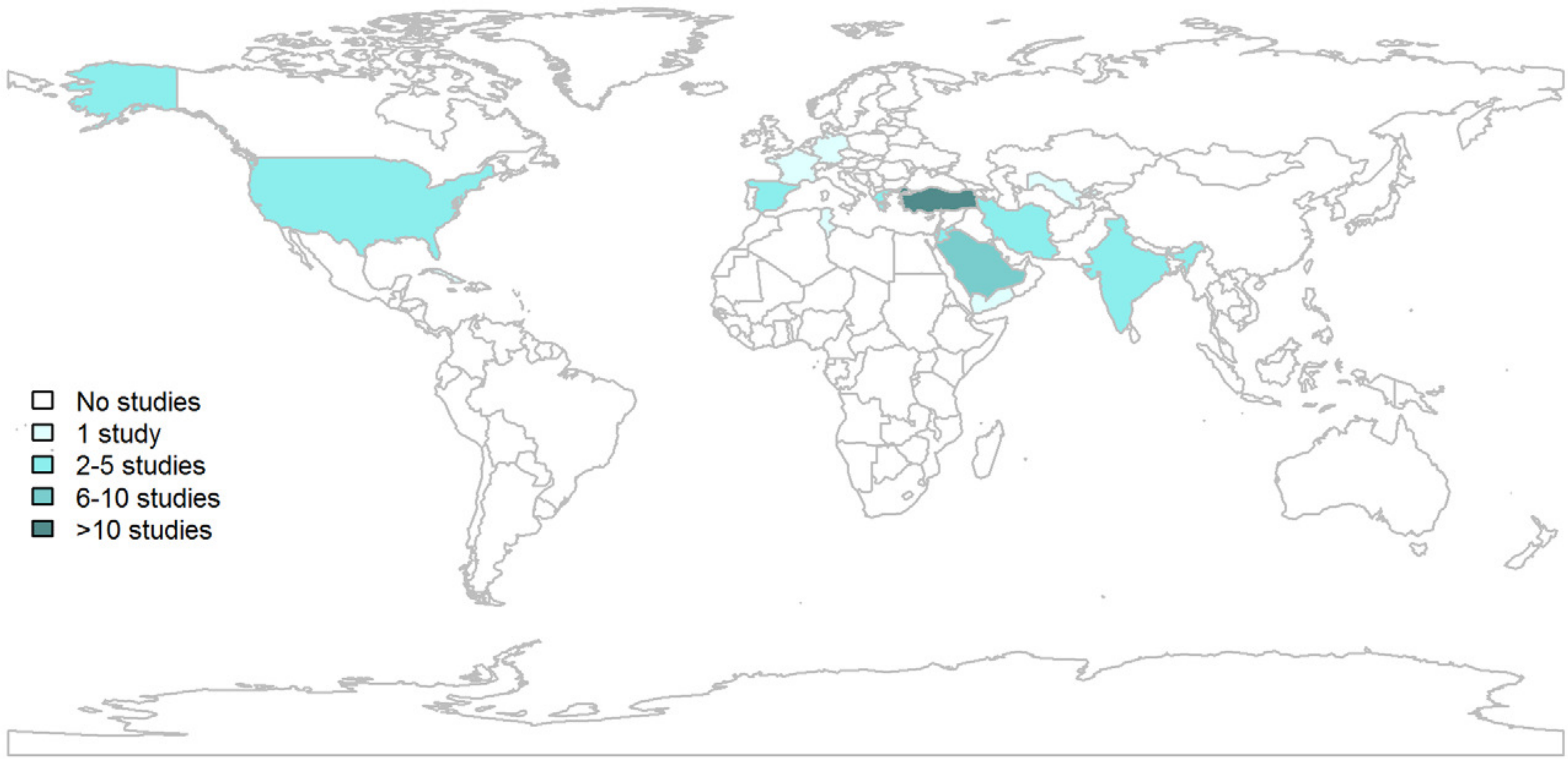

Figure 8: Geographical distribution of selected studies

In terms of study type, 37 were classified as retrospective case series with data retrieved from medical records, and 19 as prospective case series. One study was a case-control. Seventeen studies provided detailed information about cases with specific syndromes, e.g. neurological brucellosis (*McLean et al. 1992*; *Bodur et al. 2003*; *Yetkin et al. 2006*; *Karaoglan et al. 2008*), epididymoorchitis (*Bayram & Kervancioglu 1997*; *Yetkin et al. 2005*; *Akinci et al. 2006*; *Colmenero et al. 2007*), osteoarticular complications (*Khateeb et al. 1990*; *Colmenero et al. 1991*; *Tasova et al. 1999*; *Pourbagher et al. 2006a*), spondylitis (*Bodur et al. 2004*; *Yilmaz et al. 2004*), pulmonary brucellosis (*Hatipoglu et al. 2005*), pancytopaenia (*M. Karakukcu et al. 2004*), and pregnant women (*Khan et al. 2001*). As these studies also provided some information about proportions of general brucellosis cases with specific symptoms/syndromes, they were included in the review.

Twenty-three studies included both children and adult participants (*Khateeb et al. 1990*; *Shehabi et al. 1990*; *Colmenero et al. 1991*; *McLean et al. 1992*; *Zaks et al. 1995*; *Colmenero et al. 1996*; *Neau et al. 1997*; *Al Shamahy & Wright 2001*; *Khan et al. 2001*; *Gur et al. 2003*; *Al Dahouk et al. 2005*; *Fallatah et al. 2005*; *Troy et al. 2005*; *Pourbagher et al. 2006a*; *Pourbagher et al. 2006b*; *Andriopoulos et al. 2007*; *Colmenero et al. 2007*; *Savas et al. 2007*; *Bukharie 2009*; *Earhart et al. 2009*; *Buzgan et al. 2010*; *Alsubaie et al. 2005*). Twelve studies investigated only children (*Galanakis et al. 1996*; *Gottesman et al. 1996*; *Issa & Jamal 1999*; *Shaalan et al. 2002*; *Tsolia et al. 2002*; *Mantur et al. 2004*; *M. Karakukcu et al. 2004*; *Giannakopoulos et al. 2006*; *Afsharpaiman & Mamishi 2008*; *Shen 2008*; *Tanir et al. 2009*; *Citak et al. 2010*), with an upper age limit ranging from 13 years to 18 years. Of the 19 studies with an adult population of 15 years or older (*Bayram & Kervancioglu 1997*; *Pérez et al. 1997*; *Tasova et al. 1999*; *Memish et al. 2000*; *Elbeltagy 2001*; *Aygen et al. 2002*; *Bodur et al. 2003*; *Tasbakan et al. 2003*; *Bodur et al. 2004*; *Yilmaz et al. 2004*; *Yetkin et al. 2005*; *Akinci et al. 2006*; *Mantur et al. 2006*; *Yetkin et al. 2006*; *Kokoglu et al. 2006*; *Abdi-Liae et al. 2007*; *Hizel et al. 2007*; *Demiraslan et al. 2009*; *Zribi et al. 2009*; *Celen et al. 2010*), five consisted of only male participants (*Bayram & Kervancioglu 1997*; *Memish et al. 2000*; *Yetkin et al. 2005*; *Akinci et al. 2006*; *Celen et al. 2010*). Three studies did not clearly state the age category (*Barroso et al. 2002*; *Hatipoglu et al. 2005*; *Karaoglan et al. 2008*) and were analysed as if containing data for both adults and children.

Data Analysis

In studies consisting of only children, 64% patients (95% CI: 60-68%) were male. The proportion of male patients in adult studies was significantly lower, at 56% (95% CI: 55-58%). In studies including both children and adult patients, 48% were male (95% CI: 46-51%). Overall, 55% patients (95% CI: 54-56%) across all studies were male.

Table 5 shows the pooled proportions of patients estimated by the random-effects model, according to clinical manifestations by age category. Forest plots are provided as Supplementary Information (Appendix 2, Figures 17-39). An analysis by study type did not show any significant changes or trends.

Documented fever was common, with an estimated 78% of patients affected across the three age categories. Estimates of the proportions of patients with self-reported symptoms of sweats, chills, fatigue, headache, and malaise, were significantly lower in children, ranging from 9-24% depending on symptom, compared to 33-81% for adults. Weight loss in children, at 13%, was also lower than the 31% reported in adults.

Abdominal-related manifestations of pain, splenomegaly and hepatomegaly were fairly uniformly distributed across age categories, with overall estimated proportions of 19%, 26% and 23%, respectively. The number of studies reporting the presence of hepatitis was small, totalling only seven, with an estimated 4% patients affected overall.

Arthralgia was common, affecting 65% patients overall, whereas arthritis affected only 26% patients. In adult patients, 56% and 49% suffered from myalgia and back pain, respectively. Only two studies reported myalgia and back pain in children. Overall, spondylitis and sacroiliitis were detected in 12-36% adults.

In relation to reproductive problems, only one study reported abortion rates as a proportion of pregnant female participants, which was 46% (*Khan et al. 2001*). Overall, 10% male patients had epididymo-orchitis.

Table 5: Meta-analysis of clinical manifestations of brucellosis by age category

Manifestation	Age Category						All studies	
	Children		Adults		All Ages			
General	*n*	*% (95% CI)*	*n*	*% (95% CI)*	*n*	*% (95% CI)*	*n*	*% (95% CI)*
Fever	7	82 (69; 91)	10	73 (59; 85)	9	79 (49; 97)	26	78 (66; 87)
Sweats	8	23 (11; 37)	14	55 (35; 74)	12	73 (60; 85)	34	54 (42; 66)
Chills	4	18 (9; 29)	5	47 (34; 60)	7	60 (34; 83)	16	45 (30; 61)
Fatigue	2	19 (13; 23)	2	33 (13; 100)	5	51 (27; 75)	9	39 (16; 65)
Headache	6	9 (5; 15)	11	34 (19; 50)	11	52 (32; 72)	28	35 (24; 46)
Malaise	2	24 (16; 34)	6	81 (71; 89)	8	74 (48; 93)	16	71 (57; 83)
Nausea/vomiting	0	-	5	16 (5; 31)	6	26 (15; 38)	11	26 (15; 38)
Weight loss	3	13(8;18)	4	31 (15; 50)	7	29 (15; 47)	14	26 (17; 36)
Abdominal								
Abdominal pain	3	14 (1; 38)	4	9 (1; 22)	9	26 (13; 41)	16	19 (11; 29)
Splenomegaly	9	31 (19; 43)	13	24 (18; 31)	14	25 (17; 34)	36	26 (21; 31)
Hepatomegaly	10	27 (15; 41)	13	22 (16; 26)	14	22 (15; 29)	37	23 (19; 27)
Hepatitis	1	1 (0; 5)*	2	8 (1; 38)	4	3 (1; 6)	7	4 (1; 9)
Musculoskeletal								
Arthralgia	9	71 (56; 84)	12	65 (49; 79)	16	62 (52; 70)	37	65 (58; 72)
Arthritis	7	41 (18; 65)	5	13 (3; 28)	14	25 (17; 34)	26	26 (19; 34)
Myalgia	2	18 (11; 26)	5	56 (38; 75)	8	49 (36; 63)	15	47 (38; 57)
Back pain	1	10 (3; 21)*	11	49 (31; 67)	11	45 (31; 60)	23	45 (34; 56)
Sacroiliitis	4	6 (3; 10)	3	32 (20; 46)	9	14 (7; 22)	16	15 (9; 22)
Spondylitis	1	18 (1; 28)*	6	12 (7, 19)	9	11 (6; 18)	16	12 (8; 17)
Specific organs								
Epididymo-orchitis	1	10 (1; 32)*	10	10 (7; 15)	10	9 (6; 13)	21	10 (7; 13)
Endocarditis	2	3 (1; 6)	6	2 (1; 3)	7	1 (1; 2)	15	2 (1; 2)
Neurological	5	2 (1; 4)	11	5 (3; 7)	10	4 (2; 6)	26	4 (3; 5)
Respiratory	3	5 (1; 14)	5	2 (1; 5)	11	9 (4; 14)	19	6 (3; 9)
Cutaneous	6	5 (2; 10)	4	4 (1; 11)	7	8 (4; 14)	17	6 (4; 9)

*** One study only, with a binomial 95% confidence interval**

Pooled proportions of patients with each manifestation are presented as percentages with 95% confidence intervals. The numbers of studies (*n*) contributing to each estimate are given.

For more severe outcomes, endocarditis was reported in an overall 1% patients, and neurological manifestations in 4%. Neurological outcomes reported included motor deficits, cranial nerve deficits, sciatica, confusion and/or psychological disturbances, meningitis and seizures. 6% of patients suffered from respiratory manifestations, including cough, bronchopneumonia, pleural adhesion and pleural adhesion. Cutaneous changes were reported in 6% patients.

As most studies were case series without a control group, an evaluation of the importance of risk factors was not possible. However, median proportions were calculated from 27 studies which provided some exposure history. Median proportions of brucellosis cases with exposure to a potential risk factor were 64% (IQR: 34-78%) for consumption of unpasteurised dairy products, 42% (IQR: 23-59%) for contact with livestock, and 6% (IQR: 3-19%) for occupational exposure, including veterinarians, butchers, and abattoir workers. From fifteen studies, the median proportion of cases with a history of brucellosis in a family member was 20% (IQR: 17-46%).

Only six studies included in the systematic review provided data regarding duration of illness prior to diagnosis and treatment (*Al Shamahy & Wright 2001*; *Shaalan et al. 2002*; *Al Dahouk et al. 2005*; *Hizel et al. 2007*; *Shen 2008*; *Zribi et al. 2009*). The age of the patient and the nature of the illness were influential factors. One study reported a longer duration of illness in adults compared to children under 15 years, averaging 8 weeks versus 4 weeks, respectively (*Al Shamahy & Wright 2001*). In another study, the average duration of illness prior to diagnosis and treatment was 40 days, but cases with osteoarticular disease generally experienced longer periods of illness, extending to 6 months (*Zribi et al. 2009*).

The GBD 2004 study estimated the disability weights for low back pain due to chronic intervertebral disc disease and osteoarthritis of the knee to be 0.121 (range 0.103-0.125) and 0.129 (range 0.118-0.147), respectively (*WHO 2004*). Given the high proportion of patients in our systematic review with joint, back, or muscular pain, a disability weight of at least 0.150 is proposed as a minimum estimate for localised, chronic brucellosis. Generalised, non-specific clinical manifestations were also common. Acute, non-localised brucellosis could be approximated by an episode of malaria, estimated to be 0.191 (range 0.172 - 0.211) by the GBD 2004 study (*WHO 2004*).

4.6 Discussion

The clinical picture of brucellosis presented in this systematic review is consistent with other literature (*Franco et al. 2007*). Although a large amount of data are available regarding clinical manifestations of brucellosis, its geographical distribution is limited. No high quality studies were identified from Sub-Saharan Africa, Central and South America or South-East Asia. This could potentially reflect either a lower disease burden or a poorer brucellosis surveillance system.

The proportion of male patients was greater than female patients amongst both children and adults. Although this difference was only small in adults, it was more pronounced in children. Possible explanations could be a greater risk of exposure amongst boys, with household responsibilities such as shepherding of livestock being preferentially delegated to boys, or gender-related differences in accessing to health care.

Given the high proportion of brucellosis cases with fever, brucellosis should be considered as a differential diagnosis for fevers of unknown origin. In malaria-endemic countries, fever patients are often diagnosed and treated for malaria based solely on clinical findings (*Amexo et al. 2004*). Improved diagnostic capacity would reduce the diagnostic delay and facilitate prompt and appropriate treatment. These health service inadequacies are compounded by socioeconomic factors, with brucellosis affecting poor, marginalised communities who often do not have the means to seek treatment. Although studies included in this systematic review did not investigate health-seeking behaviour, a study from rural Tanzania revealed that 1 in 5 patients did not present to a health centre for assessment until more than one year after the onset of illness. Once at the health centre, nearly half (45%) were not diagnosed with brucellosis at their first visit (*Kunda et al. 2007*). In children, particularly, under-diagnosis of brucellosis is likely. The lower proportions of reported general symptoms such as sweats, chills, fatigue, and headache in study populations consisting only of children in this systematic review could reflect difficulty in obtaining accurate case histories from this group.

One in 10 men experienced epididymo-orchitis, the most common genitourinary complication of brucellosis infection. This can have serious repercussions such as abscessation and infertility. Although other severe outcomes were less common, 4 neurological cases and 1 endocarditis case per 100 brucellosis patients were reported, which is substantial.

Arthralgia, myalgia, and back pain were common manifestations. The relative lower proportions of patients with sacroiliitis and spondylitis compared to those reporting back pain might reflect limitations in diagnostic capacity. Chronic pain has been shown to severely affect the quality of sufferers' social and working lives (*Breivik et al. 2006*). As the majority of the brucellosis disease burden is in less developed countries, where livelihoods are often reliant on physical activities, the impact of musculoskeletal pain and impaired function in these settings may be even more serious.

One study reported that patients with osteoarticular disease experienced a greater diagnostic delay than other cases (*Zribi et al. 2009*), reflecting the chronic debilitation that can result from brucellosis infection. Indeed, in an endemic area of Russia prior to the availability of effective antibiotic therapies approximately 40% of 1,000 brucellosis cases followed over a 20 year period continued to suffer from clinical manifestations two years after disease onset. In this study, cited by Wund in 1966, approximately 90% of cases had self-cured after 6 years. (*Wundt 1968*).

Given the complexity of the clinical manifestations of brucellosis, summarising its impact into a single disability weight risks being too reductionist. However, a disability weight is required for an assessment of the global burden of disease which is, in turn, essential for engagement of policy-makers and funding bodies. Using the disability classes formerly used by the GBD 2004 study (*Murray 1994*), a disability weight of 0.2 has been previously proposed based on Mongolian patient data (*Roth et al. 2003*). This estimate fell between Class 1 (0.096), which referred to a limited ability to perform at least one activity in the one of the following areas: recreation, education, procreation or occupation; and Class 2 (0.22), referring to a limited ability to perform most activities in one of the aforementioned areas.

Based on this systematic review and meta-analysis, better informed estimates of disability weights are proposed: at least 0.150 for chronic, localised brucellosis and 0.190 for acute brucellosis. However, as this is the first informed estimate of a brucellosis disability weight, there is a need for further debate amongst brucellosis experts and a consensus to be reached.

Research Agenda

Morbidity could vary geographically according to epidemiological setting. Well designed epidemiological studies from regions under-represented in this review would greatly contribute to an overall assessment of the global disease burden. A surveillance system amongst fever patients in malaria-endemic countries could be particularly informative. Additionally, risk factors for disease should be investigated through case-control studies. This would provide invaluable information to guide disease control interventions and policy.

Limitations

Studies for which a title or abstract was not published in a language using the Latin alphabet, such as those published only in Chinese characters or Arabic script, may not have been identified during the original database search. Of the foreign language studies that were identified, those published in languages in which the team was not competent were excluded from the analysis. It is possible that some of these studies contained data that could have contributed to this global assessment of brucellosis morbidity. Additionally, although studies in English were independently reviewed by three team members, this was not always possible for studies reviewed in other languages (German, French, Spanish).

There were likely some differences between the case definitions and diagnostic capacity of different studies. For neurological and respiratory syndromes, many studies provided only an overall aggregated estimate without details of the different disease forms. A respiratory case could potentially vary from a patient with only a cough to severe bronchopneumonia, or a neurological case from altered behaviour and confusion to nerve deficits, meningitis or seizures. All patients were positive by culture in only 3 studies. Given the complexity of brucellosis serology interpretation, it is possible that some patients in other studies were misdiagnosed as cases of active brucellosis.

The studies provide data from brucellosis patients presenting to health centres. It is possible that cases that do not present to health centres are less severe. The results of this review may, therefore, be biased towards more severe cases. As with the estimation of other disability weights, the proposed brucellosis disability weight estimate assumes that a given clinical manifestation will result in the same disability in all settings, which is unlikely (*Reidpath et al. 2003*).

4.7 Conclusion

This systematic review adds to the understanding of the global burden of brucellosis, one of the most common and important zoonotic diseases worldwide. Brucellosis is shown to have a severe, debilitating, and often chronic impact on its sufferers. Significant delays in appropriate diagnosis and treatment are the result of both health system inadequacies and socioeconomic factors. Well designed epidemiological studies from those regions identified to be lacking in data would allow a better understanding of the clinical manifestations of disease and exposure risks and provide further evidence for policy-makers. Based on the findings of this systematic review and the disability weights from the 2004 Global Burden of Disease Study, a disability weight of 0.150 is proposed as the first informed estimate for chronic, localised brucellosis and 0.190 for acute brucellosis. As this is the first informed estimate of a disability weight for brucellosis, there is a need for further debate amongst brucellosis experts and a consensus to be reached.

4.8 Acknowledgements

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5 Epidemiology of brucellosis and Q Fever in linked human and animal populations in northern Togo

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5.1 Abstract

Background: Although brucellosis (*Brucella* spp.) and Q Fever (*Coxiella burnetii*) are zoonoses of global importance, very little high quality data are available from West Africa.

Methods/Principal Findings: A serosurvey was conducted in Togo's main livestock-raising zone in 2011 in 25 randomly selected villages, including 683 people, 596 cattle, 465 sheep and 221 goats. Additionally, 464 transhumant cattle from Burkina Faso were sampled in 2012. The serological analyses performed were the Rose Bengal Test and ELISA for brucellosis and ELISA and the immunofluorescence assay (IFA) for Q Fever Brucellosis did not appear to pose a major human health problem in the study zone, with only 7 seropositive participants. *B. abortus* was isolated from 3 bovine hygroma samples, and is likely to be the predominant circulating strain. This may explain the observed seropositivity amongst village cattle (9.2%, 95%CI:4.3-18.6%) and transhumant cattle (7.3%, 95%CI:3.5-14.7%), with an absence of seropositive small ruminants. Exposure of livestock and people to *C. burnetii* was common, potentially influenced by cultural factors. People of Fulani ethnicity had greater livestock contact and a significantly higher seroprevalence than other ethnic groups (Fulani: 45.5%, 95%CI:37.7-53.6%; non-Fulani: 27.1%, 95%CI:20.6-34.7%). Appropriate diagnostic test cut-off values in endemic settings requires further investigation. Both brucellosis and Q Fever appeared to impact on livestock production. Seropositive cows were more likely to have aborted a foetus during the previous year than seronegative cows, when adjusted for age. This odds was 3.8 times higher (95%CI: 1.2-12.1) for brucellosis and 6.7 times higher (95%CI: 1.3-34.8) for Q Fever.

Conclusions: This is the first epidemiological study of zoonoses in Togo in linked human and animal populations, providing much needed data for West Africa. Exposure to *Brucella* and *C. burnetii* is common but further research is needed into the clinical and economic impact.

5.2 Résumé

Situation : Bien que la brucellose (*Brucella* spp.) et la fièvre Q (*Coxiella burnetii*) soient des zoonoses avec une importance au niveau global, très peu de données de bonne qualité existe pour l'Afrique de l'Ouest.

Méthodes / principaux résultats: Une enquête de sérologie a été menée dans la principale zone d'élevage de bétail du Togo en 2011, dans 25 villages choisis aléatoirement, comprenant 683 personnes, 596 bovins, 465 moutons et 221 chèvres. De plus, 464 bovins transhumants provenant du Burkina Faso ont été échantillonnés en 2012. Les analyses sérologiques employées comprenaient le Test de Rose Bengale et la méthode immuno-enzymatique ELISA pour la brucellose, un test d'immunofluorescence et la méthode immuno-enzymatique ELISA pour la fièvre Q. La brucellose ne semblait pas être un problème important pour la santé publique dans la zone d'étude, avec seulement 7 participants séropositifs. *B. abortus*, qui a été isolé à partir du liquide d'hygroma de 3 vaches, semble être la souche prédominante circulant dans la zone. Cela pourrait expliquer la séropositivité observée parmi les bovins sédentaires des villages (9,2%, IC95%: 4,3-18,6%) et les bovins transhumants (7,3%, IC95% : 20,6-34,7%), et l'absence de séropositivité parmi les petits ruminants. L'exposition du bétail et des humains à *C. burnetti* était fréquente, peut-être influencée par des facteurs culturels. Les individus peulhs avaient des contacts plus fréquents avec le bétail. La séroprévalence au sein de cette ethnie était également plus élevée que pour le reste de la population (peulh : 45,5%, IC95% 37,7-53,6% ; non peulh : 27,1%, IC95% : 20,6-34,7%). L'adoption de seuils de détection appropriés pour les tests diagnostiques utilisés en zone d'endémie requiert de plus amples investigations. La brucellose et la fièvre Q semblaient avoir un impact important sur la production animale. Les vaches séropositives avaient plus de chance d'avoir eu un avortement pendant l'année précédente que les vaches séronégatives. L'odds ratio était 3,8 fois plus élevé (IC95% : 1,2-12,1) pour la brucellose et 6,7 fois plus élevé (IC95% : 1,3-34,8) pour la fièvre Q.

Conclusions: Cette étude épidémiologique sur des zoonoses est la première au Togo à l'interface entre populations humaines et animales, et fournit des données importantes pour l'Afrique de l'Ouest. L'exposition à *Brucella* et *C. burnetii* est fréquente mais de

plus amples investigations portant sur leur impact clinique et économique sont nécessaires.

5.3 Introduction

Brucellosis is one of the most common zoonotic diseases globally (*Ariza et al. 2007*). *Brucella* spp are transmitted to people through the consumption of unpasteurised dairy products or direct contact with infected animals, particularly abortion materials (*Corbel 2006*; *Franco et al. 2007*). This bacterial disease causes not only a severely debilitating and disabling illness (*Dean et al. 2012a*), but also has major economic ramifications due to time lost by patients from normal daily activities (*Corbel 2006*) and losses in animal production (*Roth et al. 2003*). High quality incidence data for human brucellosis are predominantly available from the Middle East and North Africa region (*Dean et al. 2012b*) with no high quality data published from West Africa. Exposure to *Brucella* has been demonstrated in cattle populations in West Africa (*Turkson & Boadu 1992*; *Tounkara et al. 1994*; *Thys et al. 2005*; *Bankole et al. 2010*), with *B. abortus* isolated from bovine hygromas in Mali (*Tounkara et al. 1994*) and the Gambia (*Bankole et al. 2010*).

In recent years, *Coxiella burnetii* has become a bacterial zoonosis of increasing interest, due to an unprecedented, large-scale outbreak of Q Fever in the Netherlands from 2007-2010 with approximately 4,000 notified human cases (*Forland et al. 2012*). The most common route of infection for people is the inhalation of dust contaminated by infected animal fluids, followed by ingestion, particularly of unpasteurised dairy products (*Maurin & Raoult 1999*; *Porter et al. 2011*). The global importance of Q Fever is unknown. In West Africa, there is evidence of exposure to the organism amongst fever patients in Mali (*Steinmann et al. 2005*), but more representative and comprehensive seroprevalence and clinical data are needed.

A One Health approach to disease prevention and control not only fosters collaboration between human and animal health sectors, but also brings additional benefits to each (*Zinsstag et al. 2005*). The economic benefits include those generated through the sharing of resources such as vehicles or field equipment (*Schelling et al. 2005*; *Schelling et al. 2007*), or through a reduced burden on the health care system by controlling diseases in the animal reservoir (*Roth et al. 2003*; *Zinsstag et al. 2007b*). The simultaneous assessment of exposure in people and animals provides a more complete picture of the disease epidemiology, deepening our understanding of disease risk and providing an important knowledge base for policy development. In resource-poor settings, an integrated human-animal health system is particularly needed in order to increase the cost effectiveness of disease prevention and control interventions, improve the efficiency of disease surveillance and response, and strengthen the health system as a whole (*Zinsstag et al. 2009*).

In Togo, animal brucellosis was first documented in the 1960s (*Domingo 2000*). Although bovine brucellosis seroprevalence studies (*Kponmassi 1991*) and *Brucella* sp. identification (*Verger et al. 1982*) were conducted in the 1980s and early 1990s, simultaneous assessment of exposure patterns in people and livestock was lacking. The Savannah Region is Togo's principal livestock-raising zone. Being the northernmost of five administrative regions, it is bordered by Ghana to the west, Burkina Faso to the north, and Benin to the east. Cross-border livestock movements are an inherent component of livestock management practices, via largely unregulated trade routes or semi-nomadic movements known as transhumance (*IIED and SOS Sahel UK 2010b*). Official policy for transhumance in West Africa was agreed upon in 1998 by member countries of the Economic Community of West African States (ECOWAS). Togo permits the entry of foreign herds in search of pasture and watering points from January to May of each year. The Savannah Region of Togo is, therefore, a diverse epidemiological setting, with its livestock disease status likely to be linked to that of neighbouring countries.

5.4 Methods

Ethics Statement

This study was approved by the Ethics Committee for Health Research (Comité de Bioéthique pour la Recherche en Santé) of the Ministry of Health of Togo (ref. 0131/2011/MS/CAB/DGS/DPLET/CBRS) and the Livestock Division (Direction de l'Elevage) of the Ministry of Agriculture, Livestock Production and Fisheries of Togo. In Switzerland, approval was given by the Ethics Commission of the Cantons of Basel-Stadt and Basel-Land (Ethikkommission beider Basel) (ref. 146/10) and the Research

Commission of the Swiss Tropical and Public Health Institute of Basel, Switzerland. Informed written consent was obtained from all literate participants or, in the case of minors, from their parents or guardian. For illiterate participants, informed consent was recorded with a fingerprint, which was witnessed and documented in writing by a literate acquaintance of the participant. Obtaining consent in this way was approved by the aforementioned ethics commissions. The collection of blood samples from animals was always supervised by a veterinarian. Animals were handled in manner aiming to minimise stress and suffering, and were only restrained for the shortest period of time necessary.

Village Serosurvey

Using a detailed regional map and MapInfo Professional® 7.0 SCP, 25 geographical coordinates were randomly selected from the Savannah Region, excluding national parks where settlements were prohibited. The 25 villages closest to each coordinate were visited in May - June 2011 and enrolled in the study after approval from the village chief (see Figure 9). A day was allocated for the study team to return to conduct the survey in each village. The study team included nurses from local health centres, as well as veterinary technicians and veterinarians. Cluster sample sizes were calculated using the formula of Bennett (*Bennett et al. 1991*) with 95% confidence intervals according to Newcome (*Newcome 2001*).

Household and participant selection

Due to the absence of a household list, a central point in the village was identified and a direction selected by spinning a pen. The number of houses from the centre to the edge of the village was counted in this direction, and a number randomly selected between 1 and the total number of houses. The household corresponding to this number was identified by counting the doors in this direction, moving away from the central point. The study was explained in the local language and one adult and one child aged between 5-14 years of age were enrolled by randomly selecting from those household members willing to participate. The household with its door closest to the first selected household was also visited and a further two participants enrolled. The team then returned to the centre of the village to choose a second direction. An intraclass correlation coefficient of 0.1 and an expected brucellosis seroprevalence of 3% (*Schelling et al. 2003*) were assumed. A sample size of 15 people from 25 villages would achieve a precision corresponding to a 95% confidence interval not wider than 6 percentage points. Consequently, the sampling procedure above was repeated until a total of 7-8 households were enrolled in each village, i.e. 14-16 people.

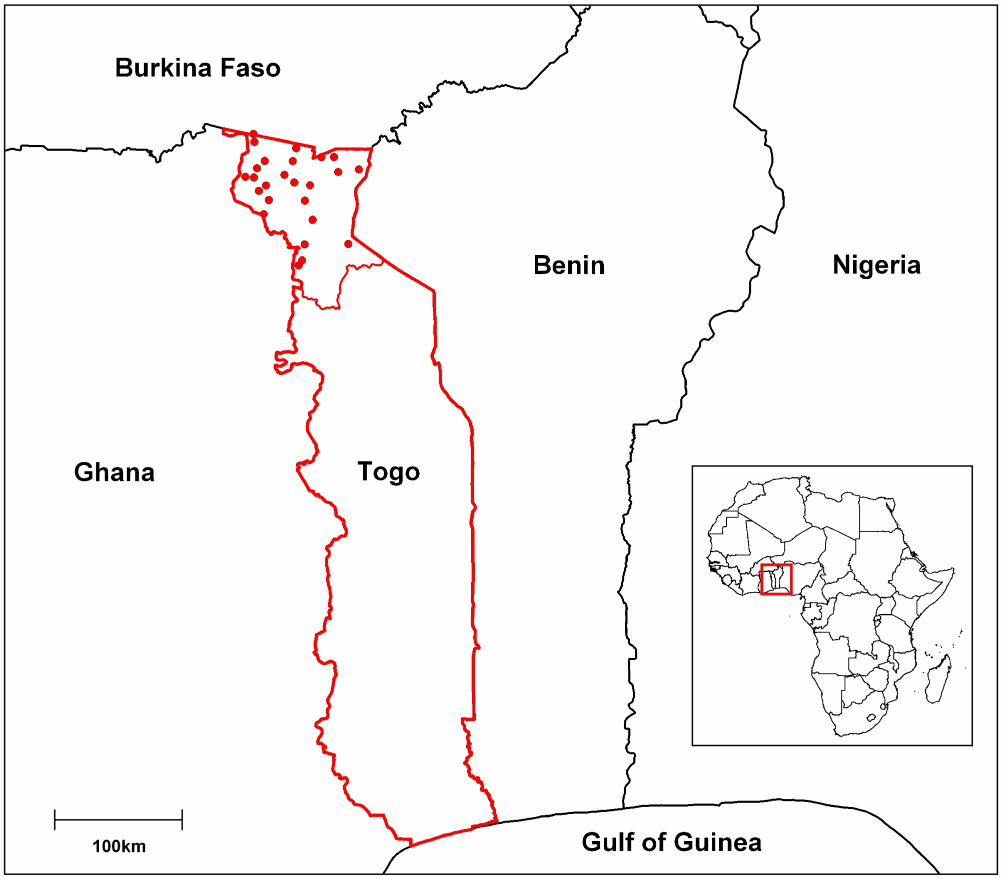

Figure 9: Map of study site

The national borders of Togo are shown in red and the boundary of the Savannah Region is indicated by the dashed red line. The red points correspond to the 25 randomly selected villages included in the survey.

The majority of cattle herds in northern Togo are managed by people of Fulani ethnicity, pastoralist communities who represent the most dispersed ethnic group in West Africa (*Oppong 2002*; *Bassett & Turner 2007*). Fulani communities represent only a very small proportion of the overall population of each village, and their households are often clustered at the edge of village. As the household selection procedure described above for the general population would not have provided an adequate sample size from the Fulani communities of each village, targeted sampling was conducted. Approval was first sought from the Fulani chief and the community members were then gathered together to explain the aims and methods of the study. Participants were selected from those willing to participant by random number allocation. The planned sample size for the Fulani was 14-16 people, thus totalling 28-30 people per village (half of which were Fulani).

Blood was collected from people by a trained nurse, in parallel with data relating to the presence of specific symptoms during the previous month (fever, headache, night-time sweats, sleeping difficulties, or muscle, joint or nerve pain). Basic data relating to demography (age, sex, ethnicity) and risk factor exposure were collected through structured interviews with participants or their parent/guardian. The risk factors assessed were the consumption of dairy products (from any species) that had not been subjected to heat treatment (boiling), and contact with either cattle or small ruminants, where contact was defined as living in a household that owned or looked after cattle or small ruminants.

Animal sampling

In each village, households owning cattle and/or small ruminants (sheep, goats) were identified through discussion with the village chief, the Fulani chief, and the local veterinary technician. In each village, these households were visited by the study team and invited to participate. Owners were requested in advance to keep their animals nearby their homes on the pre-selected sampling day. As a full census of the village's livestock population was not available, sampling was distributed across all identified herds by dividing the total desired number per village by the number of herds/flocks to obtain the number of animals to be randomly sampled per herd/flock. Animals under 3 months of age were excluded. An intraclass correlation coefficient of 0.1 (*Otte & Gumm 1997*) and an expected brucellosis seroprevalence of 5% in cattle and 1% in small ruminants (*Schelling et al. 2003*) were assumed. A sample size of 20 cattle and 25 small ruminants from 25 villages would achieve a precision corresponding to a 95% confidence interval not wider than 7 percentage points for cattle and 4 percentage points for small ruminants.

Blood collection was performed by a veterinarian and veterinary technician. Individual and herd data (age, sex, herd size, abortions during previous year, and abortions during reproductive life for transhumant cattle only) were obtained through structured interviews with herd owners or herdsmen.

Transhumant Serosurvey

In February - March 2012, information about the location of transhumant cattle herds was obtained from key informants, including local livestock owners and herders, Fulani communities, and animal health personnel. Given the available resources and time period, a sample size of 15 herds was deemed to be realistic, with 25 animals per herd based on an expected brucellosis seroprevalence of 5% (*Bennett et al. 1991*; *Newcome 2001*). Blood collection from cattle as well as structured interviews with herdsmen were conducted as per the village survey. Blood samples were not collected from the herdsmen.

Serological Analyses

After collection, all blood samples were immediately chilled on ice and centrifuged the same day. Serum was then collected and frozen at -20℃ until analysis. Sera from the village survey were transported frozen according to international biosafety standards to the Swiss Tropical and Public Health Institute in Basel, Switzerland for serological analysis. Sera from the transhumant survey were analysed at the Central Veterinary Laboratory of the Ministry of Agriculture, Livestock and Fisheries in Lomé, Togo, and training was provided to the laboratory technicians. A sub-sample of the sera tested in Switzerland was re-tested in Togo for comparison, to ensure an agreement of results by the two laboratories.

Brucellosis

All human samples were tested by the Rose Bengal Test (RBT) (Brucella Rose Bengale, Bio-Rad Laboratories, CA, USA), using a serum:reagent ratio of 1:1 for 8 minutes. Positive sera were sequentially diluted with saline to obtain dilutions from 1/2 to 1/16, then retested as per the protocol of Diaz et al (*Diaz et al. 2011*). Additionally, all sera were tested by indirect IgG ELISA. Samples positive by IgG ELISA or RBT were also tested by IgM ELISA (Serion ELISA classic Brucella IgM/IgG, Institut Virion\Serion GmbH, Würzburg, Germany), in order to identify recent infections. Samples were classified as positive or negative according to the manufacturer’s recommended cut-off ranges and doubtful samples were re-tested.

All animal samples were tested by the RBT, using a serum:reagent ratio of 1:1 for cattle and 3:1 for small ruminants for 4 minutes, as per recommendations of the World Organisation for Animal Health (OIE) (*OIE 2012*). Additionally, all samples were tested by indirect ELISA (CHEKIT Brucellose Serum ELISA Test Kit, IDEXX Laboratories, ME, USA) and classified as positive or negative according to the manufacturer's recommended cut-off ranges. Samples positive by RBT but negative by ELISA were tested by the Complement Fixation Test according to OIE standards at the Institute for Veterinary Bacteriology, University of Bern.

Q Fever

A random sub-sample of human sera was tested using an indirect IgG Phase II ELISA (*Coxiella burnetii* ELISA IgG, Vircell SL, Granada, Spain). Samples were classified as positive or negative according to the manufacturer's recommended cut-off ranges and doubtful samples were re-tested. In order to assess the suitability of the ELISA cut-off value for the Togo samples, the immunofluorescent antibody test (IFA) for Phase II IgG was performed by Vircell S.L. in Spain (*Coxiella burnetii* IFA IgG, Vircell SL, Granada, Spain) (*Blaauw et al. 2012*).

A random sub-sample of sera from village livestock was tested using an indirect IgG ELISA (CHEKIT Q-Fever Antibody Test Kit, IDEXX Laboratories, ME, USA). Samples were classified as positive or negative according to the manufacturer's recommended cut-off ranges and doubtful samples were re-tested.

Hygroma Fluid Sampling and Culture

In March 2012, cattle with joint hygromas from study villages and neighbouring villages were identified through discussion with cattle owners or herders and local veterinary technicians. Fluid samples from joint hygromas were collected from nine cattle, six of which were from herds sampled within the village serosurvey. All hygromas were involving the carpus, except for one over the hock. After aseptic collection of joint fluid, a sterile swab tip was soaked in the fluid and sealed in liquid Amies transport medium. Two swabs were harvested per animal. The swabs were stored at 4°C before transportation to Switzerland, according to international biosafety standards, for microbiological culture at the Institute for Veterinary Bacteriology, University of Bern.

The swabs were cultivated on tryptic soy agar plates supplemented with 5% sheep blood (BD, NJ, USA) and on Brucella-selective agar (BSA). The BSA contained Brucella-medium base (Oxoid, Basingstoke, UK), 5% inactivated horse serum and 7.5mL modified Brucella Selective Supplement (Oxoid, Basingstoke, UK). The plates were incubated for 10 days at 37℃ in 5% CO_2. Colonies from pure cultures were suspended in lysis buffer (0.1M Tris-HCl pH 8.5, 0.005% Tween 20, 0.24 mg/mL Proteinase K) and incubated for 60 minutes at 60℃ and for 15 minutes at 95℃. The lysates were then filter sterilised.

Identification of the genus *Brucella* was performed by realtime PCR according to the protocol of Hinic et al (*Hinic et al. 2008*). Multiple Loci Variable Number of Tandem Repeats Analysis (MLVA) was carried out over 16 loci (panel 1, panel 2A and panel 2B markers) for the isolated strains and for *B. melitensis* strain $16M^T$, as previously described (*Al Dahouk et al. 2007a*; *Le Flèche et al. 2006*). The PCR products were analysed with the Agilent 2100 Bioanalyzer using a DNA 1000 LabChip kit (Agilent Technologies, Waldbronn, Germany) according to the manufacturer's protocols, and compared with the results of De Santis et al [10]. Some of the PCR products were sequenced in order to confirm the exact size of the amplicons. The MLVA data were analysed using the *Brucella* aggregated database hosted on MLVAnet (http://mlva.u-psud.fr/) developed by University Paris Sud in Orsay, France (*Grissa et al. 2008*).

Data Analysis

Data were entered in duplicate into an Access 2003 database (Microsoft, USA), in order to detect data entry errors, and compared using the Data Compare function of Epi Info 3.5.3 (Centers for Disease Control and Prevention, USA). All statistical analyses were performed in Stata 10.1 (StataCorp LP, USA).

As animals from the same village were observed to regularly mix at common grazing and watering points, the clustering level for livestock was considered to be the village rather than the household. The apparent seroprevalence was calculated using Generalised Estimating Equations (GEE) with a binomial distribution and an independent correlation structure. To adjust for test performance, a range of plausible values for sensitivity and specificity were specified (presented in the Results section) from which a value was randomly sampled for the calculation of an adjusted seroprevalence. The model was run

for 1,000 simulations, with the random selection of sensitivity and specificity for each simulation. The mean adjusted seroprevalence was then determined.
Mixed effects logistic regression was used to calculate odds ratios to assess covariates such as demographic characteristics and potential risk factors for exposure, as well as the relationship between human and livestock seroprevalence at the village level. To assess serological test performance for brucellosis, the level of agreement between the ELISA and RBT was determined by calculating the inter-rater agreement between two unique raters. For human Q Fever, ELISA sensitivity and specificity were calculated using IFA as the gold standard reference test (*Maurin & Raoult 1999*).

5.5 Results

Human Serosurvey

From the 25 villages, 683 people participated in the survey, of which 255 (37.3%) were of Fulani ethnicity. The age and sex distribution are shown in Table 6. Consumption of non-boiled dairy products was common, particularly amongst Fulani with almost all adults (95.6%) reporting consumption compared to less than two thirds (60.7%) of the rest of the population, hereafter referred to as non-Fulani. Most households reported owning or looking after cattle, sheep, or goats, with 96.9% Fulani and 83.4% non-Fulani households reporting this livestock contact. Nearly three quarters (73.9%) of Fulani men listed livestock as a principal source of income, as opposed to 13.8% non-Fulani.

Brucella Seroprevalence

Seroprevalence results for *Brucella* spp. are presented in Table 7. Only five people were positive by IgG ELISA and three people by RBT. One person was positive to both tests. Of the three people positive by RBT, only one remained positive after sequential serum dilution. This person was also IgM ELISA positive. Overall, six of these 7 seropositive people were of Fulani ethnicity. The apparent seroprevalence for Fulani was 2.4% (95%CI: 1.0-5.6), compared to 0.2% (95%CI: 0.0-1.6) for the rest of the population, as shown in Table 7. Seropositive participants were re-assessed by the study team for symptoms compatible with active brucellosis. These participants were not clinically unwell at the time of examination and none had a clinical history supporting active disease, including the IgM seropositive participant. They were provided with information about the disease and were monitored by their local nurse.

Table 6: Characteristics of study participants from 25 villages in northern Togo

People				
Ethnicity	*n*	*Male*	*Age in years*	
			Median (range)	*≤15*
Fulani	255	118 (46.3%)	25.5 (5-85)	95 (37.3%)
Non-Fulani	428	242 (56.7%)	26 (5-90)	195 (45.6%)
Total	683	360 (52.7%)	26 (5-90)	290 (42.5%)
Animals				
Species	*n*	*Male*	*Median age in years (range)*	-
Cattle: from villages	596	201 (33.7%)	6 (0.3-16)	-
Cattle: transhumant*	464	93 (20.0%)	2 (0.5-15)	-
Sheep	465	52 (11.2%)	3 (0.3-12)	-
Goats	221	24 (10.9%)	2 (0.3-12)	-

*Transhumant cattle were sampled from 18 herds across 11 sites.

C. burnetii Seroprevalence

Two hundred and seventy-eight human sera (134 Fulani and 144 non-Fulani), representing 40.7% of the total sample, were randomly selected for IFA and ELISA. Serological evidence of exposure to *Coxiella burnetii* was common, with 100 people having Phase II IgG titres by IFA ranging from 1:32 to 1:256, in 23 of 25 villages. Assuming IFA to be the gold standard for Q Fever diagnosis (i.e. 100% sensitivity and specificity), the seroprevalence in non-Fulani was 26.9% (95%CI: 20.4-30.6%) and 45.3% (37.6-53.3%) in Fulani using a cut-off value of 1:32 as evidence of serological exposure (*de Rooij et al. 2012*). The odds of being seropositive was 2.3 times higher (95%CI: 1.4-3.8) for Fulani compared to non-Fulani when adjusted for age and sex. Only 7 people had titres of 1:256 which, as a single Phase II IgG titre, could suggest recent or active infection (*Fournier & Raoult 2003*). However, none of these cases reported symptoms during the previous month that clinically supported such a diagnosis. Owning or looking after livestock was associated with a greater odds of being seropositive using an IFA cut-off value of 1:32, although the 95% confidence intervals included 1. The odds ratios for seropositivity and contact with cattle or small ruminants were 1.6 (95%CI: 0.9-2.9) and 1.3 (95%CI: 0.7-2.3), respectively. Consumption of non-boiled milk did not increase the odds of seropositivity, with an odds ratio of 1.1 (95%CI: 0.5-2.3).

Table 7: Seroprevalence of brucellosis (*Brucella* spp) and Q Fever (*Coxiella burnetii)* in people and livestock

Brucellosis			
Population	*n*	*Seroprevalence (%) (95%CI)*	
		Unadjusted	*Adjusted for ELISA performance*
Humans: Fulani	255	2.4 (1.0-5.6)	**-**
Humans: non-Fulani	428	0.2 (0.0-1.6)	**-**
Cattle: from villages	596	9.2 (4.3-18.6)	8.9 (7.0-10.7)
Cattle: transhumant	464	7.3 (3.5-14.7)	7.1 (5.0-9.5)
Sheep	465	0	-
Goats	221	0	-
Q Fever			
Humans: Fulani	134	45.5 (37.7-53.6)*	**-**
Humans: non-Fulani	144	27.1 (20.6-34.7)*	**-**
Cattle: from villages	242	15.7 (11.6-20.9)	14.5 (10.3-19.0)
Sheep	207	16.4 (11.4-23.1)	15.0 (11.1-20.3)
Goats	198	7.1 (3.3-14.6)	6.6 (3.5-9.6)

*Using IFA cut-off of 1:32 for serological evidence of exposure

Using the manufacturer's recommended ELISA cut-off value, the sensitivity and specificity of the Phase II IgG ELISA were 84.8% and 63.0%, respectively, for evidence of serological exposure (IFA titre of 1:32). However, for IFA titres of 1:256, which can be considered the threshold for recent or active infection, sensitivity increased to 100% and specificity to 69.4%.

Animal Serosurvey

From the 25 villages, 596 cattle, 465 sheep, and 221 goats were randomly sampled. Additionally, serum was collected at 11 different sites from 464 transhumant cattle of 18 herds originating from Burkina Faso. Owners of transhumant cattle at two sites refused to participate due to time constraints. The age and sex distribution of animal subjects are shown in Table 6.

Brucella Serology

Seroprevalence results for livestock are presented in Table 7. Cattle seropositive to *Brucella* spp by indirect IgG ELISA or RBT were only detected in half of the villages (13 of 25). The apparent seroprevalence by ELISA using the manufacturer's recommended cut-off value was 9.4% (95%CI: 4.6-18.4%), whereas the seroprevalence by RBT was lower at 5.7% (95%CI: 2.4-12.8%). Based on conservative estimates of indirect ELISA sensitivity and specificity of 94-98% and 97-99% respectively (*Schelling et al. 2003*; *McGiven et al. 2003*), the seroprevalence adjusted for ELISA performance was 8.9% (95%CI: 7.0-10.7%).

In transhumant cattle, the apparent seroprevalence was slightly lower. The seroprevalence was 7.3% (95%CI: 3.5-14.7%) by ELISA and 4.5% (95%CI: 2.4-8.5%) by RBT. The seroprevalence adjusted for ELISA performance was 6.2% (95%CI: 4.4-8.2%). Overall, the two tests had an agreement of 95.9%.

The odds of being seropositive by ELISA increased with age. When adjusted for age, cows had an odds of *Brucella* seropositivity that was 5.3 times higher (95%CI: 1.5-18.7) than bulls. Results of the univariate and age-adjusted analysis of predictors of brucellosis seropositivity are shown in see Table 8. Seropositive cows had a 3.8 times greater odds (95%CI: 1.2-12.1) of having aborted a foetus during the previous year than seronegative cows, when adjusted for age.

All small ruminants were negative by indirect IgG ELISA. Eleven were positive by RBT but were negative by CFT. Thus, the apparent seroprevalence in sheep and goats was 0%.

C. burnetii Serology

For testing by indirect IgG ELISA, 242 cattle, 207 sheep, and 198 goat sera were randomly selected. In contrast to *Brucella* spp, *C. burnetii* seropositivity (meaning at least one seropositive animal) was detected in almost all villages (24 of 25). In these 24 seropositive villages, the mean village-level seroprevalence was 14.9% (range: 3.6-37.5%).

The apparent seroprevalence by ELISA using the manufacturer's recommended cut-off value was 16.1% (95%CI: 8.8-21.5%) in cattle, 16.2% (95%CI: 11.1-23.0%) in sheep, and 8.8% (95%CI: 4.4-16.9%) in goats, using a GEE model with clustering at the village level. Based on conservative estimates of ELISA sensitivity and specificity of 90-94% and 97-99% respectively (*Schelling et al. 2003*; *McGiven et al. 2003*), adjustment of seroprevalence for test performance estimated seroprevalences to be 14.8% (95%CI: 10.8-19.2%) in cattle, 14.4% (95%CI: 10.6-19.1%) in sheep, and 8.3% (95%CI: 4.2-12.4%) in goats.

Cows that had aborted a foetus during the previous year had an odds of being *C. burnetii* seropositive that was 6.7 times higher (95%CI: 1.3-34.8) than those that had not aborted (Table 8), when adjusted for age. This relationship was not evident for small ruminants. Sex and age were not predictors of seropositivity. There was only a very weak linear correlation between the village-level seroprevalence of people and livestock, with an adjusted R-squared value of 0.05.

Hygroma Fluid Culture

Although there was no growth on the BSA plates, mixed bacterial cultures grew on blood agar. *Brucella*-like colonies were then purified onto blood agar plates and isolates were recovered from hygroma samples from three cows, all of which were seropositive by RBT and ELISA. The genus *Brucella* was confirmed by realtime PCR. Assessment by MLVA-8 (panel 1) identified the isolates as *B. abortus.* Each isolate had a distinct Variable Number of Tandem Repeats (VNTR) profile by MLVA-16 (panels 1, 2A and 2B). The full biotyping results will be presented in detail in a separate publication.

Table 8: Predictors of seropositivity to *Brucella* spp and *Coxiella burnetii* by ELISA in cattle

Bovine Brucellosis (*Brucella* spp)				
Predictor	*Number of subjects (proportion seropositive)*	*Odds ratio (95%CI)*		
		Univariate Analysis	*Adjusted for sex*	*Adjusted for age*
11yrs-16yrs*°	48 (18.0%)	22.9 (7.2-73.3)	16.2 (4.9-53.4)	-
6yrs-10yrs*°	378 (12.2%)	5.7 (2.9-11.3)	4.4 (2.2-8.8)	-
3mths-5yrs*	613 (20.8%)	-	-	-
Cows	766 (11.2%)	9.8 (2.9-32.9)	-	5.3 (1.5-18.7)
Bulls	294 (1.0%)	-	-	-
Abortion during previous year+	32 (31.3%)	4.1 (1.4-12.3)	-	3.8 (1.2-12.1)
Bovine Q Fever (*Coxiella burnetii*)				
Abortion during previous year+	8 (16.0%)	6.3 (1.2-33.2)	-	6.7 (1.3-34.8)

* Excluding animals for whom age was not known (n=21)

° The reference category for the odds ratios is 3mths – 5yrs.

+ Excluding cows under 3 years of age.

5.6 Discussion

This is the first study of brucellosis and Q Fever in linked animal and human populations in Togo. Simultaneous assessment of the exposure of people and animals provides a more complete epidemiological picture and deepens our understanding of infection patterns at the animal-human interface. The findings of this study are relevant not only for Togo, but also for neighbouring countries in West Africa, given the frequent cross-border movement of livestock often occurring outside official channels.

Most published high quality data on the incidence of human brucellosis in Africa comes from northern Africa (*Dean et al. 2012b*), where *B. melitensis* has an important impact on human and animal health (*Refai 2002*). The findings of this study suggest a different disease epidemiology in northern Togo, with no evidence of circulation of *B. melitensis*. Despite the ownership/management of livestock and consumption of non-boiled dairy products being common, brucellosis does not appear to pose a major human health problem in the study zone. The disease may, however, still have a significant economic impact through a reduction in cattle productivity. The distinct VNTR profiles of the three *B. abortus* isolates obtained from the culture of bovine hygroma fluid suggests that the organism has been circulating in the study zone for some time. Cows that were seropositive for *Brucella* spp. or *C. burnetii* were more likely to have aborted a foetus during the previous year. Indeed, the Food and Agricultural Organization of the United Nations (FAO) estimated in 2002 that milk and meat production in West Africa could increase by US$56-168 million/year if brucellosis were eliminated (*Mangen et al. 2002*).

Due to the absence of a perfect test, *Brucella* serology is notoriously difficult to interpret. All serological tests have limitations when used as screening tools (*OIE 2009a*). In this study, the apparent seroprevalence has been adjusted for the performance of the serological tests and the influence of age and sex. However, it remains only an approximation of the true disease seroprevalence in the population. Exposed but seronegative animals, for example, are not captured in the adjusted seroprevalence estimates. The OIE guidelines for brucellosis state that both the RBT and ELISA are suitable screening tests for the control of brucellosis at the local or national level. Positive reactions should be tested by a confirmatory strategy, such as the CFT (*OIE 2009a*; *OIE 2012*). Given that the 11 small ruminants in this study that were seropositive by RBT and seronegative by ELISA remained negative when tested by CFT, the ELISA may be a more specific test than the RBT for brucellosis. It may also have a higher sensitivity, with more cattle diagnosed seropositive by ELISA than by RBT. However, an additional confirmatory test would be required in order to investigate this further. The levels of seropositivity of village and transhumant cattle were similar. Given the frequent cross-border movements and mixing of livestock in West Africa, these findings suggest that the livestock disease status in one country may be potentially linked to that of its neighbour.

Exposure of animals and people to *C. burnetii* was common, although the proportion of seroconversions representing clinical disease is not known. More research is needed into identifying appropriate cut-off values for diagnostic tests in endemic settings. However, given that 5.0% of 870 acute febrile patients presenting to health centres in Tanzania were diagnosed with acute Q Fever (*Prabhu et al. 2011*), it is indeed likely that the pathogen causes substantial morbidity in Togo.

Cultural practices may influence zoonotic disease risk. The seroprevalence of both diseases were higher amongst the Fulani compared to the rest of the population, possibly reflecting their greater ownership or management of livestock and their increased consumption of non-boiled dairy products. However, a positive association between the consumption of raw milk and seropositivity to *C. burnetii* could not be demonstrated in this study. The role of milk consumption in *C. burnetii* transmission is not fully understood, with asymptomatic seroconversion without clinical illness reported (*Cerf & Condron 2006*; *Benson et al. 1963*).

General Recommendations

This is the first joint human and animal zoonotic disease study in Togo, which is an important component of the broader One Health concept. A One Health approach to zoonotic diseases research should be encouraged in Togo, as it may guide disease prevention and control strategies, bring economic benefits to both sectors and strengthen the health system as a whole (*Zinsstag et al. 2005*).

Community awareness of practices risking exposure to zoonotic diseases is needed. Given their higher levels of exposure, this is particularly important for Fulani communities. Boiling milk before consumption would reduce the risk of exposure to foodborne pathogens in general. The importance of avoiding contact with aborted materials, as well as basic hygiene practices including handwashing and environmental cleanliness (*Bonfoh et al. 2006*), should also be communicated. Given the low levels of literacy amongst study participants, appropriate methods include verbal communication of health messages by the local nurse or the village and Fulani chiefs, or the use of visual tools. In order to successfully engage communities, the support of both the village chief and the Fulani chief is essential. However, information, education and communication campaigns are costly to implement. These costs would need to be assessed in relation to the expected combined benefits for human health and livestock productivity (*Zinsstag et al. 2007b*).

Diagnosing and treating zoonotic diseases is particularly challenging in malaria-endemic countries (*Amexo et al. 2004*; *D'Acremont et al. 2010*). The screening of fever cases for differential diagnoses, including Q Fever, should be carried out in order to determine their role in febrile illness (*Steinmann et al. 2005*; *Prabhu et al. 2011*). This would first require training of health workers and laboratory staff, and strengthening of laboratory capacity. In terms of animal health, the higher odds of abortion by seropositive cows suggests that livestock productivity is affected by both brucellosis and Q Fever. Further investigation into the economic impact of these diseases would allow the identification and tailoring of cost-effective control strategies, such as vaccination. These strategies must be assessed in the context of overall benefits to public health, the livestock production sector, and household economies (*Roth et al. 2003*). There may be opportunities to combine the selected interventions with other livestock health programs, such as the anthrax vaccination campaigns which take place annually in the study zone.

Study Limitations

There are several limitations to this study. Firstly, the proportion of the total population sampled in each location is unknown, due to the unavailability of recent, comprehensive census data. The age distributions of the human and animal study populations are unlikely to reflect the true age distribution of the general population. Secondly, the village survey was conducted during the transition period between the dry and wet seasons (May-June 2011), before road access became too difficult due to flooding. In some particularly dry areas, livestock left the village early in the morning in search of watering points and grazing pasture. These animals were not captured by the sampling strategy of the field team. Given the practicalities of conducting a field survey in remote, rural communities, this potential source of selection bias was unfortunately unavoidable. There may have also been temporal variations in pathogen exposure and clinical symptoms that were not captured by the cross-sectional study design. Finally, more detailed information about risk factors, including the dairy products consumed and the level of contact with livestock and abortion materials, would have allowed a more thorough assessment of exposure risk.

5.7 Conclusion

This is the first epidemiological study of brucellosis and Q Fever in linked human and animal populations in Togo, providing much needed data for the West African region. *Brucella* spp and *C. burnetii* are shown to be circulating in the study zone, but further research is needed into their clinical and economic impact. Education of the community about practices risking exposure to zoonotic diseases is needed.

5.8 Acknowledgements

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6 Deletion in the gene BruAb2_0168 of *Brucella abortus* strains: diagnostic challenges

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6.1 Abstract

Three *Brucella abortus* strains were isolated from joint hygromas from cows in northern Togo. Two deletions in the 5' side of the gene BruAb2_0168 were identified. As this gene is used for species identification, these deletions have consequences for diagnostic procedures. Multiple Locus Variable Number of Tandem Repeat (VNTR) Analysis was therefore performed for species identification. The strains showed unique VNTR profiles, providing some of the first genotypic data from West Africa. More molecular and epidemiological data are needed from the region, in order to better understand transmission patterns and develop suitable diagnostic assays.

6.2 Résumé

Trois souches de *Brucella abortus* ont été isolées à partir du liquide d'hygroma de vaches au nord du Togo. Deux délétions du côté 5' du gène BruAb2_0168 ont été identifiées. Ce gène étant important pour l'identification de l'espèce, ces délétions ont des conséquences importantes pour les procédures diagnostiques. Par la suite, la procédure d'Analyse de plusieurs locus de répétition en tandem polymorphe (VNTR) a été réalisée afin d'identifier l'espèce. Ces souches ont montré des profils VNTR uniques, qui constituent quelques-unes des premières données génotypiques pour l'Afrique de l'Ouest. Des données moléculaires et épidémiologiques supplémentaires sont nécessaires afin de mieux comprendre la transmission de ce pathogène dans cette région, et élaborer des tests diagnostiques plus appropriés.

6.3 Main Text

Although brucellosis is one of the world's most common zoonoses, there is a lack of data from sub-Saharan Africa (*Dean et al. 2012b*; *Pappas et al. 2006*). Brucellosis impacts on human and animal health (*Dean et al. 2012a*) and has important economic consequences (*Roth et al. 2003*). This report describes the isolation and genetic characterisation of *Brucella abortus* strains from Togo, which posed a diagnostic challenge due to deletions in a gene targeted by PCR and the inability to grow on selective medium.

During a brucellosis serosurvey in Togo (2011-2012) (*Dean et al. 2013a*), joint fluid was aspirated aseptically from 9 seropositive cows from 5 herds with hygromas in the carpi/hocks. Sterile swab tips were soaked in the fluid and sealed in liquid Amies medium. After storage and transportation at 4°C according to international biosafety standards, they were cultivated in Switzerland on tryptic soy agar plates supplemented with 5% sheep blood (BD, Allschwil, Switzerland) and on *Brucella*-medium base agar supplemented with 5% inactivated horse serum and modified *Brucella* selective supplement (Oxoid, Basingstoke, UK). The plates were incubated for 10 days at 37°C in 5% CO_2. All culture manipulations were performed under Biosafety Level 3 containment. Although there was no growth on *Brucella*-selective medium, mixed cultures grew on blood agar. *Brucella*-like colonies were purified and isolates were recovered from three cows from three different herds.

Phenotypic testing of the strains was performed. The strains did not require CO_2 for growth and grew in the presence of thionin (0.04 mg/ml and 0.02 mg/ml) and basic fuchsine (0.02 mg/ml). They produced H_2S, as detected by hydrogen sulfide test strips (Sigma Aldrich, Buchs, Switzerland) and were urease positive (Oxoid). These results indicated *B. abortus* biovar 3 (*WHO et al. 2006*).

Molecular identification of the genus *Brucella* was performed by real-time PCR, as previously described (*Hinic et al. 2008*). As a species-specific signal for *abortus* or *melitensis* could not be obtained but *B. abortus* was suspected, it was decided to amplify a larger DNA segment of the target locus of the real-time PCR, which was specific for the species *abortus*, the BruAb2_0168 gene (BAbS19_II01580 in vaccine strain S19). The primer pair used was 0168_Babo_1F and 0168_Babo_1R. The PCR products were sequenced (primers in Table 9). Two deletions of 2678 and 263 base pairs were identified, from nucleotide positions 984-3662 and 4295-4558 of the sequence of *B. abortus* strain S19 (Figure 10) (GenBank accession number: KC847095).

Table 9: Primers used for the amplification and sequencing of the BruAb2_0168 gene

Primers	Sequences (5' to 3')
0168_Babo_1F	GGCGTGTATGTTGTTGGTAA
0168_Babo_2F	AACATATCGGCGACGCAGTA
0168_Babo_3F	GTGACGGGGACGGGTTGGAC
0168_Babo_4F	TGCGGATAATAATCTGGGTGA
0168_Babo_5F	CGGTTAATGGCACGCTTGAA
0168_Babo_6F	CCTCAATGGTGCGTGGGACAA
0168_Babo_7F	CGGTTCTGGGTGGCACGGTTA
0168_Babo_8F	GCACGGGCAGTCTGACGAAG
0168_Babo_9F	GGCGGCACGACGACGGTTGATG
0168_Babo_11F	GGATACTGACGGCACGCTTGA
0168_Babo_14F	GAACTTCATATCGGTACTGGTG
0168_Babo_15F	CTTGGTGATGACAATTCCAAGA
0168_Babo_16F	GTCTTTTGTGCTCAAGAACAATC
0168_Babo_17F	TGTCGTCAATGGCGGGCGATGGA
0168_Babo_18F	GGGAGTGCAGGCAATCACAG
0168_Babo_19F	CAGGCACGCTGACGCTGA
0168_Babo_20F	ATACACTGACGCTTCAGAACA
0168_Babo_1R	ATCGCCACCAACCATCAGC
0168_Babo_2R	CGCCGTCAGACTGCCCTCCA
0168_Babo_3R	ACCGTCGTTGCGCCCGTA
0168_Babo_4R	AAGTGCCGCCGTTAAACGTCA
0168_Babo_5R	GTGCCTGTGCCGCTCTTCA
0168_Babo_6R	AATTATTCGCCGCATCCTCA

The isolated strains displayed features that complicated diagnosis. Firstly, none grew on *Brucella* selective medium. As some *B. melitensis* and *B. abortus* strains are sensitive to the antibiotic concentrations in modified *Brucella* selective supplement, it is therefore advisable to concurrently inoculate a standard growth medium or modified Thayer-Martin plate (*OIE 2009a*). Secondly, the deletions in the BruAb2_0168 gene suggest that this may not be a suitable target for an *abortus* species-specific PCR. Samples for which a species-specific signal cannot be obtained should always be tested for a genus-specific signal to ensure that *Brucella* sp is not missed. False negatives due to deletions in targets of diagnostic assays can have important public health consequences. In Sweden, a deletion of 377 base pairs in the target of a commercial PCR for the diagnosis of *Chlamydia trachomatis* was identified following a decrease in the number of human cases detected (*Ripa & Nilsson 2006*). Genomes can undergo modifications such as deletions, insertions or rearrangements, and this aspect of bacterial evolution must always be considered when performing PCR assays for detection and/or identification. Ideally, more than one target should be tested and unexpected results investigated.

Loci with a Variable Number of Tandem Repeats (VNTR) can be used as markers to identify species belonging to the *Brucella* genus (panel 1 markers) and to discriminate among *Brucella* strains (panel 2A and 2B markers). Molecular typing by Multiple Locus VNTR Analysis (MLVA) over 16 loci can be used as a molecular epidemiology tool to assess brucellosis transmission patterns (*Al Dahouk et al. 2007a*; *Le Flèche et al. 2006*).

MLVA was performed on the three culture lysates and on *B. melitensis* strain $16M^T$. For most loci, sequencing confirmed the exact PCR product length. However, five loci could not be sequenced (bruce06, bruce07, bruce09, bruce30, bruce55) and their length was determined by comparing the PCR product bands with those of *B. melitensis* $16M^T$ on an agarose gel, and by the Agilent 2100 Bioanalyzer using a DNA 1000 LabChip kit (Agilent Technologies, Waldbronn, Germany) and comparing with the results of De Santis et al (*De Santis et al. 2009*). The MLVA data were analysed using the *Brucella* aggregated database on MLVAnet (http://mlva.u-psud.fr/) hosted by Université Paris-Sud (*Grissa et al. 2008*). This database compares queried strains to described strains and performs a clustering analysis using the categorical coefficient and unweighted pair group method with arithmetic mean (UPGMA). The resultant Newick strings were imported into R statistical software Version 2.12.2 (http://www.R-project.org) and a dendogram was drawn using the package 'ape' (http://ape.mpl.ird.fr). The three strains showed distinct VNTR profiles (Table 10) clustering with African strains of *B. abortus* biovar 3 (Figure 11), confirming the phenotypic results. There is only one other genotyped strain of *B. abortus* reported from West Africa, isolated from the Gambia (*Bankole et al. 2010*). Furthermore, the three strains were obtained from a small geographical zone, the sampling sites being only 13-42km from one another. This demonstrates the diversity of the circulating strains. Given the importance of semi-nomadic herd management and cross-border trade in Togo (*Dean et al. 2013b*), livestock movements likely play a role in this genetic diversity.

Table 10: MLVA results for three *Brucella abortus* strains from Togo over 16 loci

Strain	MLVA-16 loci*															
	06	08	11	12	42	43	45	55	18#	19	21	04	07	09	16#	30
1	3	5	3	11	2	2	3	3	8	41	8	4	2	3	6	4
2	3	5	3	11	2	2	3	3	10	41	8	4	2	3	8	4
3	3	5	3	11	2	2	3	3	8	41	8	4	2	3	5	4

*The name of each locus begins with "bruce", followed by the corresponding number.
#The three strains differ at loci bruce18 and bruce16.

In developing countries, culture is often not feasible due to inadequate laboratory biosafety. At the time of joint fluid collection, several drops were also added to 1mL of a chaotropic lysis buffer (*Bürki et al. 2011*). DNA extraction was performed in Switzerland as previously described (*Cheng et al. 1995*). Real-time PCR (*Hinic et al. 2008*) confirmed the *Brucella* genus for six cows, three of which were culture positive. The use of inactivated, buffered samples is therefore both safer and more sensitive than culture, and is recommended for resource-poor, remote areas. Given that DNA extraction and PCR were performed 10 months after collection, rapid sample processing is not required.

More molecular data are needed from Sub-Saharan Africa, in order to develop more suitable diagnostic assays for brucellosis and better understand the epidemiology of this important disease. Incoherent diagnostic results should always be further investigated.

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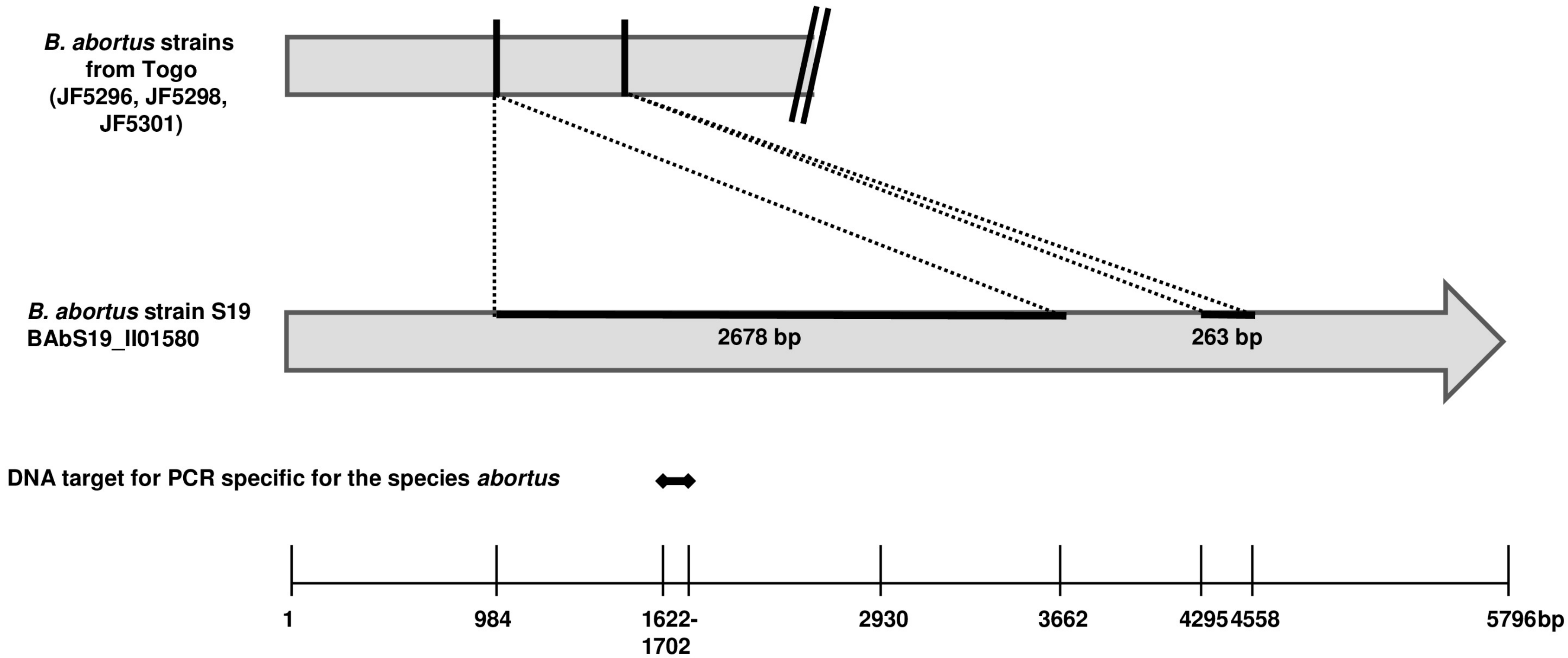

Figure 10: Locations of deletions

Location of two deletions in the BruAb2_0168 gene of *Brucella abortus* strains from Togo compared to *B. abortus* vaccine strain S19. The DNA target for the PCR specific for the *abortus* species is shown to fall in the range of the first deletion.

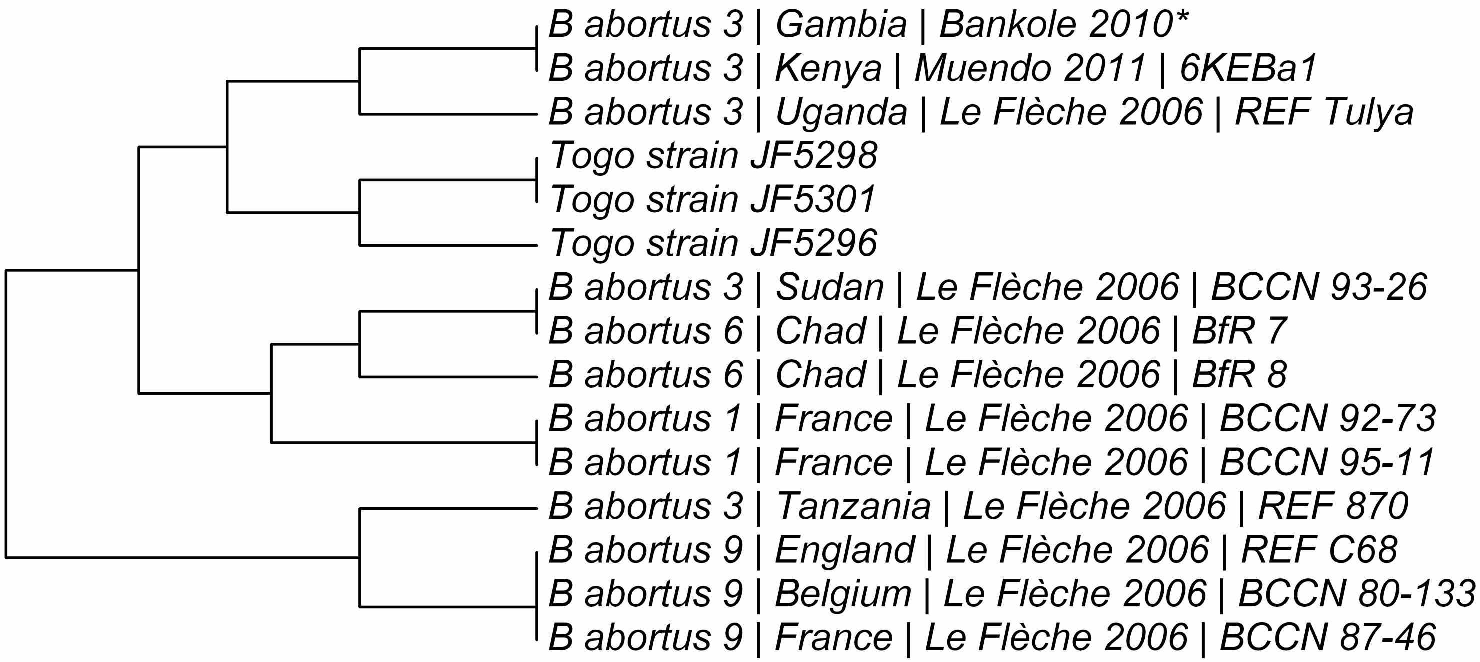

Figure 11: Dendogram showing the genetically closest strains to the three Togo strains

The species and biovar are given, followed by the country in which the strain was isolated, the author and the strain reference from the MLVAnet. This analysis was performed using MLVA-8 (panel 1) loci plus bruce18 and bruce21, in order to assess large-scale population structure.

* This strain is not listed in MLVAnet (*Bankole et al. 2010*).

7 Potential risk of regional disease spread in West Africa through cross-border cattle trade

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7.1 Abstract

Background: Transboundary animal movements facilitate the spread of pathogens across large distances. Cross-border cattle trade is of economic and cultural importance in West Africa. This study explores the potential disease risk resulting from large-scale, cross-border cattle trade between Togo, Burkina Faso, Ghana, Benin, and Nigeria for the first time.

Methods and Principal Findings: A questionnaire-based survey of livestock movements of 226 cattle traders was conducted in the 9 biggest cattle markets of northern Togo in February-March 2012. More than half of the traders (53.5%) operated in at least one other country. Animal flows were stochastically simulated based on reported movements and the risk of regional disease spread assessed. More than three quarters (79.2%, range: 78.1-80.0%) of cattle flowing into the market system originated from other countries. Through the cattle market system of northern Togo, non-neighbouring countries were connected via potential routes for disease spread. Even for diseases with low transmissibility and low prevalence in a given country, there was a high risk of disease introduction into other countries.

Conclusions: By stochastically simulating data collected by interviewing cattle traders in northern Togo, this study identifies potential risks for regional disease spread in West Africa through cross-border cattle trade. The findings highlight that surveillance for emerging infectious diseases as well as control activities targeting endemic diseases in West Africa are likely to be ineffective if only conducted at a national level. A regional approach to disease surveillance, prevention and control is essential.

7.2 Résumé

Situation: Les mouvements transfrontaliers des animaux facilitent la dissémination des pathogènes sur de longues distances. Le commerce transfrontalier de bovins est important économiquement et culturellement en Afrique de l'Ouest. Cette étude évalue le risque potentiel de dissémination de maladies suite au commerce transfrontalier de bovins entre le Togo, le Burkina Faso, le Ghana, le Bénin et le Nigéria pour la première fois.

Méthodes et principaux résultats: Une enquête de questionnaire portant sur les mouvements commerciaux de bovins a été menée dans les neuf plus grands marchés de bovins du nord du Togo entre février et mars 2012. Deux cent vingt-six commerçants de bovins ont été interviewés. Plus de la moitié (53,5%) d'entre eux achetaient et/ou vendaient des bovins dans au moins un autre pays. Les mouvements d'animaux ont été simulés stochastiquement en utilisant les mouvements rapportés par les participants. Le risque de dissémination de maladies dans la région a été évalué. Plus de trois quarts (79,2%, gamme : 78,1-80.0%) des bovins entrant dans le système de marchés de la région des Savanes provenaient d'autres pays. A travers ce système de marchés, des pays non voisins étaient connectés par des routes le long desquelles des maladies pourraient se disséminer. Même des maladies caractérisées par une faible transmissibilité et une faible prévalence dans un pays donné, avaient un risque élevé de se répandre dans d'autres pays.

Conclusions: A travers la simulation stochastique des données récoltées lors d'entretiens menés avec des commerçants de bovins dans le nord du Togo, cette étude a permis d'identifier les risques potentiels de dissémination régionale de maladies en Afrique de l'Ouest suite au commerce transfrontalier de bovins. Ces résultats soulignent le fait que la surveillance des maladies émergentes et les interventions ciblant les maladies endémiques en Afrique de l'Ouest seront inefficaces si elles sont conduites au niveau national. Une approche régionale pour la surveillance, la prévention et le contrôle des maladies est essentielle.

7.3 Introduction

Animal movements within countries and across borders can facilitate the rapid spread of pathogens across large distances (*Fèvre et al. 2006*). Recent examples include the spread of highly pathogenic avian influenza H5N1 globally (*Kilpatrick et al. 2006*) and of *Trypanosoma brucei rhodesiense*, one of the agents of Human African Trypanosomiasis, through cattle trade in Uganda (*Hutchinson et al. 2003*). In industrialised countries, this risk can be mitigated through strict importation controls. However, in developing countries, cross-border trade of live animals is often an important component of livestock production systems from both an economic and cultural perspective.

In West Africa, long-distance, cross-border cattle trade was estimated to be worth US$150 million in 2000 (*Williams et al. 2006*). Traditionally, livestock have been raised in the semi-arid Sahel zone and traded with countries in the southerly forested zones (*De Haan & van Ufford 1999*; *Williams et al. 2006*). These cross-border movements continue to occur frequently and often outside of official veterinary control. Similar trends exist in East Africa, where cross-border trade involving Somalia, Ethiopia, Sudan, Kenya, and Tanzania was estimated to be worth US$61 million per annum in 2009, with only 10% of trade occurring through official channels (*Little 2009*). In South-East Asia, interviews with cattle traders uncovered trade routes involving Thailand, Laos, Cambodia, and Vietnam. Almost half (45%) of the 60 Cambodian traders interviewed admitted to trading animals which they suspected to be infected with Foot-and-Mouth Disease (FMD) virus (*Kerr et al. 2010*).

Understanding the potential pathways and risk for regional spread of infectious diseases is essential for tailoring appropriate control interventions in West Africa. In this context, a questionnaire-based survey was undertaken with cattle traders operating in cattle markets in northern Togo in 2012, and the potential risk of regional disease spread through trade routes was assessed through stochastic simulations.

7.4 Methods

Ethics statement

This research was a component of a larger study of zoonotic disease epidemiology in Togo (*Dean et al. 2013a*), and was approved by the Ethics Committee for Health Research (Comité de Bioéthique pour la Recherche en Santé) of the Ministry of Health of Togo. In Switzerland, approval was given by the Ethics Commission of the Cantons of Basel-Stadt and Basel-Land and the Research Commission of the Swiss Tropical and Public Health Institute of Basel, Switzerland. The information to be communicated to participants was provided as a written document to the interviewers and they received training regarding the consent process. Prior to interviewing, the study objectives, procedures and questionnaire content were explained to participants in their local language and they were assured that the questionnaire data would be treated anonymously. As the interviews were conducted in the cattle traders' busy, outdoor workplaces, obtaining written consent was determined to be impractical in this setting.

Similar to previous cross-sectional surveys conducted with traders in marketplaces (*Van Kerkhove et al. 2009*; *Fournié et al. 2012*), all participants provided informed verbal consent before the interview, as approved by the aforementioned ethics and research commissions. The informed consent of each participant was recorded by the interviewer on the questionnaire form at the time of interview, and refusals to participate were recorded on a separate sheet.

Study site

The study was conducted in the northernmost region of Togo, the Savannah Region, which is bordered by Burkina Faso, Ghana, and Benin. The Savannah Region is a pastoral zone important for livestock raising, with approximately half of Togo's cattle population found in this region, estimated to be 138,000 in 2011 (Direction de l'Elevage - Togo, personal communication). The area also receives a large number of transhumant (i.e. semi-nomadic) herds each dry season, the official period of transhumance being from January-May. These herds are mainly from Burkina Faso, as well as from Benin and Niger.

Questionnaire survey

Through discussion with regional veterinary services and livestock traders, the nine biggest cattle markets in the region were identified. Markets were open 1-2 days per week. The target population was traders of live cattle operating in markets in northern Togo. The survey was conducted in February-March 2012 and, in order to capture as many traders as possible, larger markets were visited up to 5 times.

Structured questionnaire-based interviews were conducted by two trained interviewers. Although the questionnaire was in French, the official language of Togo, interviews were also conducted in four local languages. Traders were asked to name all of the sites that they visited to purchase or sell cattle during the current dry season and the previous wet season. The information recorded by the interviewers included the nature of the site (market or informal trading place), its full location (village, district, province and country), and the type of the stakeholders with whom they were trading (such as traders, farmers, or butchers). If the traders reported visiting markets, the frequency of their visits was recorded. For each location, traders were asked to specify the minimum and maximum number of cattle sold or purchased per month, if they visited that location every month in a given season. If a location was visited only sporadically, they instead specified a minimum and maximum per season. Additionally, traders were asked whether they sold animals from, or bought animals for, herds that they personally owned. The locations of these herds, the minimum and maximum number of cattle sold/purchased and the frequencies of sales/purchases were recorded. As the definition of dry and wet seasons may vary between individuals, participants were asked to define the months corresponding to these periods.

Additionally, the manager of each market was asked to estimate the number of traders operating in the market each open day in both seasons. In order to minimise data entry errors, all data were entered twice into a pre-designed Microsoft Access 2003 database and cross-checked for discrepancies using EpiInfo 3.5.3 (Centers for Disease Control and Prevention, USA).

Market catchment area

Locations where cattle were bought or sold by traders operating in the Savannah market system were visualised using MapInfo Professional Version 7.0. The centroid of each province was plotted in order to show the geographic distribution.

Cattle flows through market system

The analysis was conducted using R statistical software Version 2.12.2 (*R Development Core Team 2012*). Characterisation of the flows of cattle into and out of the market system of the Savannah Region could not be directly deduced from the empirical data, due to two constraints. Firstly, it was not possible to sample every trader operating in the market system during the course of the survey. Secondly, although the number of cattle purchased and sold in each location was known for each trader, information about the actual origin or destination of these animals was missing. In other words, the number of animals purchased by a trader from locations A and B was known, as was the number sold to locations C and D, but the proportions of cattle purchased in A or B that were then sold to C or D were unknown. Consequently, it was necessary to estimate the flows of cattle into and out of the Savannah market system through stochastic simulations. The simulation algorithm is described below, which was repeated over 10,000 simulations.

In order to assess the proportion of the total trader population in the Savannah market system that was captured by the survey, the number of trader-days per season per market was first calculated from the market visit frequencies reported by the traders. This was compared with the estimate of the number of traders provided by the market manager.

If the trader interview data gave a lower estimation of the number of trader-days per season than the market manager's estimation for a given market, it was assumed that the trader sample did not capture the full trader population of the market. In these markets, the trader data were completed by randomly re-sampling from the group of traders visiting this market until the number of trader-days estimated by the manager was reached. This modified dataset was then used to reconstruct the flow of cattle between locations.

Cattle movements were simulated for each individual trader as follows. The minimum and maximum numbers of cattle reported to be sold or purchased at each site by a given trader were summed over one season (dry or wet). These minimum and maximum values accounted for the monthly variability reported by traders as well as the uncertainty associated with the recall of the number of cattle traded. As traders were often livestock owners who bought and sold cattle for their own herds, the location of their herd was considered as the sale location for cattle which they purchased for themselves. For those traders selling cattle from their own herds, the location of the herd was considered as the purchase location. For each trader *t*, the number $S_{t,j}$ of cattle sold in location *j* (with $1 \leq j \leq m$) and the number $B_{t,i}$ of cattle purchased in location *i* (with $1 \leq i \leq n$) were then randomly drawn from their respective ranges extending from the minimum to maximum value. In order to ensure that a given simulated trader *t* sold as many cattle as he purchased over an entire season, the total number N_t of cattle traded was randomly drawn from the range extending from the total of number sold, $\sum_j S_{t,j}$, and the total number purchased, $\sum_i B_{t,i}$. The simulated number of cattle sold and purchased in each location was then defined as $S^*_{t,j} = S_{t,j} N_t \Big/ \sum_l S_{t,l}$ and $B^*_{t,i} = B_{t,i} N_t \Big/ \sum_l B_{t,l}$, respectively.

The sold animals were attributed to an origin by exploring two different scenarios, Location Scenarios 1 and 2. In the first scenario, Location Scenario 1, the probability of an animal being sold in any of the sale locations did not depend on its purchase location. For each animal sold by a trader, an origin was allocated by randomly sampling from the locations given for the animals purchased by this trader, without replacement. In other words, each animal sold by a given trader was randomly matched with a unique animal purchased by this trader. The flows of cattle resulting from each individual trader were then summed. However, given the possibility that cattle may have been more likely to be sold at a site closer to the purchase location, an assumption of dependence of purchase and sale sites was also explored. In this second cattle flow scenario, Location Scenario 2, cattle were preferentially sold in the same country as where they were purchased; or, for purchases in Togo, they were preferentially sold in the same administrative region.

For each simulation, the number of cattle entering the market system did not necessarily equal the number leaving. The number of cattle flowing through the Savannah market system in each simulation was, therefore, assumed to be the greater of these two values. After running the algorithm over 10,000 simulations, the mean, minimum and maximum numbers of cattle flowing between locations was assessed.

Market network

A network of contacts between markets in the Savannah Region was simulated using R package sna (*Schwarzer 2012*), with markets as nodes and animal movements as edges, which were simulated according to the algorithm described above. The network was directed, meaning that the direction of animal movements was accounted for. Each directed edge connecting two markets was given a weight, equal to the number of cattle traded between these two locations. Network connectivity was assessed via the giant strongly connected component (GSCC) and giant weakly connected component (GWCC). The GSCC refers to the part of the network within which all nodes can reach one another through directed paths, whereas the GWCC includes nodes that can reach one another through undirected paths. The in- and out-degree distributions of the binary and weighted networks were assessed. For the binary network, the in- and out-degree referred to the number of Savannah markets sending cattle to and receiving cattle from a given Savannah market, respectively. For the weighted network, the in-degree referred to the number of cattle being moved to a given Savannah market from other Savannah markets, with the out-degree being the converse.

Cattle in the network were moved directly from their place of purchase to their place of sale based on the results of the cattle flow simulations described above. It is possible, however, that a trader visiting several markets may conduct his visits in a particular order. For example, a trader may purchase all of his cattle in market A, move these cattle to market B where some would be sold, and then finally visit market C to sell those remaining. Information about the order of market visits was not available, but could influence the distribution of links between markets. In order to explore this, an algorithm which stochastically ordered the market visits of each trader was run over 1,000 simulations. Further information is given in Appendix 3.

Risk of disease spread through market system

The risk of cross-border disease spread through the simulated cross-border livestock flows was assessed for Location Scenario 1. The risk was defined as the probability of a disease invading an area *j* through importation of cattle from a disease-endemic area *i* within a one year period. The pathway was divided into two steps: firstly, the introduction of an infectious animal into area *j* from area *i*; secondly, the spread of disease within the cattle population of area *j* due to the introduction of this infectious animal. Given that border crossings rarely involve veterinary assessment, it is assumed that infectious animals would be able to cross into neighbouring countries without detection and quarantine. Therefore, the probability p_i of exporting an infectious animal from an area *i* can be approximated by the prevalence of the disease in area *i*. The number $n_{i,j}$ of animals moved from area *i* to *j* within the one year period was taken from the results of the simulations previously described.

When an infectious animal has been introduced into an area *j*, the disease may either fade out or spread within the cattle population of area *j*. The basic reproduction number of a disease (R_0) is the number of secondary cases resulting from the introduction of one infectious case into a fully susceptible population. It refers to a pathogen's potential to spread in a given population. The cattle population in area *j* was assumed to be fully susceptible and to mix homogeneously. With R_{0_j} being the reproduction number in area *j*, the likelihood of disease extinction soon after the introduction of one infected animal into this population was equal to $1/R_{0_j}$ (*Keeling & Rohani 2008*), and the probability of a sustained outbreak was $p_j = 1 - 1/R_{0_j}$. The risk *P* of disease invasion was therefore:

$$P = 1 - \left(1 - p_i p_j\right)^{n_{i,j}}$$

By varying R_{0_j} and p_i, the risk of an exotic disease invading the cattle population of the Savannah Region in Togo through cattle trade from Burkina Faso was explored. Here, p_i refers to the disease prevalence in Burkina Faso, and R_{0_j} refers to the potential of a disease to invade the Savannah cattle population following the introduction of an infectious animal into one of its herds through the Savannah market system. The Savannah cattle population was assumed to mix homogeneously.

Additionally, the probability of a disease invading at least three other countries through cattle trade from Savannah herds was investigated. Here, p_i refers to the disease prevalence in the cattle population in the Savannah region, and R_{0_j} refers to the potential of a disease to invade a cattle population in a given country after the introduction of an infectious animal from the Savannah Region into the market system of this country. R_{0_j} was assumed to be the same in all countries trading with the Savannah Region and, in each given country, the local cattle population and cattle traded through the local market system were assumed to mix homogeneously. This assumption was necessary because data relating to flow of cattle from the market systems into herds in these countries were not available.

7.5 Results

Descriptive analysis of empirical data

Two hundred and twenty-six traders were interviewed, with a refusal rate of 12%, mainly due to lack of time to participate. In each of the nine markets, 9-55 traders were interviewed (median 20, IQR: 11-36). For three markets, the number of trader-days calculated from the trader interview data accounted for only 24-77% of the trader-days estimated by the market manager, requiring modification (inflation) of their datasets. For the other six markets, the market manager estimated less trader-days than the trader interview data and modification of the dataset was not required. Only 8.4% of traders (19 of 226) transported their animals between locations solely by vehicle, with the remainder herding their animals by foot, either exclusively or in combination with road transport. The majority of traders defined the dry season as extending from October – April, and the wet season from May – September.

Given the higher number of cattle traded at a greater number of locations during the dry season compared to the wet season, these data will be presented in detail below. The corresponding information for the wet season is provided in Appendix 4. Most interviewees (193/226) not only acted as traders, but also bought and sold cattle for their own private herds. However, the proportions of their purchases and sales that involved their own herds were small. In the dry season, the median proportion of their purchases that represented cattle taken from their own herds was only 1.1% (IQR: 0.7-1.8%), and the proportion of their sales corresponding to cattle being added to their own herds was 1.5% (IQR: 1.0-2.3%). In the dry season, cattle were sold from 179 herds. Most of these herds (146 of 179) were located in the Savannah Region, as well as other areas of Togo (5 of 179), Burkina Faso (23 of 179) and Ghana (5 of 179). Similarly, most of the 175 herds which received purchased cattle during the dry season were in the Savannah Region (141 of 175), as well as other areas of Togo (5 of 175), Burkina Faso (23 of 175), Ghana (4 of 175), Benin (1 of 175) and Niger (1 of 175).

The distribution of the average number of cattle traded per trader during the dry season was right-skewed. While the median was 500 (IQR: 173-639), as many as 2,916 cattle were reported to be traded by a single trader during the season. The median numbers of purchase and sale locations of the traders were 4 (IQR:3-5) and 3 (IQR:2-4), respectively, regardless of season, with a maximum of 8. Most of these sites were cattle markets within the Savannah Region. The median number of Savannah markets in which traders operated for purchase or sale was 3 (IQR: 2-4) in the dry season.

Nearly three quarters of traders (166 of 226, 73.5%) in the dry season bought cattle in at least one Savannah market and sold cattle in at least one other Savannah market. Around half of the cattle purchases and sales reported by traders (55.2% and 51.4%, respectively) took place in Savannah markets, whilst 35.8% and 21.6% of purchases and sales, respectively, took place in another country. Of all the cattle purchases and sales taking place in the 28 Savannah markets, 83% and 82% respectively took place in only 4 markets. Outside of Togo, most of the foreign cattle purchases (85.3%) were conducted in Burkina Faso, and 38.4% of foreign cattle sales took place in Nigeria. The number of traders operating at different purchase and sale locations as well as the numbers of cattle traded at these sites are summarised for the dry season in Table 11.

The market catchment area is shown in Figure 12, with traders operating in the Savannah market system also operating in Burkina Faso, Ghana, Benin, Nigeria, and Niger, as far as 500km from the Savannah Region. Overall, more than half of the traders (121 of 226, 53.5%) operated in at least one other country outside of Togo. Among those traders operating in multiple countries, only one quarter (32 of 121, 26.4%) conducted both purchase and sale activities in at least two countries. In the dry season, almost two thirds of the traders operating in Burkina Faso (49 of 81, 60.5%) conducted only purchases in Burkina Faso, without any sales. Half (11 of 22, 50.0%) of traders operating in the dry season in Ghana, three quarters (27 of 37, 73.0%) of traders operating in Benin and all (31 of 31, 100%) of the traders operating in Nigeria only sold cattle in these countries, without purchasing.

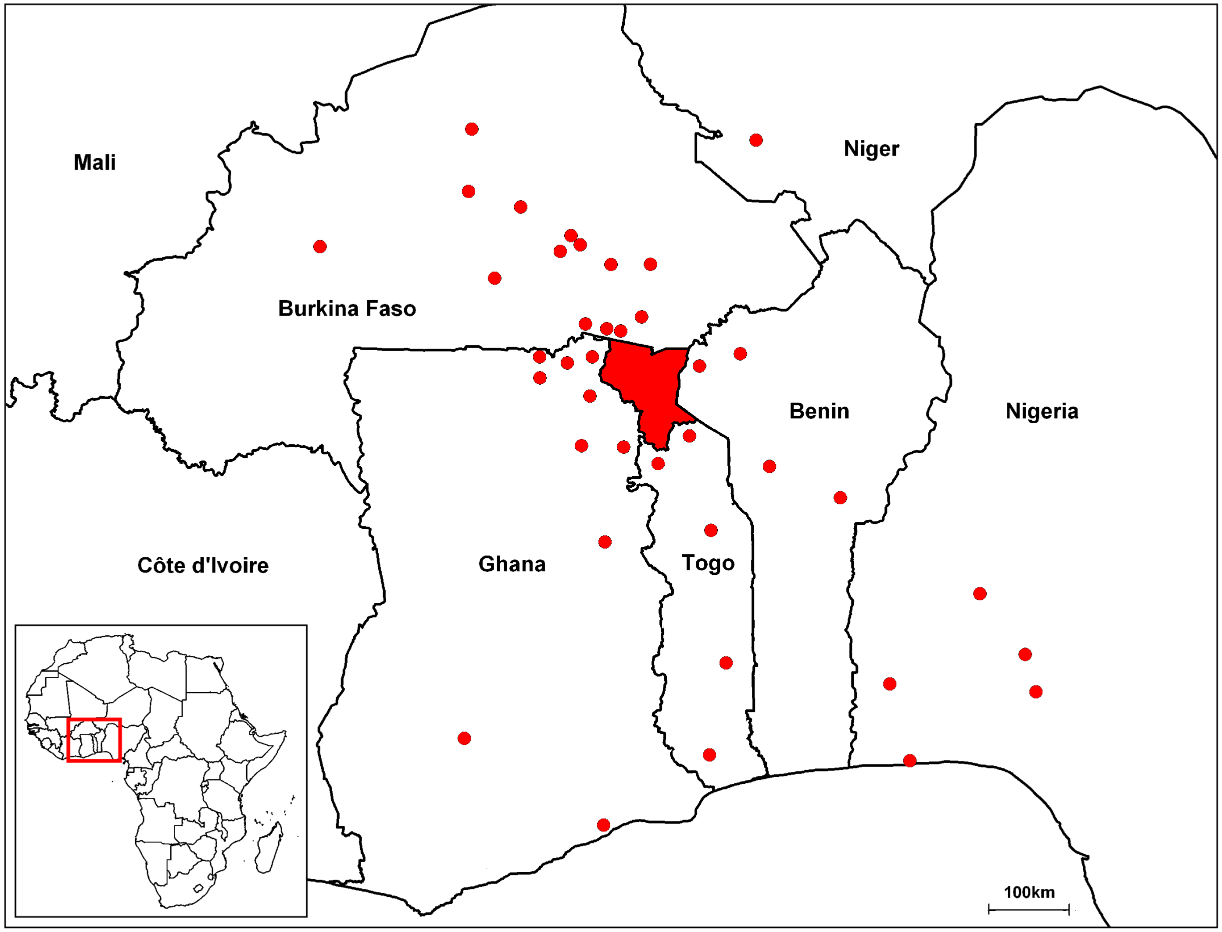

Figure 12: Study Zone in West Africa

The study zone, the Savannah Region of northern Togo, is shaded red. The centroids of the other districts where the interviewed traders also bought or sold cattle are shown as red dots.

Table 11: Empirical data from interviews with 226 cattle traders - dry season

	Purchase and sale locations									
	Savannah markets	Savannah herds	Savannah butchers	Other Togo markets	Other Togo herds	Benin	Burkina Faso	Ghana	Niger	Nigeria
No. of traders purchasing or selling	226 (100%)	172 (76.1%)	39 (17.3%)	91 (40.3%)	7 (3.1%)	37 (16.4%)	81 (35.8%)	22 (9.7%)	2 (0.09%)	31 (13.7%)
No. of traders purchasing	202 (89.4%)	163 (72.1%)	0	16 (7.1%)	6 (2.7%)	10 (4.4%)	79 (35.0%)	11 (4.9%)	0	0
No. of traders selling	190 (84.1%)	152 (67.3%)	39 (17.3%)	80 (35.4%)	5 (2.2%)	34 (15.5%)	32 (14.2%)	18 (8.0%)	2 (0.09%)	31 (13.7%)
No. of cattle purchased, ranging from min. to max.	58334-66285 (55.2%)	6605-7370 (6.2%)	0	2761-3094 (2.6%)	299-327 (0.3%)	2480-2908 (2.4%)	32776-36067 (30.5%)	3120-3391 (2.9%)	0	0
Median proportion (%) of total cattle purchased per trader* (IQR)	77 .0 (53.0-99.0)	2 (1.0-18.5)	0	28.5 (19.0-34.5)	2.5 (1.3-13.5)	33.5 (27.0-38.0)	55 (29.0-95.0)	29 (18.5-49.5)	0	0
No. of cattle sold, ranging from min. to max.	54675-61312 (51.4%)	2519-2806 (2.4%)	2961-3388 (2.8%)	23177-26084 (21.8%)	67-72 (0.1%)	6422-6997 (5.9%)	2933-3533 (2.9%)	4796-5282 (4.5%)	36-36 (<0.1%)	8779-9914 (8.3%)
Median proportion (%) of total cattle sold per trader^{+} (IQR)	83.5 (50.0-99.0)	2.0 (1.0-4.0)	15.0 (10.0-22.0)	45.0 (30.8-61.3)	2.0 (1.0-2.0)	26.5 (21.0-32.8)	5.0 (1.0-27.0)	26.0 (20.3-39.3)	6.5 (4.3-8.8)	28.0 (19.0-35.0)

This table shows the number of traders purchasing from, and selling to, different locations over the dry season. The proportion of the total number of traders interviewed is given in brackets as a percentage. The minimum and maximum numbers of cattle purchased and sold in these locations are presented for the dry season, expressed in brackets as percentages of the average number of cattle purchased or sold. Markets and herds located outside of the study zone, the Savannah Region, are referred to as “Other Togo markets” and “Other Togo herds”. No butchers were located outside of the Savannah Region.

*This is the median value of the proportion of each trader’s purchases or sales^{+} taking place in the given locations, as a percentage. Traders that did not purchase in a given location were excluded.

Simulated livestock flows

The 10,000 model simulations gave means of 32,370 cattle (range: 31,070-34,180) flowing into the Savannah market system during the dry season and 28,860 (range: 27,940-29,970) during the wet season. Location Scenario 2 with non-independence of buying and selling locations did not have a notable impact on the results, with a mean cattle flow into the Savannah market system during the dry season of 28,260 (range: 26,810-29,500) and 24,900 (range: 23,670-25,930) in the wet season. Overall trends of animal flows were the same regardless of season. Given the greater cattle flows during the dry season, these data will be presented in detail below. Results from the wet season are provided in Appendix 4. A summary of results for Location Scenarios 1 and 2 for both the dry and wet seasons is provided as a table in Appendix 5.

Figure 13 shows the mean proportions of animals entering into and leaving the Savannah market system obtained by the simulations for the Location Scenario 1 for the dry season. More than three quarters (79.2%, range: 78.1-80.0%) of cattle flowing into the Savannah market system during the dry season originated from other countries, with 68.0% (range: 65.2-70.2%) of inflow coming from Burkina Faso, 7.6% (range: 7.2-8.0%) from Ghana and 3.6% (range: 3.1-4.7%) from Benin. Half of the cattle leaving the Savannah market system in the dry season (49.3%, range: 47.0-51.7%) were sent to Togolese markets outside of the Savannah Region. The majority (93.7%, range: 92.7%-94.5%) of these were sent to one large market near the coastal capital city of Lomé, where animals are generally slaughtered for meat consumption. One third of the cattle (38.8%, range: 36.7-40.7%) leaving the Savannah market system moved into other countries, with 7.8% (range: 7.3-8.4%) of outflow into Ghana, 12.3% (range: 11.1-13.8%) into Benin and 14.7% (range: 13.5-15.9%) into Nigeria. The results of Location Scenario 2 did not demonstrate any major differences in flow, as detailed in Appendix 5.

In the dry season, 2,992 (range: 2775-3284) cattle flowed into herds in the Savannah Region, equating to 2.2% (range: 2.0-2.4%) of the estimated total cattle population size in the Savannah Region. Given that most herds likely breed their own replacement animals, many more animals flowed in the reverse direction from Savannah herds into the market system. There was a mean of 13,633 cattle (range: 9,096-24,506) leaving herds in the dry season, equating to 9.9% (range: 6.6-17.8%) of the estimated total cattle population size. Location Scenario 2 produced similar results with 2,989 cattle (range: 2,733-3,305) flowing into herds and 13,730 (range: 9,408-23,940) leaving herds. Flows into and out of the Savannah herds followed the same trends as the aforementioned market system flows.

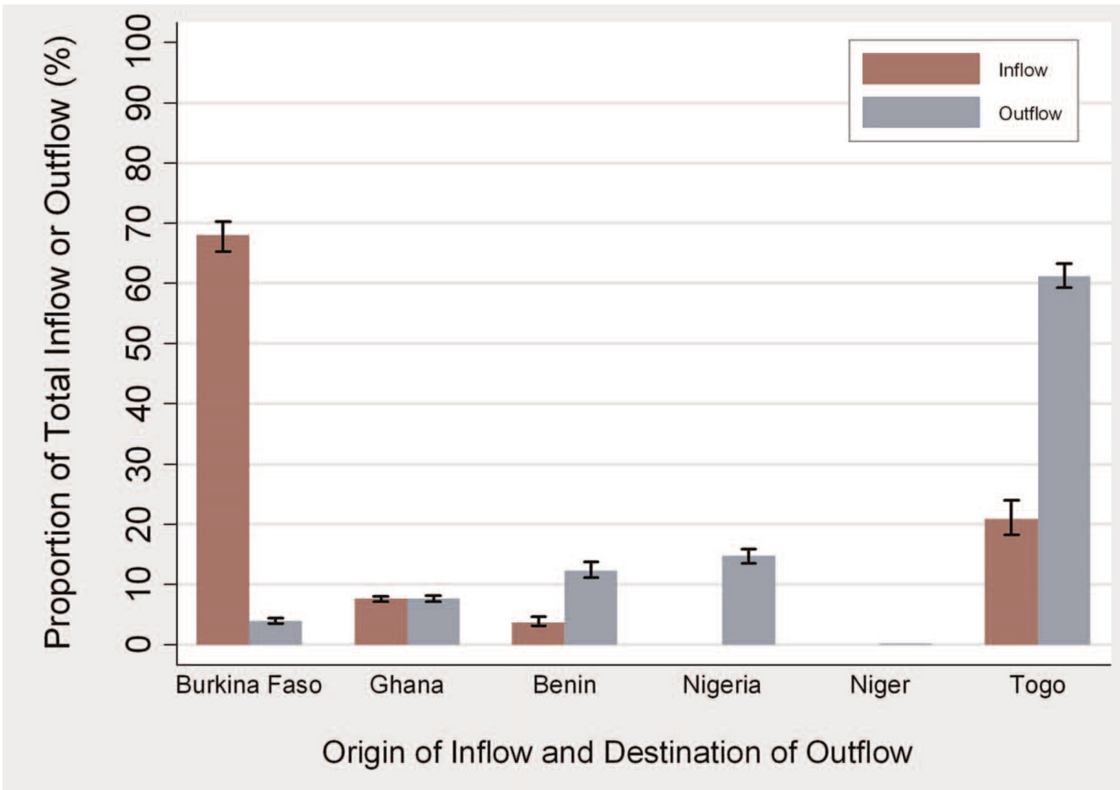

Figure 13: Simulated flows of cattle into and out of the market system of Savannah Region, Togo.
These simulated mean flows are shown for the six month dry season period, as a proportion of total flow into or out of the Savannah market system. The range of simulated values from minimum to maximum are shown as black bars.

Market network

In the dry season, the market system consisted of 28 markets. They formed a well connected network incorporating all but one of the markets. The GWCC, estimating the upper bound of the maximum epidemic size, was 27. Nearly half of these markets (13) formed the GSCC, estimating the lower bound of the maximum epidemic size. When using the alternative algorithm for reconstructing the order of market visits, the GSCC was even higher, with a median of 20 markets (range: 14-23). Further details of this algorithm are provided in Appendix 3.

Figure 14 shows the distribution of markets during the dry season as a function of their binary and weighted in- and out-degrees. The majority of markets (17 of 28) received cattle from at least two other markets, with a maximum of 13 other markets. Approximately half of the markets (15 of 28) sent cattle to at least two other markets, with a maximum of 14 other markets. However, most cattle movements within the Savannah market network were mediated by a small number of markets: 4 markets accounted for 73.7% and 78.6% of the total weighted in- and out-degrees, respectively. The markets with the highest degrees were those included in the survey, shown as blue circles in Figure 3. The Savannah market network for the wet season is presented in Appendix 4.

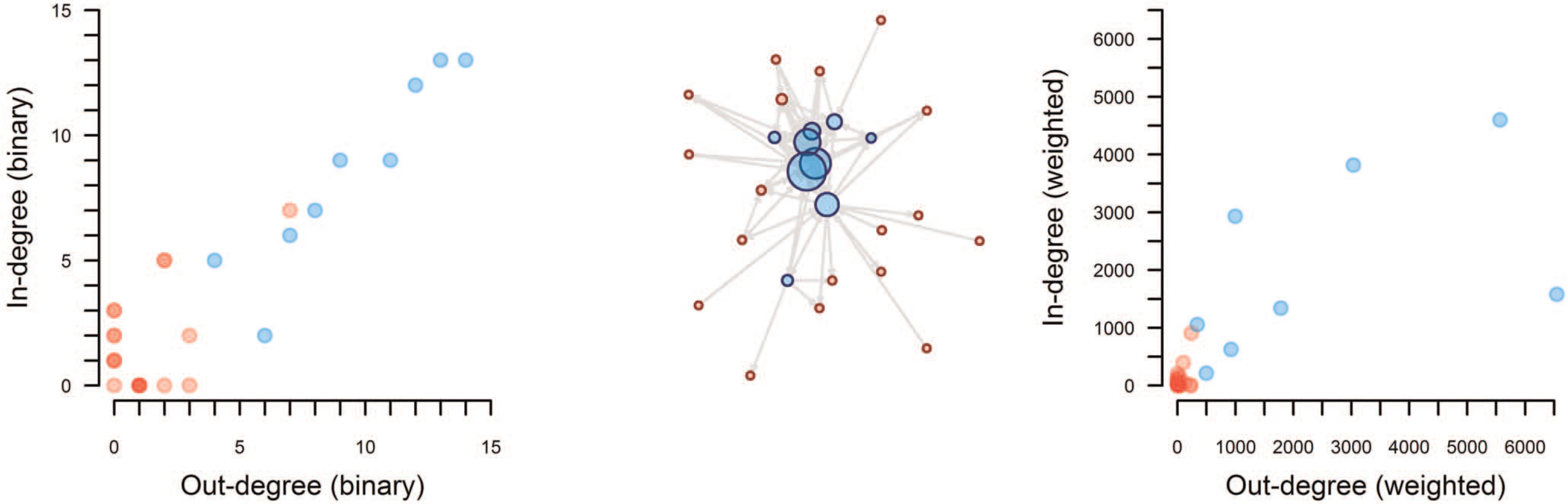

Figure 14: Market network of the Savannah Region of Togo and degree distributions

The graph on the left shows the binary in-degree as a function of the binary out-degree, and the graph on the right shows the weighted in-degree as a function of the weighted out-degree, during the dry season. The 9 markets where the survey was conducted are coloured blue and the other 19 are red.

Disease risk

Figure 15 shows the average probability over the course of one year that a disease present in Burkina Faso will be introduced through the Savannah market system into Togolese herds and result in an outbreak. For a hypothetical disease, even at a low prevalence of less than 1% in Burkina Faso and low transmissibility with an R_0 of around 1.25, there was a high probability (80%) of an outbreak in Togo. When disease prevalence is higher, between 1-10%, this probability reaches 100%. Similarly, if a hypothetical disease with an R_0 of around 1.25 is present in Savannah herds at a prevalence of less than 1%, the probability of disease being introduced into at least 3 other countries in the region through the Savannah market system is also 80% (Figure 16).

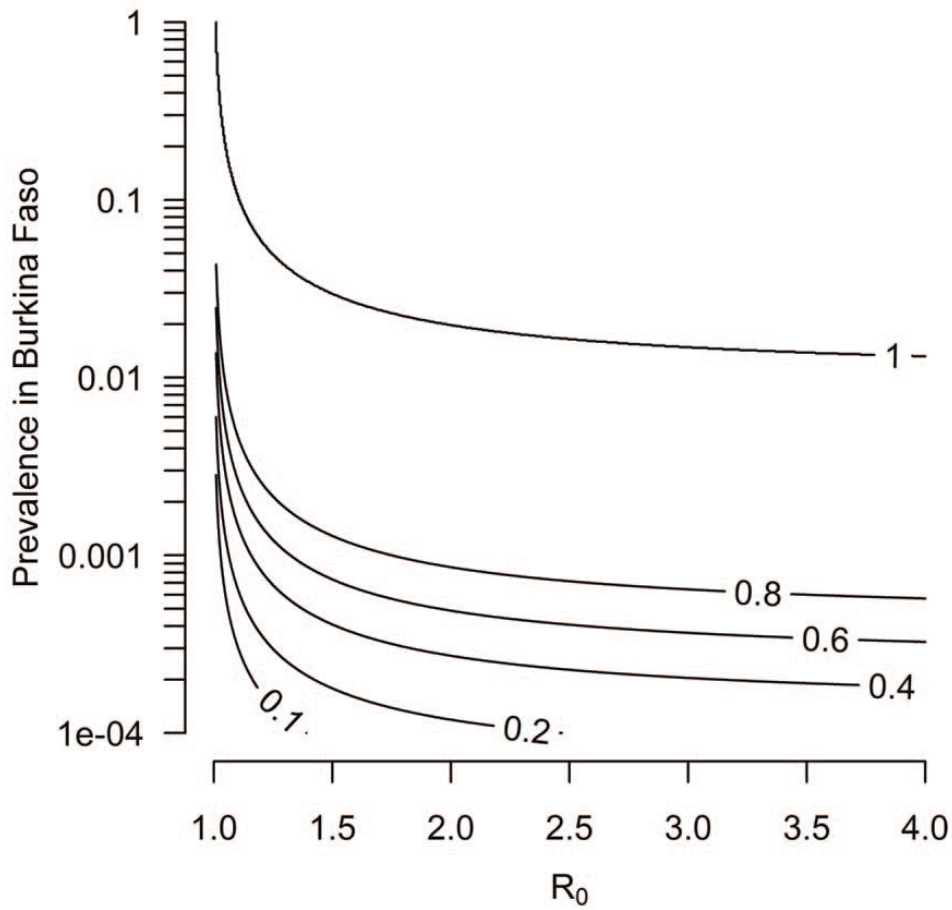

Figure 15: Probability of a disease invading the cattle population of the Savannah Region in Togo through cattle trade from Burkina Faso.
The probability of a disease invading the cattle population of the Savannah Region is shown as a function of the disease prevalence in Burkina Faso and the basic reproduction number of the disease, R_0. Here, R_0 relates to the potential of a disease to invade the Savannah cattle population following the introduction of an infectious animal into one of its herds.

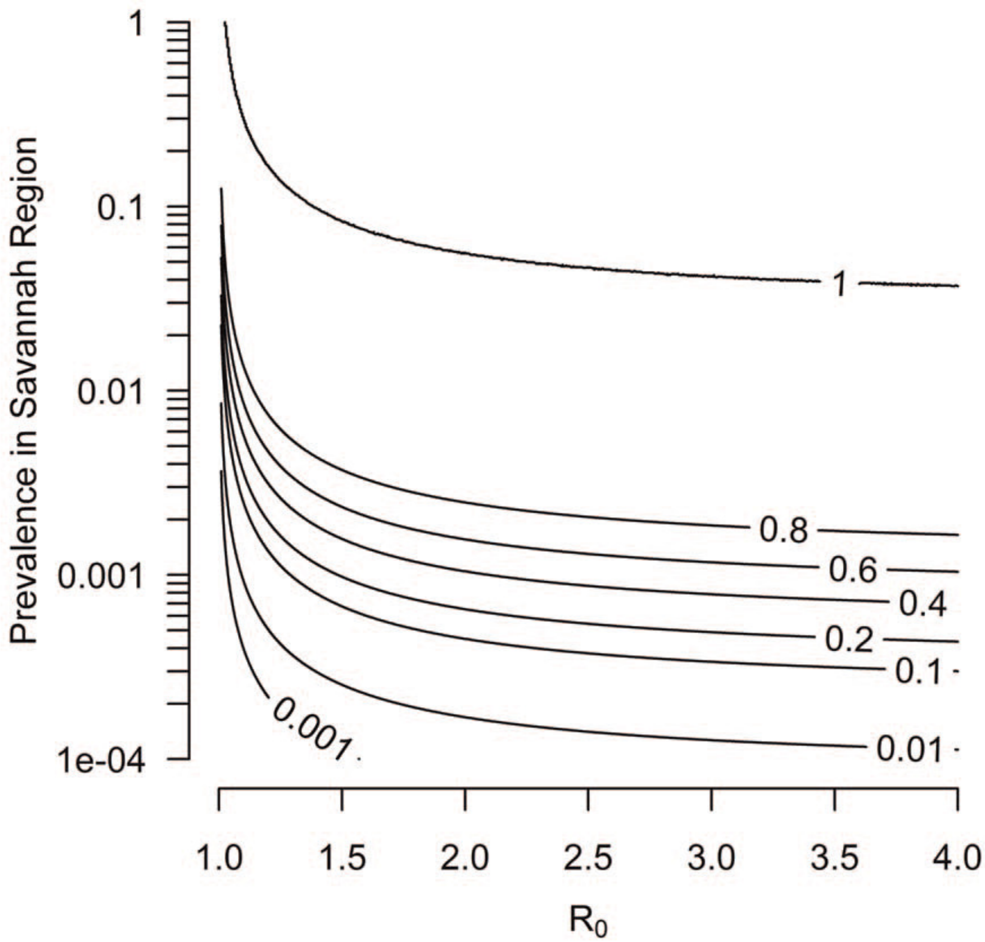

Figure 16: Probability of a disease invading at least three other countries through cattle trade from Savannah herds
The probability of disease invasion is shown as a function of the disease prevalence in the cattle population in the Savannah region and the basic reproduction number of the disease, R_0. Here, R_0 relates to the potential of a disease to invade a cattle population in a given country, after the introduction of an infectious animal into the market system of this country.

7.6 Discussion

Although livestock market networks and implications for disease spread have been described in detail in developed countries (*Bigras-Poulin et al. 2006*; *Natale et al. 2009*), few data are available from developing countries and are not captured by official datasets such as FAOSTAT from the Food and Agricultural Organization of the United Nations (FAO) (*FAO 2012*). This study quantitatively captures the potential disease risk resulting from large-scale, cross-border cattle trade between Togo, Burkina Faso, Ghana, Benin, and Nigeria for the first time. The findings will serve as the basis for further research hypotheses and should lead to strengthened collaboration at the regional level in the planning of disease control measures. The number of animals flowing into the northern Togolese Savannah market system during the dry season equals nearly one quarter of the resident cattle population. Although more than half of these animals were reported by sellers as having been purchased in Burkina Faso, it is possible that some animals originated from further afield, such as Mali or Niger. Through the cattle market system of northern Togo, non-neighbouring countries are potentially epidemiologically connected via trade routes.

The GSCC of a network is an estimate of the lower bound of the maximum epidemic size for a given disease, whilst the GWCC estimates the upper bound (*Kao et al. 2006*). The Savannah market network displayed high connectivity, with nearly half of the markets being incorporated into the GSCC and all but one forming the GWCC. In the alternative algorithm for reconstructing the sequence of market visits (Appendix 3), the GSCC was higher, incorporating the majority of markets. This suggests that a disease introduced into one market could rapidly spread to other markets. The market network of northern Togo is, therefore, a potential conduit for disease spread between West African countries. Although no data are available, it is likely that the scale of cross-border trade through the Togolese market network is not a unique scenario, but is rather the norm for West Africa.

These findings are relevant not only to the surveillance and control of newly emerging diseases, but also endemic diseases. Cross-border cattle trade and transhumance may have contributed to the genetic diversity of *Brucella abortus* strains circulating in the study zone (*Dean et al. 2013a*). These cross-border movements could also potentially explain the overlapping distribution of FMD serotypes O, A, SAT1 and SAT2 across West African countries (*Perry et al. 2011*; *The Pirbright Institute 2012*; *Tounkara et al. 2012*), particularly given that wildlife play a less important role in disease transmission than in East Africa. The results of this study are of direct relevance to the effective implementation of the Global FMD Control Strategy of the FAO and the World Animal Organisation for Animal Health (OIE) announced in 2012, a 15 year program which seeks to reduce the global impact of this devastating livestock disease (*FAO & OIE 2012*). Estimates of the R_0 of FMD in sub-Saharan Africa are not available. However, the R_0 of Rinderpest, a viral disease of cattle officially eradicated in 2011, has been estimated to range between 1.2 - 4.4 in Somalian and Sudanese cattle populations (*Mariner et al. 2005*; *Tempia et al. 2010*). The R_0 of Contagious Bovine Pleuropneumonia, a severe respiratory disease of cattle in Africa, is estimated to fall between 3.2 - 4.6 in pastoral herds of southern Sudan (*Mariner et al. 2006*). Therefore, the R_0 range of 1 - 4 used in this study to assess the risk of disease spread through regional cattle trade in West Africa is appropriate.

In addition to animal movements through trade, the Savannah Region of northern Togo also receives a large number of transhumant herds from the Sahel zone in search of grazing pasture and water sources during the dry season. Members of the Economic Community of West African States (ECOWAS) are legally bound to permit seasonal cross-border movements of herds (*Ly 2007*). In 2011, 47,000 transhumant cattle entered the Savannah Region through official Togolese government check points (Direction de l'Elevage - Togo, personal communication), although it is likely that an even greater number entered the country unofficially. The risk of regional disease spread in West Africa through trade is, therefore, further compounded by transhumance. In northern Togo, there is evidence that anthrax outbreaks occur along the routes followed by transhumant herds. As many herds do not follow the official routes designated by the Togolese authorities, conducting disease surveillance in this zone is particularly challenging (*Kulo & Kada 2011*). According to the market managers, the higher cattle flow through markets during the dry season reflected contributions from transhumant herds temporarily visiting Togo.

This study highlights the importance of a regional approach to disease control activities in West Africa. Prior to the annual childhood polio vaccination campaign implemented in 20 countries in Central and West Africa in March 2012, health care providers of border districts in the Savannah Region met and discussed with their counterparts in neighbouring countries. The animal health sector should also invest in cross-border disease prevention activities, such as synchronised vaccination campaigns or formal systems for the communication of unusual animal health events.

Limitations

The questionnaire was written in French, the official language of Togo, Burkina Faso, and Benin. However, interviews were predominantly conducted orally in local languages by two trained multilingual interviewers. Occasionally, interpreters were also required. Due to the linguistic complexity of the study zone, data errors due to incorrect interpretation are possible. As the survey results are based on estimations of cattle transactions by traders, rather than witnessed transactions, there is a risk of recall bias.

The flow of cattle through the Savannah market system was reconstructed using simulations. While it is possible that the complexities of the market system have not been fully captured, these are unlikely to have a major impact on the results: estimates obtained when assuming independence or dependence of purchase and sale locations (Location Scenarios 1 and 2) were similar. This is likely due to the fact that when operating in locations outside of the Savannah region, most traders either purchased or sold cattle, not both. However, the data only capture cattle trade through the formal market system. Given that informal trade also occurs, the scale of cross-border cattle trade is likely to have been underestimated. Furthermore, small ruminant cross-border trade has not been considered in this study. Although further data collection would improve the accuracy and applicability of the study findings, substantial investment of resources would be required.

The order in which traders visited markets in the Savannah Region was not known and the network of cattle movements between these markets had to be reconstructed. While the GSCC size varied, it was always greater than 45% of all markets, meaning that all simulated networks displayed high connectivity. Moreover, most of the purchases and sales were always mediated by only a few markets. These dominant markets were those where the interviews were conducted. This may suggest that the sampling approach may have potentially introduced bias. However, given that these markets were identified by the local veterinary services as the largest markets in the Savannah Region, the constructed network may indeed reflect the true structure.

The estimations of the risk of disease spread through the market system assumed a homogenously mixing population, simplifying animal contact patterns and the underlying disease dynamics. Collecting further information on cattle population dynamics in this region would be useful for refining this estimate. Moreover, pathogen amplification within herds and within markets was not accounted for. If such amplification did occur, this would only serve to further increase the risk of disease spread.

7.7 Conclusions

By stochastically simulating data collected by interviewing cattle traders in northern Togo, this study identifies potential risks for regional disease spread in West Africa through cross-border cattle trade. The findings highlight that surveillance for emerging infectious diseases as well as control activities targeting endemic diseases in West Africa, such as FMD, are likely to be ineffective if only conducted at a national level. A regional approach to animal disease surveillance, prevention and control is essential.

7.8 Acknowledgements

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8 Mobility and access to health care and social services: an exploratory study in northern Togo

Working paper

8.1 Abstract

Background: Seasonal, transhumant movements are important to livestock production in West Africa. The Fulani are the most dispersed ethnic group in West Africa and are the main livestock keepers in Togo. There is no high quality information available regarding the access of communities in Togo to health care and other social services, and the role of mobility.

Methods/Principle Findings: Semi-structured interviews were conducted with 406 adults in 25 randomly selected villages in northern Togo, to investigate migration trends and the utilisation of health, veterinary and education services. Around one quarter of Fulani adults were born outside of Togo, significantly more than the rest of the population (referred to as non-Fulani). In general, most new arrivals to villages were women. Most adults reported having accessed health care for themselves or their children within the previous year, with no difference between Fulani and non-Fulani. Satisfaction with the local health centre was generally high, except for the cost of services. More women of child-bearing age had used the services of skilled birth attendants than older women, but the distance to the health centre was an influential factor. Significantly less Fulani than non-Fulani sent their children to school, at 61.1% (95%CI: 52.0-69.7%) and 86.6% (96%CI: 81.1-91.0%) respectively. Few livestock herds had vaccination certificates from the previous 3 years. Around one third of Togolese herds undertook transhumance. Additionally, herdsmen from 18 transhumant herds from Burkina Faso were interviewed about their movements. Ten of these herds returned to the same zone annually because of pre-existing social contacts.

Conclusions: This study provides a useful evidence base on which to develop research questions for a more in-depth assessment of mobility and access. Topics for further investigation include the needs of mobile sub-groups, such as women or transhumant herdsmen, the influence of the cost of services and distance to the health centre on health care access, and reasons for the scarcity of livestock vaccination certifications.

8.2 Résumé

Situation: Les mouvements transhumants et saisonniers sont un aspect important de l'élevage en Afrique de l'Ouest. Les Peuls forment l'ethnie la plus dispersée en Afrique

de l’Ouest et sont les principaux éleveurs de bétail au Togo. Il n’existe pas de donnée sur l’accès de ces communautés aux soins médicaux et autres services sociaux au Togo, ni sur le rôle de la mobilité.

Méthodes / Principaux résultats: Des entretiens semi-structurés ont été réalisés avec 406 adultes dans 25 villages sélectionnés aléatoirement dans le nord du Togo, afin d’étudier la migration et l’utilisation des services de santé, des services vétérinaires et la scolarisation des enfants. Environ un quart des adultes peuls étaient nés en dehors du Togo, ce qui est considérablement plus élevé que pour le reste de la population (non peul). En général, la plupart des individus récemment arrivés dans un village étaient des femmes. La majorité des adultes a rapporté avoir utilisé les services de santé pour eux-mêmes ou pour leurs enfants pendant l’année précédente, sans différence entre peuls et non peuls. Les personnes interrogées étaient généralement satisfaites des centres de santé, mais pas du coût des services qu’elles jugeaient trop élevé. La proportion de femmes en âge d’avoir un enfant ayant déjà eu recours aux services d’un assistant d’accouchement était plus élevé que parmi les femmes plus âgées, néanmoins, la distance au centre de santé était un facteur influant. La proportion de peuls qui envoyaient leurs enfants à l’école, (61,1% ; IC95% : 52,0-69,7%) étaient plus faible que pour les non peuls (86,8% ; IC95% : 81,1-91,0%). Peu d’élevages avaient des certificats de vaccination pour les trois dernières années. Environ un tiers des troupeaux togolais pratiquaient la transhumance. De plus, les bouviers de 18 troupeaux transhumants du Burkina Faso ont été enquêtés. Dix de ces troupeaux retournaient à la même zone chaque année au vu des contacts sociaux préexistants.

Conclusion: Cette étude fournit une base d’évidences pour développer des questions de recherche qui permettraient une évaluation plus précise de la mobilité et de son impact sur l’accès aux soins et la scolarisation. Les sujets qui pourraient faire l’objet de plus amples investigations comprennent les besoins des sous-populations mobiles, telles que les femmes ou les bouviers transhumants, l’influence des coûts des services et la distance au centre de santé sur l’accès aux soins médicaux, et les raisons de la rareté des certificats de vaccination du bétail.

8.3 Introduction

More than half of the world's nomadic and semi-nomadic populations live in Africa, with poorer access to health services than settled communities *(Sheik-Mahomed and Velema 1999; Fokou et al. 2004).* Combining outreach veterinary and human health services has been shown to be increase coverage amongst pastoral nomadic communities (*Schelling et al. 2005*). There is a need for more practical interventions addressing the priorities of communities beyond the reach of conventional services.

A novel framework for analysing and improving access to health care has been developed in the context of malaria treatment and care in Tanzania. The Health Access Livelihood Framework considers access within the broader context of livelihood assets and the policies, institutions, and processes which govern health services (*Obrist et al. 2007*). The following five dimensions are included:

- Availability – the existing services and goods meet the client's needs, with adequate supplies and appropriately skilled personnel
- Accessibility – the location of services is convenient
- Affordability – the prices of services meet the client's ability to pay both direct and indirect costs
- Adequacy – the organisation of health care meets client's expectations in terms of opening hours, structure and cleanliness
- Acceptability – the characteristics of health care providers match those expected by the client, in terms of social values and personality

Togo is one of the world's poorest countries, with a Gross National Income (GNI) at Purchasing Power Parity (PPP) per capita ranked at 201 out of 214 countries in 2011 (*The World Bank 2013*). There is no high quality information available regarding the access of communities to health care and other social services in this low income, resource-poor setting. The Fulani are the most dispersed ethnic group in West Africa and are traditionally associated with livestock production activities. The previous 30 years have seen significant migration of Fulani pastoral communities from the Sudano-Sahelian zone into the more humid Sudano-Guinean savannahs of West Africa, as a result of social and agroecological adjustments (*Bassett & Turner 2007*; *Oppong 2002*). In Togo, the Fulani are the main keepers of livestock, either as hired caretakers or as part or full owners of herds. They have been shown to have a greater level of exposure to zoonoses than the rest of the population (*Dean et al. 2013a*).

Most of Togo's livestock production takes place in the northernmost of five administrative regions, the Savannah Region, bordered by Ghana, Burkina Faso and Benin. This a dynamic zone where cross-border cattle trade and seasonal, semi-nomadic movements referred to as transhumance are integral to livestock production (*Tezike & Dewa-Kassa 2008*; *Grolimund 2010*; *Kulo & Kada 2011*; *Dean et al. 2013a*; *Dean et al. 2013b*). The aim of this study was to assess the mobility of northern Togolese communities and their access to health care and other social services, including the way in which mobility influences access.

8.4 Methods

Survey

This questionnaire-based survey was conducted in parallel with a brucellosis and Q Fever serosurvey in 25 villages in the Savannah Region of northern Togo in May-June 2011. The random selection of villages and study participants, as well as ethics considerations, are described in Chapter 5. Trained interviewers conducted semi-structured interviews with the adult participants (over 15 years of age) of the serosurvey, in the local language of the participant (Fulani, Moba, Gourma, Tchokossi, Gangan, or Yanga). Both open and closed questions were included, relating to migration as well as to the utilisation and opinions of health, veterinary and education services. Questions relating to all five dimensions of the Health Access Livelihood Framework were included. Additionally, 18 transhumant herds from Burkina Faso were identified through discussion with the local veterinary services and included in a brucellosis serosurvey in February-March 2012, as described in Chapter 5. The accompanying herdsmen, all of whom were young men, were posed open questions about the organisational structure of their transhumance activities.

Data Analysis

Data were entered in duplicate into an Access 2003 database (Microsoft, USA), in order to detect data entry errors, and compared using the Data Compare function of Epi Info 3.5.3 (Centers for Disease Control and Prevention, USA). The data were analysed in

Stata 10.1 (StataCorp LP, USA). This included a descriptive analysis and logistic regression for binary outcomes. Confidence intervals for proportions were calculated using the normal approximation to the binomial.

8.5 Results

Mobility

Questionnaire data were available for 178 Fulani adults and 231 adults from the rest of the population, referred to hereafter as non-Fulani. The non-Fulani were predominantly from the Moba, Gourma, Tchokossi, and Gangan ethnic groups, as well as small numbers of Mossi, Yanga and Haoussa people. The sex and age distribution of the study participants is shown in Table 10. The Fulani communities were fairly well settled, with more than three quarters of interviewees born in Togo (78.4%, 95%CI: 71.6-84.2%). The majority of those not born in Togo were born in Burkina Faso. Moreover, half of the Fulani (50.8%, 95%CI: 43.3-58.4%) were either born in the village where they were interviewed or had been living there for over 20 years, compared with 83.3% (95%CI: 77.9-87.9%) of the non-Fulani. Nearly all of the non-Fulani (92.2%, 95%CI: 88.0-95.3%) were born in Togo, with most of the remainder born in Ghana. Overall, most of the new arrivals to the villages within the previous 5 years were women (72.7%, 95%CI: 54.5-86.7%).

Ten of the 18 transhumant herdsmen interviewed reported that their cattle herd had returned to the same zone each year for the previous 4-9 years. The owners of these herds had long-standing relationships with local families, whose agricultural productivity benefited from the fertilisation of their fields by livestock manure. Livestock owners in Burkina Faso often sent their herds on transhumance together with the herds of their relatives or friends, rather than the herd travelling alone.

Table 12: Sex and age distribution of 409 study participants, by ethnicity

Sex	Number interviewed	Median age in years (range)
Fulani		
Men	85	40 (36-45)
Women	93	32 (30-36)
Non-Fulani		
Men	145	48 (46-53)
Women	86	40 (36-42)

Access to health care

Most adults (81.3%, 95%CI: 77.1-85.0%), reported having accessed health care for themselves or for their children within the previous year, with no difference by ethnicity. Most people (89.4%, 95%CI: 86.0-92.2%) had visited the local health centre at least once. The level of satisfaction with the local health centre was high. Less than 5% (range: 0.6-4.7%) of people who had visited the local health centre were dissatisfied with one of the following features: opening hours, quality of medication, competence of staff, or the welcome received from staff. However, 38.4% (95%CI: 33.2-43.5%) of people were dissatisfied with the cost of services.

Nearly all (97.2%) of the interviewed women had previously given birth. Of these, nearly half (46.0%, 95%CI: 36.7-55.6%) of the women of child-bearing age (under 45 years of age) did not do so in the presence of a skilled attendant, either at a health centre or in the village with a midwife. Women from the 14 villages without a health centre had a greater odds of not giving birth with the assistance of a skilled attendant compared to women from the 11 villages equipped with health centres, when adjusted for age (OR 3.4, 95%CI: 1.8-6.4). Of these 14 villages, women from the 7 villages that were more than 5km from a health centre had an odds 4.7 (95%CI: 1.8-12.2) times greater of not receiving assistance that women within 5km from a health centre. No differences were noted according to ethnicity or the amount of time living in the village where the interview was conducted. For older mothers of at least 60 years of age, the majority had not used the services of a skilled attendant (80.8%, 95%CI: 60.6-93.4%).

Access to education

Significantly less Fulani sent all of their children aged from 7-15 years of age to school, at 61.1% Fulani (95%CI: 52.0-69.7%) compared with 86.6% non-Fulani (96%CI: 81.1-91.0%). The most common reason given by Fulani for not sending their children to school was that they were needed to herd livestock, whereas non-Fulani generally reported a lack of financial means. Other reasons provided were that children were needed to help in the home or in the agricultural fields, or that the child did not want to attend school.

Access to veterinary services

Approximately one third (35.2%, 95%CI: 26.9-44.2%) of owners or managers of milk-producing cows reported selling some of their milk. The income was used to buy meat, soap and other household items, and to grind grain at the mill.

Half of the livestock owners (54.2%, 95%CI: 45.3-62.9%) reported having used the animal health services during the previous year. However, only 6.9% could provide a vaccination certificate from any of the three previous years. Approximately one quarter (27.4%, 95%CI: 19.8-36.2%) of cattle owners reported that their cattle undertook seasonal transhumance locally within northern Togo.

8.6 Discussion

This exploratory study has some limitations in its design. The sampling strategy did not capture the most mobile sub-groups of the population, and the data collection tools were only able to investigate the topics of interest at a fairly superifical level. This research does, however, raise further research questions for a more in-depth assessment of mobility and access.

In general, the Fulani communities in northern Togo were fairly well settled, with most of the interviewees born in Togo. Half were even born in the village where the interview was conducted, although this is nonetheless a significantly lower proportion than amongst non-Fulani. There may be specific sub-groups of the Fulani and non-Fulani populations which are more mobile but were not captured by this survey. Given that most of the recent arrivals to the study villages were women, migration within the study zone may be primarily driven by marriage alliances. However, a more detailed exploration of the underlying drivers is needed.

Togo receives many transhumant cattle herds from neighbouring countries from January to May each year, during the dry season. Most of these herds are managed by young Fulani men who travel without their families and remain in contact with the livestock owner via mobile phone (*Grolimund 2010*). Some of the interviewed Burkinabé herdsmen had a good network of long-standing social contacts, but others did not. During the implementation of the survey, the arrest and incarceration of several transhumant herdsmen was observed by the author, which was claimed to be due to unpaid taxes for accessing grazing pasture during the night. Resource-sharing between transhumant and local populations in limited geographical zones can result in conflict of varying degrees (*Fokou et al. 2004*; *Grolimund 2010*), and there is a need for more open dialogue between stakeholders regarding mitigation strategies. Additionally, the health, economic, and social needs of the visiting herdsmen was not assessed in this study and warrants further investigation. This is also a relevant question in terms of internal transhumance within Togo, undertaken by one quarter of Togolese cattle owners interviewed.

Although more than three quarters of interviewees reported having accessed health care during the previous year for themselves or for their children, the underlying reasons are not known. This figure could, therefore, include door-to-door childhood vaccination campaigns rather than the active seeking of treatment. The high proportion of study participants who had ever visited their closest health centre suggests reasonable accessibility in the study zone. Based on an assessment using the five dimensions of access, the health system in northern Togo performed well. These results should, however, be treated somewhat cautiously. Questions were posed in a closed manner without probing and the nurse from the local health centre was often nearby during the interview, risking the introduction of reporting bias. The cost of health care was the main concern raised by participants. However, the actual financial barrier to accessing appropriate health care, including different treatment options, can not be quantified. Careful consideration of the objectives and design of any future studies assessing access is important, in order to ensure that the value of the research outputs is maximised.

Birthing practices have likely become safer in recent decades, with many more women aged under 45 years using skilled birth attendants compared to those over 60 years of age. Distance to the nearest health centre was shown to be an important factor in determining whether births were supervised by skilled attendants. Possible contributing factors such as a lack of transport should be explored.

Although the Fulani communities are fairly settled and do not appear to be marginalised in terms of access to health care, cultural perceptions may influence school attendance. The poorer school attendance of Fulani compared to non-Fulani children is predominantly due to livestock herding activities, which is important both culturally and economically. The Food and Agricultural Organization of the United Nations (FAO) recognises that livestock herding activities undertaken by children can impair normal physical and social development and interfere with education. Under certain circumstances, such activities can be classified as a form of child labour (*FAO 2013*). There is a need to raise awareness regarding the importance of education, but communication activities must be conducted in a culturally appropriate manner.

The scarcity of livestock vaccination certificates could reflect either low vaccination coverage or poor record-keeping. It would be useful to compare owner-reported and formally documented vaccination histories, and to validate findings by discussing with local veterinarians. In the Savannah Region, the government veterinary service organises annual anthrax vaccination campaigns in collaboration with veterinarians in the private sector, the latter being responsible for vaccine delivery. However, the campaigns are not subsidised by the state and livestock owners must pay for this service. Potential barriers to vaccination should be investigated, such as insufficient veterinary personnel, the high cost of vaccines, or a lack of owner awareness. Given that one third of cattle owners or managers sell milk for income generation, the organisation and structure of the milk production system for both small and large ruminants should be investigated, including aspects relating to local economies, social networks, and public health.

8.7 Conclusions

This exploratory study is the first investigating the mobility of northern Togolese communities and their access to health care and social services. It provides useful background information on which to develop further research questions and observational studies.

9 Discussion

9.1 Overall Significance of Research

This research has direct practical applications as well as providing a much needed evidence base on which to develop more targeted research questions and public health interventions and policy. The content of the thesis is broad, incorporating global disease burden, regional mobility of livestock and people, local zoonotic disease epidemiology, and access to health care and social services. A variety of methods were employed, including desk-based systematic review, field epidemiology (see photos in Appendix 7), serological and molecular analyses, and statistical analyses and modelling. There was an almost complete absence of pre-existing high quality information from Togo across all areas considered in this thesis. Consequently, this research makes an important public health contribution to Togo. Furthermore, given the ECOWAS policy of free movement of people and livestock across borders of member countries (*Adepoju 2005*; *Adepoju et al. 2007*), the findings are of direct relevance to the West African region. Cultural, economic, and epidemiological factors influencing human and animal health are not confined by national boundaries.

9.2 Innovation, Validation, Application

This work was conducted in the context of the public health continuum of "innovation, validation, application", which forms the foundation of Swiss TPH's research activities. Innovation refers to novel ideas, methods or approaches; validation refers to the testing of such an innovation; and application refers to the practical implementation of a validated innovation. This PhD thesis contributes to all three areas, as shown in Table 13.

Table 13: Classification of research in terms of innovation, validation and application

Ch	Title	Innovation	Validation	Application
3	Global burden of human brucellosis: a systematic review of disease frequency			Systematic review methodology was used to assess global brucellosis incidence
4	Clinical manifestations of human brucellosis: a systematic review and meta-analysis	The first informed brucellosis disability weight was calculated.		Systematic review methodology was used to asses brucellosis morbidity
5	Epidemiology of Brucellosis and Q Fever in Linked Human and Animal Populations in Northern Togo	The One Health concept was translated into a practical, in-field approach to assessing the epidemiology zoonoses.	Important zoonoses were confirmed to be circulating in northern Togo for the first time. The impact on livestock production and differences in human exposure according to ethnicity were demonstrated.	
6	Deletion in the gene BruAb2_0168 of *Brucella abortus* strains: diagnostic challenges	A gene deletion with important diagnostic and possibly epidemiological consequences for West Africa was identified. A simpler, safer approach to *Brucella* diagnosis was trialled.	PCR on *Brucella* DNA extracted from hygroma fluid preserved in a chaotrophic buffer was shown to be more sensitive than culture.	

Ch	Title	Innovation	Validation	Application
7	Potential risk of regional disease spread in West Africa through cross-border cattle trade	Quantitative data were captured from the cross-border cattle market system in West Africa for the first time.	Simulations demonstrated the risk of regional disease spread through the market system.	
8	Mobility and access to health care and other social services: an exploratory study in northern Togo			The Health Access Livelihood Framework was used to assess access in the context of livelihoods and mobility in communities northern Togo.

9.3 Research Outputs versus Objectives

Despite the aforementioned achievements of this PhD research, not all of the objectives presented in Chapter 2 could be achieved. The contribution of this thesis to each research objective is considered below.

9.3.1 Global burden of human brucellosis (Objective 1)

The systematic review undertaken in the framework of the Global Burden of Disease Study 2010 highlighted the severe and chronic impact of brucellosis on the lives of its sufferers. The first informed estimates of acute and chronic disability weights for human brucellosis presented in Chapter 4 have led us one step closer to the calculation of the DALYs for brucellosis, which would in turn be a valuable advocacy tool for a greater investment of resources. However, Chapter 3 uncovered major knowledge gaps regarding the incidence of brucellosis. Due to heterogenous and patchy data, it was not possible to aggregate the data into regional estimates. The calculation of the global DALYs for brucellosis based on this data could risk significant bias. Herein lies a difficult question: whether it is better to produce a possibly biased DALYs estimate in order to advocate on behalf of those communities around the world affected by brucellosis, or whether strict scientific principles should always be upheld.

9.3.2 Burden and epidemiology of brucellosis and Q Fever in northern Togo (Objective 2)

By translating the One Health concept into a practical approach to zoonotic disease epidemiology in the field, the first high quality human and animal data for Togo were captured. Serological surveys are a useful starting point for investigating disease burden, providing information about the exposure of human and animal populations to pathogens. However, the interpretation of results is often not straight-forward. Seropositivity does not equate to clinical disease and, consequently, the impact of brucellosis and Q Fever on human and animal health and the economy could not be assessed by this research. Furthermore, most commercially available diagnostic kits have only been validated in countries with a low disease burden. Recommended positive and negative cut-off values may not be appropriate in different epidemiological settings, as was demonstrated by the assessment of human Q Fever seropositivity in Togo by

ELISA and IFA, presented in Chapter 5. There is a need to validate diagnostic tests for neglected zoonoses in developing countries, in order to improve their performance in the regions where they are most needed.

The association between seropositivity and abortion in cows presented in Chapter 5 indicates that these diseases likely have an important impact on livestock production. The absence of any small ruminants seropositive for brucellosis suggests that, surprisingly, *B. melitensis* is not circulating in northern Togo. The use of multiple testing methods was particularly valuable here, with RBT-positive small ruminants proving to be false positives when tested by ELISA and CFT. The RBT alone would have failed to capture this important epidemiological finding. People of Fulani ethnicity were shown to have greater exposure to zoonoses, highlighting the way in which cultural factors can influence disease epidemiology. This serosurvey therefore raises some important research questions, but does not provide enough information on which to plan disease control interventions or develop policy.

Apart from one genotyped *B. abortus* strain from Gambia (*Bankole et al. 2010*), the three strains isolated from bovine hygromas in Togo are among the first genotypic data available from West Africa. The identification of three unique VNTR profiles from only a small geographical area reflects the dynamic nature of the study zone. African strains of *Bacillus anthracis*, the causative organism of anthrax, display some unexpected molecular characteristics compared with strains from other regions of the world (*Tamborrini et al. 2011*), and this also appears to be true for *Brucella*. The deletion detected in the BruAb2_0186 gene in Chapter 6 has important diagnostic implications, with the need for new species-specific PCR targets. Additionally, there may be epidemiological consequences in West Africa, in terms of the role of the BruAb2_0186 gene in pathogen virulence. The use of the chaotrophic buffer to preserve and inactivate *Brucella* DNA in hygroma fluid was shown to be a safer and more sensitive diagnostic method than culture, and of particular relevance to countries with poor laboratory biosafety. There is a real need for more molecular epidemiological research in Africa, not just in relation to *Brucella*, but also other zoonoses.

9.3.3 Livestock movements in northern Togo (Objective 3)

The first quantitative assessment of the risk of disease spread in West Africa through cross-border cattle trade, presented in Chapter 7, highlighted the importance of a regional, rather than national, approach to disease control. This is essential not only for the control of endemic diseases, including the Global Foot and Mouth Disease Control Strategy of the FAO and OIE, but also for rapid detection and containment of emerging and re-emerging diseases, such as Contagious Bovine Pleuropneumonia. The epidemiological system of one country was shown to be potentially linked to that of its neighbours through cattle trading activities. This research uncovered important biosecurity concerns in the region, although the role of informal trade was not accounted for.

9.3.4 One Health in Togo (Objective 4)

The research was conducted using a One Health approach, an important component of the broader concept of Ecohealth. Nurses and animal health workers from the study zone gained valuable experience working as part of a multidisciplinary field team. An intersectoral workshop was held in Lomé in November 2012 to discuss the research findings and consult stakeholders in relation to public health priorities and recommendations for future research. The workshop was attended by 35 representatives from the health services, veterinary services, universities and government administration in Togo, as well as researchers from the CSRS in Abidjan, including a sociologist.

The high level of participation at the workshop reflects the stakeholders' interest in integrating One Health into research and public health in Togo. The enthusiasm of the administrative heads of the five provinces of the study zone to attend a technical, science-based workshop was particularly encouraging. Conversely, the health and veterinary personnel valued the opportunity to establish dialogue with the authorities regarding public health priorities and needs. The workshop received significant media attention (see Togo-Presse newspaper article in Appendix 6).

9.3.5 Mobile populations and access to health care in northern Togo (Objective 5)

The ability of the research to meet this objective was compromised by the relocation of the study site to northern Togo. As mentioned in Chapter 2, this PhD research was initially planned to be conducted in northern Côte d'Ivoire. As a result of the political unrest in Côte d'Ivoire at the end of 2010, the study site was shifted to northern Togo in February 2011. Due to the time lost during this transition, the preliminary research that had been conducted in northern Côte d'Ivoire in May-August 2010, including focus group discussions and in-depth interviews with communities and other stakeholders, could not be repeated in Togo. Once all of the necessary permits and authorisations were obtained in late May 2011, rapid collection of the serum samples was a priority. This urgency was not only due to the need to now conduct the PhD research within only two years instead of three, but also because of climatic factors. The rainy season beginning in June results in much of the study zone becoming unreachable by road.

Although the field work was ultimately successful with many important research findings for Togo and the West African region, the value of the outputs relating to mobility and access was compromised by inadequate planning. The Fulani in northern Togo were fairly well settled and integrated into the local communities, usually living in villages alongside other ethnic groups. In contrast, the Fulani in Côte d'Ivoire tended to form separate communities outside of villages. In this sense, a study interested in mobility in Togo may have been more fruitful if it had targeted pre-identified sub-groups for whom mobility was important, e.g. young men responsible for livestock, or women moving for marriage or other reasons. Secondly, the assessment of access to health care and other social services would have benefited from the input of social scientists, in order to refine the methodology. Nonetheless, the research outcomes presented in Chapter 8 do still provide useful background information about mobility and access, on which further research questions could be developed. Of particular interest were findings relating to the impact of the distance to the nearest health centre on birthing practices, concerns raised over the cost of health care, and differences in school attendance according to ethnicity.

9.4 Applications of Research

The direct practical applications of the research are considered below.

- The brucellosis disability weight estimation and the Togolese field data will strengthen advocacy efforts. This is of particular relevance to ADVANZ, an EU-funded initiative to advocate for neglected zoonoses, of which Swiss TPH is a partner (*ADVANZ 2012*).
- The findings relating to brucellosis and Q Fever seroprevalence in Togo, including the performance of the serological tests in an endemic setting, will guide the design of future population-based, epidemiological studies of neglected zoonoses in sub-Saharn Africa.
- The deletion detected in the BruAb2_0186 gene of the *B. abortus* isolates will encourage further molecular studies of *Brucella* in sub-Saharan Africa and the development of more suitable diagnostic assays.
- The simple, safe, and sensitive method developed and trialled in Togo for the diagnosis of active brucellosis in livestock can be applied to other developing country settings.
- The strengthening of collaborative relationships between the animal health services of Togo and those of neighbouring countries will be encouraged by the findings relating to cross-border cattle trade. Additionally, this work will guide the design of future livestock mobility studies in West Africa.
- By drawing on findings related to ethnicity (e.g. greater exposure to zoonoses and poorer school attendance of the Fulani) as well as more general community practices (e.g. the high level of consumption of unboiled milk), community health education activites in northern Togo can be more appropriately targeted.
- By addressing concerns of the communities (e.g. the cost of health services) as well as identified influential factors (e.g. distance to the nearest health centre), strategies for improving the health care access of communities in northern Togo can be developed.

9.5 General Recommendations and Future Research

Recommendations arising from this research, as well as areas for further investigation, are considered below for each research objective.

9.5.1 Global burden of human brucellosis (Objective 1)

General recommendations:

- Advocacy targeting governments, international organisations, and funding bodies for greater investment in brucellosis prevention and control
- Strengthening of health systems, leading to improved brucellosis surveillance and treatment
- Integrated approach to brucellosis involving collaboration of human and animal health sectors
- Epidemiological and statistical training of researchers from developing countries in order to ensure the design and implementation of high quality population-based research studies

Further research:

- Regional disease incidence in Eastern Europe, Sub-Saharan Africa, Asia-Pacific, Central and South America
- DALYs estimate for brucellosis, including an assessment of the uncertainty associated with the available data and an exploration of different scenarios

9.5.2 Burden and epidemiology of brucellosis and Q Fever in northern Togo (Objective 2)

General recommendations

- Education of communities regarding ways in which to minimise the risk of exposure to zoonoses and to improve general hygiene, including boiling milk before consumption and avoiding contact with animal birthing materials. Verbal communication of health messages by the local nurse or the village chief or visual tools would be appropriate, given the low level of literacy.

Further research:

- Proportion of fevers associated with zoonotic diseases, including Q Fever, in northern Togo
- Impact of brucellosis and Q Fever on livestock production and the economy in northern Togo

- Livestock serosurveys in West Africa, including investigation of whether *B. melitensis* is circulating
- Genotyping of *B. abortus* strains from West Africa
- Development of *B. abortus* diagnostic assays using new targets
- Validation of serological tests in developing countries

9.5.3 Livestock movements in northern Togo (Objective 3)

General recommendations:

- Formal process for exchange between animal health departments of West African countries, ensuring a joint approach to disease surveillance, disease control interventions (e.g. vaccination) and border control. This could be mediated through ECOWAS or the African Union, in collaboration with non-governmental organisations.

Further research:

- Impact of season, informal trade networks and population dynamics on cross-border livestock flows and disease risk
- West African trade networks extending beyond Togo and its immediate neighbours
- Socioeconomic drivers of livestock trade
- Association between transboundary animal trade and transboundary disease spread, potentially incorporating phylogenic data
- Suitability and feasibility of interventions to improve border security

9.5.4 One Health in Togo (Objective 4)

General recommendations:

- Increased collaboration between researchers and practitioners of human health, animal health, social science, and natural science in Togo

Further research:

- Appropriate mechanisms or tools for operationalising intersectoral collaboration in Togo, such as frameworks or formal agreements

- Resource sharing, including consideration of the carrying capacity of grazing lands, in relation to transhumance

9.5.5 Mobile populations and access to health care in northern Togo (Objective 5)

Further research:

- Drivers of the greater mobility observed in women, as well as needs and challenges after arrival in a new community
- Mobility of herdsmen during transhumance, including needs, social contacts, conflict with local communities, and access to health care and other services
- Impact of distance to the health centre on access to health care and the role of outreach health services, particularly for birthing assistance
- Vaccination coverage of livestock and barriers such as insufficient veterinary personnel, the high cost of vaccines, or a lack of owner awareness
- Organisation and structure of the milk production system, including aspects relating to local economies, social networks, and public health
- The role of child labour in the livestock sector and its impact on education and development

9.6 Transitioning from Côte d'Ivoire to Togo

As previously mentioned, this research was initially planned to be conducted in northern Côte d'Ivoire, in collaboration with the CSRS. Shifting the study site two countries eastward led to certain challenges as well as lessons learned.

This PhD research commenced in January 2010 and four months were spent in the field during the first year of the PhD to prepare for data collection (May-August 2010 and November 2010). Although the CSRS is no stranger to "research in a war zone" (*Bonfoh et al. 2011b*), the post-electoral crisis in December 2010 posed a serious security concern. All foreign students were evacuated from the CSRS, but most were able to return by mid-2011 to continue their research. However, given the uncertainty of the situation, the remoteness of the study site from the CSRS, and the need to progress with data collection, the decision was made in February 2011 to completely relocate this PhD project to northern Togo. This was a joint decision made by the supervisors of this thesis, Esther Schelling and Bassirou Bonfoh, in discussion with the author and a Togolese collaborator, Kulo Abalo.

By March 2011, the author had already arrived in Togo to conduct the first field visit. This speedy relocation of the study site to northern Togo was only possible due to a pre-existing, formal collaboration between the CSRS in Côte d'Ivoire and ESA in Togo. This highlights the importance of partnerships between institutions in developing countries, referred to as south-south partnerships. The two countries share similar ecological and social characteristics and, despite not being direct neighbours, are linked by the ECOWAS policy of free movement of people and goods. South-south partnerships are important not only for the sharing of ideas, research findings, and technical capacity, but also for a combined approach to the planning of interventions and policy. Health, mobility livestock production and trade are issues that should be considered at a regional level, rather than a purely national level.

Swiss TPH has only limited experience conducting research activities in Togo. The local collaborating partner, ESA, had previously assisted a Masters student co-supervised by Swiss TPH and the Institute of Social Anthropology at the University of Basel. ESA provided invaluable administrative support for this PhD research. However, certain procedures, such as obtaining ethics approval from the Ministry of Health, were unknown to the school and proved to be a learning experience. The organisation of the fieldwork itself was predominantly the responsibility of the author, including finding accommodation in the study zone, obtaining a rental car, identifying and training a suitable field team, and managing other logistic issues such as establishing a cold chain. The materials and equipment needed for the fieldwork were, for a period of time, distributed across three countries along the Gulf of Guinea - Côte d'Ivoire, Ghana and Togo. Through a combination of air and long-distance road travel, much of which was personally carried out by Bassirou Bonfoh, all materials eventually arrived in Togo.

Data collection began in May 2011. The first round was successfully completed by July 2011, due to a delayed start to the rainy season. Although this was very fortunate for the study, the livelihoods and food security of the communities suffered from this extended dry season. This highlighted the precarious situation in which many local people live from day to day. A second field visit was conducted between January-April 2012, completing the data collection. Ultimately, this research was successful but, as mentioned in section 9.3.5, time constraints did limit the planning of the study and the value of some of the research outputs. Photos taken during the field work in Togo are provided in Appendix 7.

9.7 Final Conclusions

This PhD thesis addresses global disease burden, regional mobility of livestock and people, local zoonotic disease epidemiology and access to health care and social services. The research was conduced across the continuum of innovation-validation-application that forms the basis of the activities of Swiss TPH. The findings provide a public health contribution that is valuable from global to regional and national levels. In addition to the direct, pratical applications of this research, it also provides a much needed evidence base on which to advocate on behalf of communities, develop more targeted research questions, and, eventually, design public health interventions and policy.

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Appendix 1: Supplementary tables for Chapter 3

Table 14: Selected brucellosis seroprevalence studies by region

Country, Author	Study design	Study level	Study population	Diagnostic tests	Seroprevalence (%), (95% CI)	Number of subjects
North Africa and Middle East						
Egypt (*El Sherbini et al. 2007*)	Cross-sectional	Sub-district	General population	STAT	0.03 (0-3)	616
Iran, (*Alavi et al. 2007*)	Cross-sectional	District	Nomadic community	RBT; sequential testing of all positives with WAT; sequential testing of all positives to both tests with 2ME	8.0 (7.1-8.9) (RBT); 7.9 (7.0-8.8) (RBT + Wright); 6.3 (5.5-7.1) (RBT + Wright + 2ME)	3,594
Iran (*Khorasgani et al. 2008*)	Unknown	Provincial	Healthy blood donors	RBT; sequential testing of all positives with STAT	0.08 (0-0.1) (RBT); 0.06 (0-0.1) (RBT + STAT)	10,500
Iraq (*Yacoub et al. 2006*)	Cross-sectional	District	General population: rural	RBT	5.7 (3.5-7.8)	439
Iraq (*Yacoub et al. 2006*)	Cross-sectional	District	General population: urban	RBT	12.2 (9.1-15.3)	435
Iraq (*Yacoub et al. 2006*)	Cross-sectional	District	General population: semi-rural	RBT	29.3 (25.1-33.3)	465
Jordan (*Al-Ani et al. 2004*)	Longitudinal	National	Healthy blood donors and healthy people undergoing routine health exams	MAT	4.1 (2.7-5.5)	800

Country, Author	Study design	Study level	Study population	Diagnostic tests	Seroprevalence (%), (95% CI)	Number of subjects
Oman (*Idris et al. 1993*)	Cross-sectional	District	School children	MAT, followed by testing of positives by STAT	1.2 (0.1-2.3)	373
Saudi Arabia (*Alballa 1995*)	Cross-sectional	Provincial	General population	MAT, followed by STAT of positives	16.3 (15.3-17.3)	4794
Saudi Arabia (*Al Sekait 1999*)	Cross-sectional	National	General population	CFT	15 (14.5-15.5)	23,613
Sub-Saharan Africa						
Chad (*Schelling et al. 2003*)	Cross-sectional	Provincial	Nomadic community	Parallel ELISA + RBT	3.8 (2-5)	860
Western Europe						
Turkey (*Cetinkaya et al. 2005*)	Cross-sectional	District	General population	RBT	4.8 (3.5-6.1)	1052
Turkey (*Karabay et al. 2004*)	Cross-sectional	Provincial	General population	Parallel RBT and STAT	Overall:1.3 (0.8-1.8); Urban:1.7 (1.0-2.4); Rural:1.0 (0.3-1.7)	Overall:2,098 Urban:1,298 Rural:800
Turkey (*Vancelik et al. 2008*)	Cross-sectional	District	General population	STAT, RBT	5.4 (3.5-7.3) (STAT); 11.9 (9.2-14.6) (RBT)	573
Central Asia						
Kyrgyzstan (*Bonfoh et al. 2011a*)	Cross-sectional	National	General population	Parallel ELISA + RBT + Huddleson	8.8 (4.5-16.5)	1,774
Central and South America						
Argentina (*Marder et al. 2005*)	Routine data	District	Healthy blood donors	Huddleson	1.4 (1.3-1.5)	35,388
Mexico (*Hernandez et al. 1999*)	Longitudinal	Provincial	Healthy blood donors	RBT	2.8 (2.5-3.1)	9590

Table 15: Selected brucellosis incidence studies by region

Country, Author	Study design	Sampling methods	Study level	Reference population type and size	Diagnostic tests	Incidence (cases per 100,000 per year)	Number of cases
North Africa and Middle East							
Egypt (*Crump et al. 2003*)	Longitudinal	Non-random purposive sampling; active case-finding	District	General population: 664,000; Acute febrile illness population: 449	STAT and/or culture	18.0	31
Egypt 2002-2003 Jennings (*Jennings et al. 2007*)	Longitudinal	Non-random purposive sampling; active case-finding	Provincial	General population: 2,347,249; Acute febrile illness population: 4490	STAT and/or culture	64.0 (2002) 70.0 (2003)	135 (2002) 186 (2003)
Iran (*Haghdoost et al. 2007*)	Routine data	Non-random sampling; passive case-finding	District	General population: 50,000	Serology	141.6	97
Iran (*Hosseini et al. 2008*)	Routine data	Non-random sampling; passive case-finding	Provincial	General population	Wright Agglutination and 2ME	13.1	145

Country, Author	Study design	Sampling methods	Study level	Reference population type and size	Diagnostic tests	Incidence (cases per 100,000 per year)	Number of cases
Jordan (*Abu Shaqra 2000*)	Routine data	Non-random sampling; passive case-finding	National	General population	Rose Bengal followed by STAT of positives	16.7 (1988) 21.4 (1989) 24.1 (1990) 29.9 (1991) 22.3 (1992) 22.6 (1993) 22.6 (1994) 27.3 (1995) 26.1 (1996) 25.7 (1997)	7,842
Jordan (*Gargouri et al. 2009*)	Longitudinal	Non-random sampling; passive case-finding	National	General population	STAT	130	31 (Sept. 2003); 9 (May 2004)
Palestine (*Awad 1998*)	Longitudinal	Non-random sampling; passive case-finding	National	General population: 860,000	RBT	8	69
Saudi Arabia (*Elbeltagy 2001*)	Routine data	Non-random sampling; passive case-finding	Provincial	General population: 40,000	Sympto-matically or culture or paired serology or CFT	34	137
Saudi Arabia (*Al Tawfiq & AbuKhamsin 2009*)	Routine data	Non-random sampling; passive case-finding	District	Company employees: 37,000	ELISA or STAT	13 (1983) 33.3 (1985) 51.3 (1986) 70.7 (1987) 26.3 (1983-1992) 6 (1993-2007)	913

Country, Author	Study design	Sampling methods	Study level	Reference population type and size	Diagnostic tests	Incidence (cases per 100,000 per year)	Number of cases
Western Europe							
Germany (*Al Dahouk et al. 2007b*)	Routine data	Non-random sampling; passive case-finding	National	General population	Serology and/or culture	0.03 (1998-2001) 0.6 (1962-1965)	245
Greece (*Jelastopulu et al. 2010*)	Routine data	Non-random sampling; passive case-finding	Provincial	General population: 322,790	Unknown	4	86
Greece (*Avdikou et al. 2005*)	Longitudinal	Non-random sampling; passive case-finding	Provincial	General population: 353,820	ELISA or RBT or STAT	17.3	152
Greece (*Minas et al. 2007*)	Longitudinal	Non-random sampling; passive case-finding	Provincial	General population: 278,000	ELISA, CFT, STAT, IFA or Coombs	32.5	821
Italy (De Massis et al. 2005)n}	Routine data	Non-random sampling; passive case-finding	National	General population	Unknown	1.4 (2002) 2.7 (1990) 3.3 (1996)	-
North America							
USA (*Doyle & Bryan 2000*)	Routine data	Non-random sampling; passive case-finding	National	General population: 322,790; Zone 1: 9,871,639; Zone 2: 45,572,965; Zone 3: 203,067,933	Unknown	Zone 1: 0.18 Zone 2: 0.09 Zone 3: 0.02	Zone 1: 158 Zone 2: 360 Zone 3: 398

Appendix 2: Supplementary forest plots for Chapter 4

Study	n	Proportion	95%-CI
1. Children			
Giannakopoulos, 2006	52	0.808	[0.675; 0.904]
Gottesman, 1996	88	0.909	[0.829; 0.960]
Issa, 1999	68	0.750	[0.630; 0.847]
Mantur, 2004	93	0.527	[0.421; 0.631]
Shaalan, 2002	115	0.913	[0.846; 0.958]
Shen, 2007	20	0.800	[0.563; 0.943]
Tsolia, 2002	39	0.949	[0.827; 0.994]
Random effects model (for age category)		**0.816**	**[0.691; 0.914]**
2. Adults			
Aygen, 2002	480	0.390	[0.346; 0.435]
Bodur, 2004	86	0.616	[0.505; 0.719]
Hizel, 2007	163	0.607	[0.528; 0.683]
Kokoglu, 2006	138	0.406	[0.323; 0.493]
Mantur, 2006	495	0.842	[0.807; 0.873]
Memish, 2000	160	0.862	[0.799; 0.912]
Tasbakan, 2003	109	0.972	[0.922; 0.994]
Tasova, 1999	238	0.815	[0.760; 0.862]
Yetkin, 2006	305	0.695	[0.640; 0.746]
Zribi, 2009	45	0.933	[0.817; 0.986]
Random effects model (for age category)		**0.733**	**[0.593; 0.853]**
3. All ages			
Al-Shamahy, 2001	235	1.000	[0.984; 1.000]
Al Dahouk, 2005	30	0.633	[0.439; 0.801]
Andriopoulos, 2007	144	1.000	[0.975; 1.000]
Bukharie, 2009	84	0.726	[0.618; 0.818]
Buzgan, 2010	1028	0.288	[0.260; 0.317]
Fallatah, 2005	159	0.792	[0.721; 0.853]
Neau, 1997	59	0.525	[0.391; 0.657]
Savas, 2007	140	0.529	[0.442; 0.613]
Shehabi, 1990	106	1.000	[0.966; 1.000]
Random effects model (for age category)		**0.786**	**[0.492; 0.972]**
Random effects model (overall)		**0.775**	**[0.661; 0.872]**

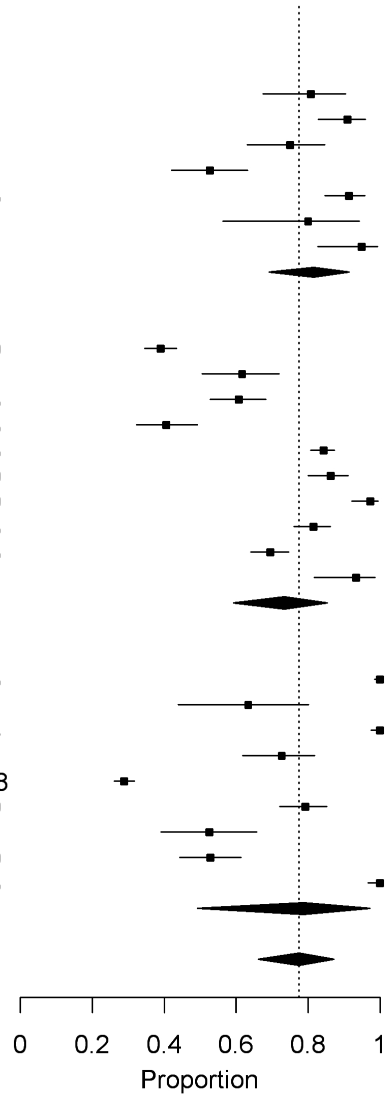


Figure 17: Forest plot for fever

Study	n	Proportion	95%-CI
1. Children			
Afsharpaiman, 2008	44	0.500	[0.346; 0.654]
Galanakis, 1996	52	0.192	[0.096; 0.325]
Giannakopoulos, 2006	52	0.154	[0.069; 0.281]
Gottesman, 1996	88	0.045	[0.013; 0.112]
Issa, 1999	68	0.603	[0.477; 0.720]
Shaalan, 2002	115	0.087	[0.042; 0.154]
Tanir, 2009	90	0.189	[0.114; 0.285]
Tsolia, 2002	39	0.205	[0.093; 0.365]
Random effects model (for age category)		**0.229**	**[0.111; 0.375]**
2. Adults			
Akinci, 2006	134	0.075	[0.036; 0.133]
Aygen, 2002	480	0.844	[0.808; 0.875]
Bodur, 2004	86	0.442	[0.335; 0.553]
Celen, 2010	143	0.734	[0.654; 0.805]
Elbeltagy, 2001	131	0.786	[0.706; 0.853]
Hizel, 2007	163	0.540	[0.460; 0.618]
Kokoglu, 2006	138	0.725	[0.642; 0.797]
Mantur, 2006	495	0.038	[0.023; 0.059]
Memish, 2000	160	0.188	[0.130; 0.257]
Pérez, 1997	241	0.299	[0.242; 0.361]
Tasbakan, 2003	109	0.917	[0.849; 0.962]
Tasova, 1999	238	0.630	[0.566; 0.692]
Yetkin, 2006	305	0.689	[0.633; 0.740]
Zribi, 2009	45	0.822	[0.679; 0.920]
Random effects model (for age category)		**0.546**	**[0.349; 0.736]**
3. All ages			
Al-Shamahy, 2001	235	0.919	[0.877; 0.951]
Al Dahouk, 2005	30	0.400	[0.227; 0.594]
Andriopoulos, 2007	144	0.958	[0.912; 0.985]
Barroso, 2002	565	0.958	[0.937; 0.973]
Buzgan, 2010	1028	0.648	[0.618; 0.677]
Colmenero, 1996	530	0.849	[0.816; 0.878]
Fallatah, 2005	159	0.201	[0.142; 0.272]
Gür, 2003	283	0.792	[0.739; 0.837]
Neau, 1997	59	0.424	[0.296; 0.559]
Pourbagher(b), 2006	251	0.530	[0.466; 0.593]
Savas, 2007	140	0.857	[0.788; 0.911]
Shehabi, 1990	106	0.877	[0.799; 0.933]
Random effects model (for age category)		**0.732**	**[0.599; 0.846]**
Random effects model (overall)		**0.537**	**[0.417; 0.656]**

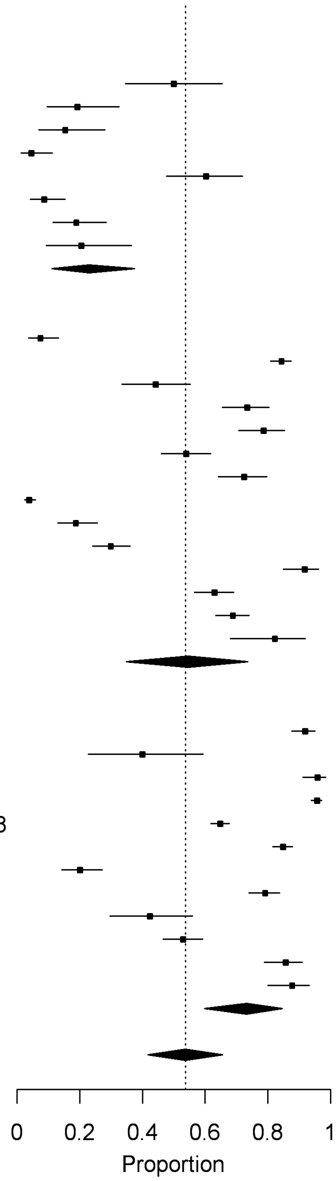


Figure 18: Forest plot for sweats

Study	n	Proportion	95%-CI
1. Children			
Giannakopoulos, 2006	52	0.058	[0.012; 0.159]
Shaalan, 2002	115	0.183	[0.117; 0.265]
Shen, 2007	20	0.250	[0.087; 0.491]
Tsolia, 2002	39	0.282	[0.150; 0.449]
Random effects model (for age category)		**0.183**	**[0.094; 0.293]**
2. Adults			
Celen, 2010	143	0.699	[0.617; 0.773]
Elbeltagy, 2001	131	0.389	[0.305; 0.478]
Kokoglu, 2006	138	0.442	[0.358; 0.529]
Memish, 2000	160	0.488	[0.408; 0.568]
Pérez, 1997	241	0.328	[0.269; 0.391]
Random effects model (for age category)		**0.469**	**[0.343; 0.596]**
3. All ages			
Al Dahouk, 2005	30	0.433	[0.255; 0.626]
Barroso, 2002	565	0.906	[0.879; 0.929]
Buzgan, 2010	1028	0.339	[0.310; 0.368]
Colmenero, 1996	530	0.860	[0.828; 0.889]
Gür, 2003	283	0.809	[0.758; 0.853]
Neau, 1997	59	0.169	[0.084; 0.290]
Troy, 2005	28	0.536	[0.339; 0.725]
Random effects model (for age category)		**0.597**	**[0.341; 0.827]**
Random effects model (overall)		**0.452**	**[0.300; 0.608]**

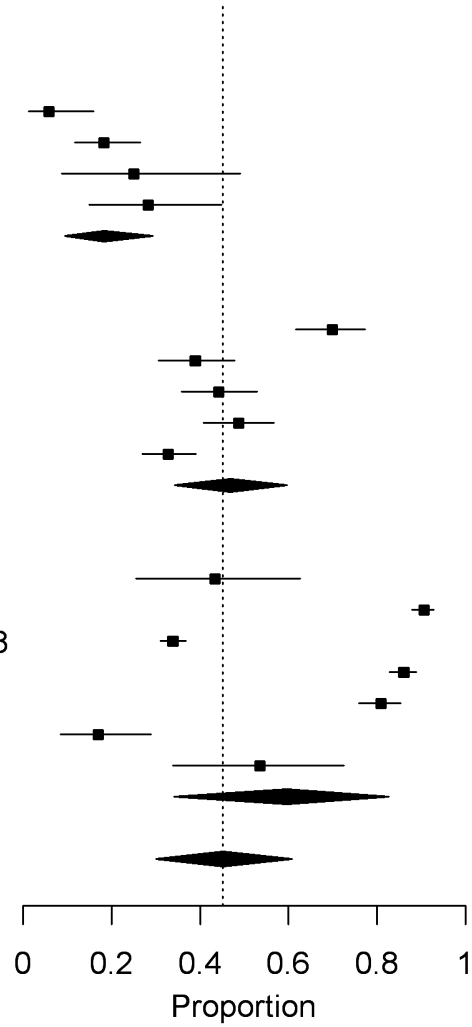


Figure 19: Forest plot for chills

Study	n	Proportion	95%-CI
1. Children			
Shaalan, 2002	115	0.183	[0.117; 0.265]
Tsolia, 2002	39	0.179	[0.075; 0.335]
Random effects model (for age category)		**0.186**	**[0.129; 0.250]**
2. Adults			
Elbeltagy, 2001	131	0.809	[0.731; 0.873]
Mantur, 2006	495	0.012	[0.004; 0.026]
Random effects model (for age category)		**0.332**	**[0.128; 0.999]**
3. All ages			
Al-Shamahy, 2001	235	0.702	[0.639; 0.760]
Barroso, 2002	565	0.488	[0.447; 0.531]
Buzgan, 2010	1028	0.712	[0.683; 0.740]
Fallatah, 2005	159	0.013	[0.002; 0.045]
Pourbagher(b), 2006	251	0.765	[0.708; 0.816]
Random effects model (for age category)		**0.508**	**[0.269; 0.745]**
Random effects model (overall)		**0.392**	**[0.160; 0.654]**

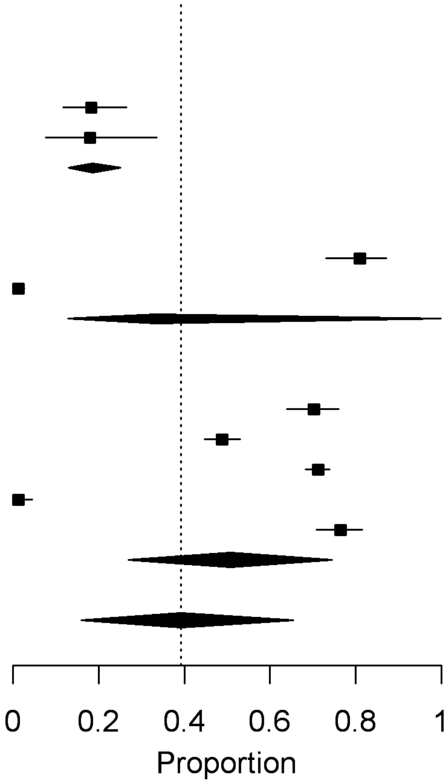


Figure 20: Forest plot for fatigue

Study	n	Proportion	95%-CI
1. Children			
Galanakis, 1996	52	0.212	[0.111; 0.347]
Giannakopoulos, 2006	52	0.019	[0.000; 0.103]
Gottesman, 1996	88	0.080	[0.033; 0.157]
Shaalan, 2002	115	0.070	[0.031; 0.132]
Shen, 2007	20	0.150	[0.032; 0.379]
Tsolia, 2002	39	0.051	[0.006; 0.173]
Random effects model (for age category)		**0.091**	**[0.048; 0.146]**
2. Adults			
Akinci, 2006	134	0.373	[0.291; 0.461]
Aygen, 2002	480	0.190	[0.155; 0.228]
Celen, 2010	143	0.566	[0.481; 0.649]
Elbeltagy, 2001	131	0.771	[0.689; 0.840]
Kokoglu, 2006	138	0.514	[0.428; 0.600]
Mantur, 2006	495	0.016	[0.007; 0.032]
Memish, 2000	160	0.162	[0.109; 0.229]
Pérez, 1997	241	0.502	[0.437; 0.567]
Tasbakan, 2003	109	0.110	[0.058; 0.184]
Tasova, 1999	238	0.429	[0.365; 0.494]
Yetkin, 2006	305	0.308	[0.257; 0.363]
Random effects model (for age category)		**0.336**	**[0.192; 0.499]**
3. All ages			
Al-Shamahy, 2001	235	0.740	[0.679; 0.795]
Al Dahouk, 2005	30	0.300	[0.147; 0.494]
Alsubaie, 2005	34	0.588	[0.407; 0.754]
Andriopoulos, 2007	144	0.660	[0.576; 0.737]
Barroso, 2002	565	0.835	[0.802; 0.865]
Buzgan, 2010	1028	0.144	[0.123; 0.167]
Fallatah, 2005	159	0.258	[0.192; 0.333]
Gür, 2003	283	0.661	[0.602; 0.716]
Pourbagher(b), 2006	251	0.506	[0.442; 0.569]
Savas, 2007	140	0.743	[0.662; 0.813]
Shehabi, 1990	106	0.292	[0.208; 0.389]
Random effects model (for age category)		**0.522**	**[0.322; 0.718]**
Random effects model (overall)		**0.346**	**[0.238; 0.464]**

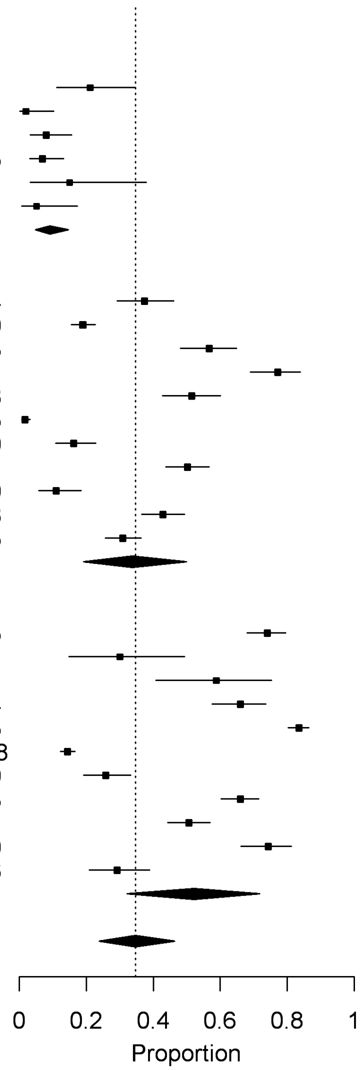


Figure 21: Forest plot for headache

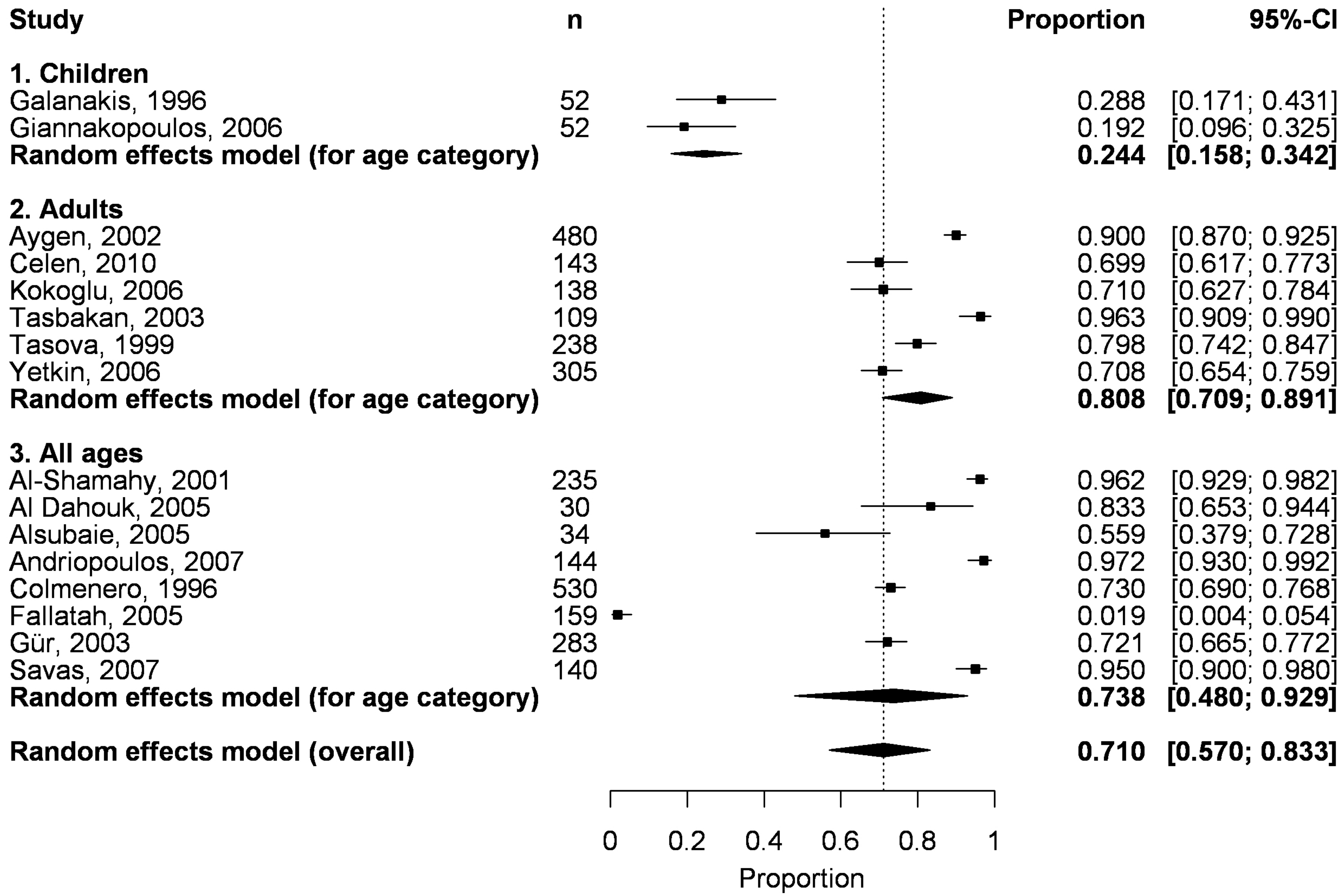


Figure 22: Forest plot for malaise

Study	n	Proportion	95%-CI
2. Adults			
Akinci, 2006	134	0.157	[0.100; 0.230]
Aygen, 2002	480	0.323	[0.281; 0.367]
Kokoglu, 2006	138	0.254	[0.183; 0.335]
Mantur, 2006	495	0.026	[0.014; 0.044]
Yetkin, 2006	305	0.105	[0.073; 0.145]
Random effects model (for age category)		**0.157**	**[0.049; 0.311]**
3. All ages			
Al-Shamahy, 2001	235	0.596	[0.530; 0.659]
Buzgan, 2010	1028	0.249	[0.223; 0.277]
Gür, 2003	283	0.180	[0.137; 0.230]
Pourbagher(b), 2006	251	0.295	[0.239; 0.355]
Savas, 2007	140	0.800	[0.724; 0.863]
Shehabi, 1990	106	0.075	[0.033; 0.143]
Random effects model (for age category)		**0.352**	**[0.186; 0.538]**
Random effects model (overall)		**0.257**	**[0.148; 0.384]**

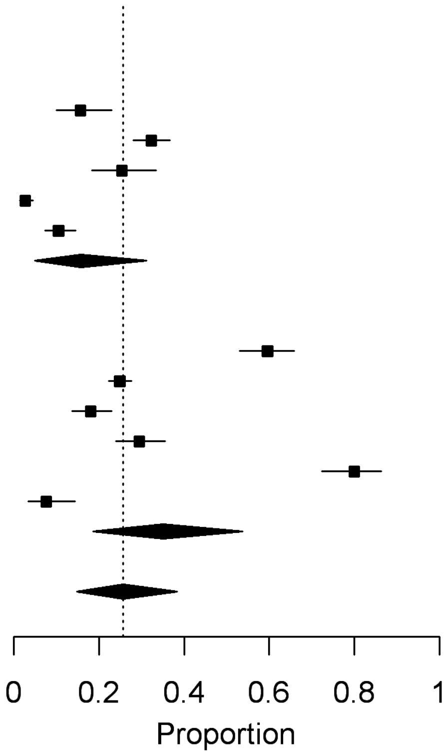


Figure 23: Forest plot for nausea/vomiting

Study	n	Proportion	95%-CI
1. Children			
Giannakopoulos, 2006	52	0.077	[0.021; 0.185]
Tanir, 2009	90	0.156	[0.088; 0.247]
Tsolia, 2002	39	0.103	[0.029; 0.242]
Random effects model (for age category)		**0.126**	**[0.082; 0.177]**
2. Adults			
Akinci, 2006	134	0.313	[0.236; 0.399]
Aygen, 2002	480	0.444	[0.399; 0.489]
Elbeltagy, 2001	131	0.427	[0.341; 0.517]
Tasova, 1999	238	0.109	[0.073; 0.156]
Random effects model (for age category)		**0.313**	**[0.152; 0.503]**
3. All ages			
Al Dahouk, 2005	30	0.333	[0.173; 0.528]
Buzgan, 2010	1028	0.424	[0.394; 0.455]
Fallatah, 2005	159	0.006	[0.000; 0.035]
Pourbagher(b), 2006	251	0.215	[0.166; 0.271]
Savas, 2007	140	0.450	[0.366; 0.536]
Shehabi, 1990	106	0.358	[0.268; 0.457]
Troy, 2005	28	0.464	[0.275; 0.661]
Random effects model (for age category)		**0.294**	**[0.149; 0.465]**
Random effects model (overall)		**0.258**	**[0.171; 0.357]**

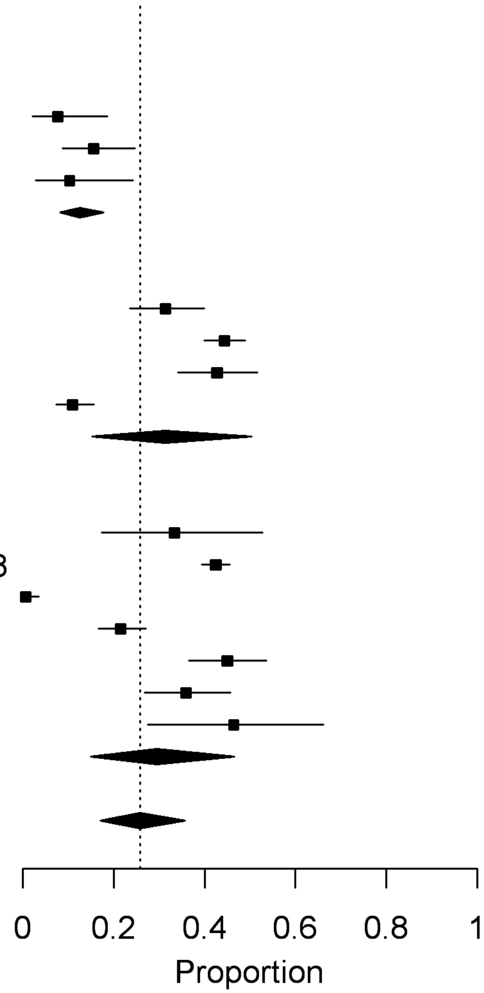


Figure 24: Forest plot for weight loss

Study	n	Proportion	95%-CI
1. Children			
Galanakis, 1996	52	0.192	[0.096; 0.325]
Shaalan, 2002	115	0.017	[0.002; 0.061]
Shen, 2007	20	0.300	[0.119; 0.543]
Random effects model (for age category)		**0.143**	**[0.013; 0.376]**
2. Adults			
Akinci, 2006	134	0.015	[0.002; 0.053]
Aygen, 2002	480	0.210	[0.175; 0.250]
Kokoglu, 2006	138	0.159	[0.103; 0.231]
Mantur, 2006	495	0.026	[0.014; 0.044]
Random effects model (for age category)		**0.087**	**[0.013; 0.215]**
3. All ages			
Al-Shamahy, 2001	235	0.609	[0.543; 0.671]
Barroso, 2002	565	0.382	[0.342; 0.424]
Bukharie, 2009	84	0.012	[0.000; 0.065]
Buzgan, 2010	1028	0.067	[0.053; 0.084]
Fallatah, 2005	159	0.113	[0.068; 0.173]
Gür, 2003	283	0.191	[0.147; 0.242]
Pourbagher(b), 2006	251	0.283	[0.228; 0.343]
Savas, 2007	140	0.450	[0.366; 0.536]
Troy, 2005	28	0.500	[0.306; 0.694]
Random effects model (for age category)		**0.260**	**[0.132; 0.414]**
Random effects model (overall)		**0.188**	**[0.107; 0.285]**

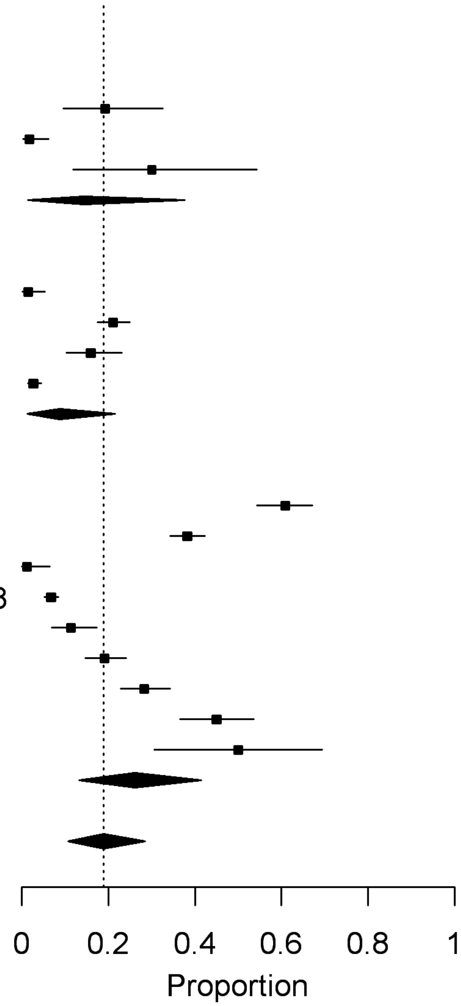


Figure 25: Forest plot for abdominal pain

Study	n	Proportion	95%-CI
1. Children			
Afsharpaiman, 2008	44	0.432	[0.283; 0.590]
Galanakis, 1996	52	0.519	[0.376; 0.660]
Giannakopoulos, 2006	52	0.481	[0.340; 0.624]
Gottesman, 1996	88	0.420	[0.316; 0.530]
Issa, 1999	68	0.338	[0.228; 0.463]
Mantur, 2004	93	0.097	[0.045; 0.176]
Shaalan, 2002	115	0.122	[0.068; 0.196]
Tanir, 2009	90	0.111	[0.055; 0.195]
Tsolia, 2002	39	0.385	[0.234; 0.554]
Random effects model (for age category)		**0.307**	**[0.193; 0.434]**
2. Adults			
Abdi-Liae, 2007	85	0.094	[0.042; 0.177]
Akinci, 2006	134	0.179	[0.118; 0.255]
Aygen, 2002	480	0.142	[0.112; 0.176]
Bodur, 2004	86	0.291	[0.198; 0.399]
Celen, 2010	143	0.224	[0.158; 0.301]
Elbeltagy, 2001	131	0.252	[0.180; 0.335]
Kokoglu, 2006	138	0.362	[0.282; 0.448]
Mantur, 2006	495	0.192	[0.158; 0.229]
Memish, 2000	160	0.062	[0.030; 0.112]
Pérez, 1997	241	0.170	[0.125; 0.224]
Tasbakan, 2003	109	0.596	[0.498; 0.689]
Tasova, 1999	238	0.218	[0.168; 0.276]
Zribi, 2009	45	0.511	[0.358; 0.663]
Random effects model (for age category)		**0.238**	**[0.176; 0.306]**
3. All ages			
Al-Shamahy, 2001	235	0.383	[0.321; 0.448]
Al Dahouk, 2005	30	0.567	[0.374; 0.745]
Andriopoulos, 2007	144	0.507	[0.422; 0.591]
Barroso, 2002	565	0.375	[0.335; 0.417]
Bukharie, 2009	84	0.155	[0.085; 0.250]
Buzgan, 2010	1028	0.145	[0.124; 0.168]
Colmenero, 1996	530	0.223	[0.188; 0.260]
Fallatah, 2005	159	0.038	[0.014; 0.080]
Gür, 2003	283	0.269	[0.218; 0.324]
Neau, 1997	59	0.119	[0.049; 0.229]
Pourbagher(b), 2006	251	0.084	[0.053; 0.125]
Savas, 2007	140	0.121	[0.072; 0.187]
Shehabi, 1990	106	0.613	[0.514; 0.706]
Troy, 2005	28	0.179	[0.061; 0.369]
Random effects model (for age category)		**0.251**	**[0.174; 0.336]**
Random effects model (overall)		**0.259**	**[0.213; 0.307]**

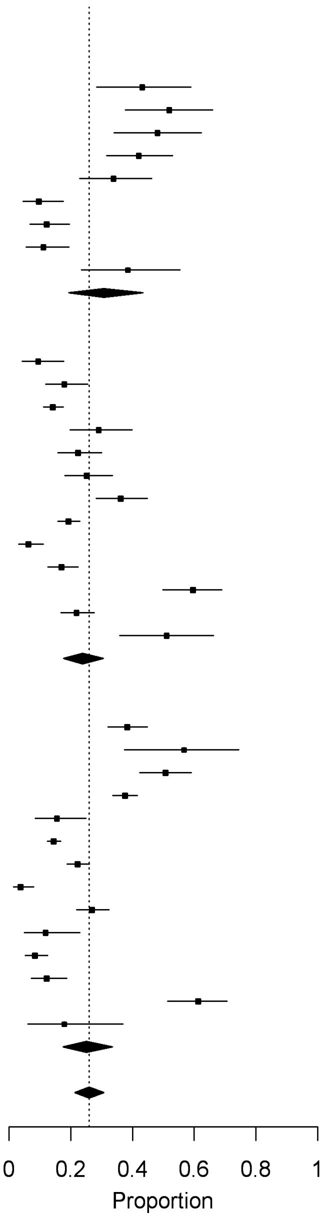


Figure 26: Forest plot for splenomegaly

Study	n	Proportion	95%-CI
1. Children			
Afsharpaiman, 2008	44	0.341	[0.205; 0.499]
Galanakis, 1996	52	0.327	[0.203; 0.471]
Giannakopoulos, 2006	52	0.692	[0.549; 0.813]
Gottesman, 1996	88	0.432	[0.327; 0.542]
Mantur, 2004	93	0.054	[0.018; 0.121]
Shaalan, 2002	115	0.130	[0.075; 0.206]
Shen, 2007	20	0.150	[0.032; 0.379]
Tanir, 2009	90	0.156	[0.088; 0.247]
Tsolia, 2002	39	0.282	[0.150; 0.449]
Random effects model (for age category)		**0.273**	**[0.152; 0.413]**
2. Adults			
Abdi-Liae, 2007	85	0.118	[0.058; 0.206]
Akinci, 2006	134	0.187	[0.125; 0.263]
Aygen, 2002	480	0.212	[0.177; 0.252]
Bodur, 2004	86	0.419	[0.313; 0.530]
Celen, 2010	143	0.266	[0.195; 0.346]
Elbeltagy, 2001	131	0.229	[0.160; 0.311]
Kokoglu, 2006	138	0.268	[0.196; 0.350]
Mantur, 2006	495	0.113	[0.087; 0.144]
Memish, 2000	160	0.069	[0.035; 0.120]
Pérez, 1997	241	0.133	[0.093; 0.182]
Tasbakan, 2003	109	0.376	[0.285; 0.474]
Tasova, 1999	238	0.231	[0.179; 0.290]
Zribi, 2009	45	0.156	[0.065; 0.295]
Random effects model (for age category)		**0.206**	**[0.159; 0.258]**
3. All ages			
Al-Shamahy, 2001	235	0.285	[0.228; 0.347]
Al Dahouk, 2005	30	0.200	[0.077; 0.386]
Andriopoulos, 2007	144	0.250	[0.182; 0.329]
Barroso, 2002	565	0.317	[0.279; 0.357]
Bukharie, 2009	84	0.107	[0.050; 0.194]
Buzgan, 2010	1028	0.206	[0.182; 0.232]
Colmenero, 1996	530	0.381	[0.340; 0.424]
Fallatah, 2005	159	0.013	[0.002; 0.045]
Gür, 2003	283	0.279	[0.228; 0.335]
Neau, 1997	59	0.102	[0.038; 0.208]
Pourbagher(b), 2006	251	0.060	[0.034; 0.097]
Savas, 2007	140	0.207	[0.143; 0.284]
Shehabi, 1990	106	0.538	[0.438; 0.635]
Troy, 2005	28	0.286	[0.132; 0.487]
Random effects model (for age category)		**0.217**	**[0.152; 0.290]**
Random effects model (overall)		**0.225**	**[0.186; 0.268]**

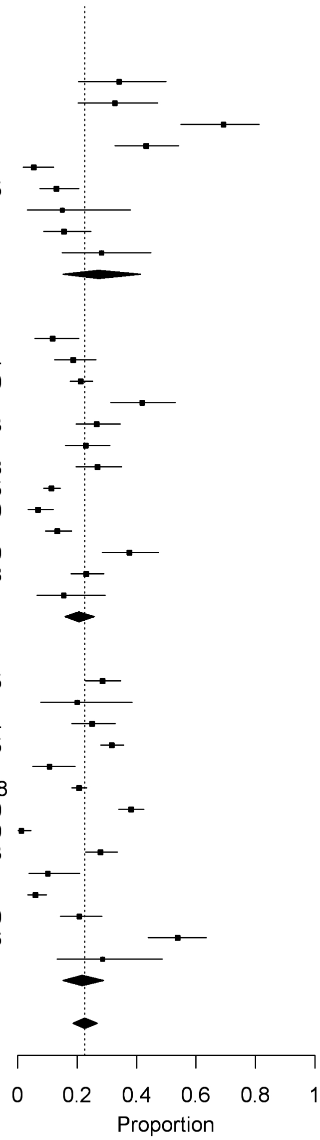


Figure 27: Forest plot for hepatomegaly

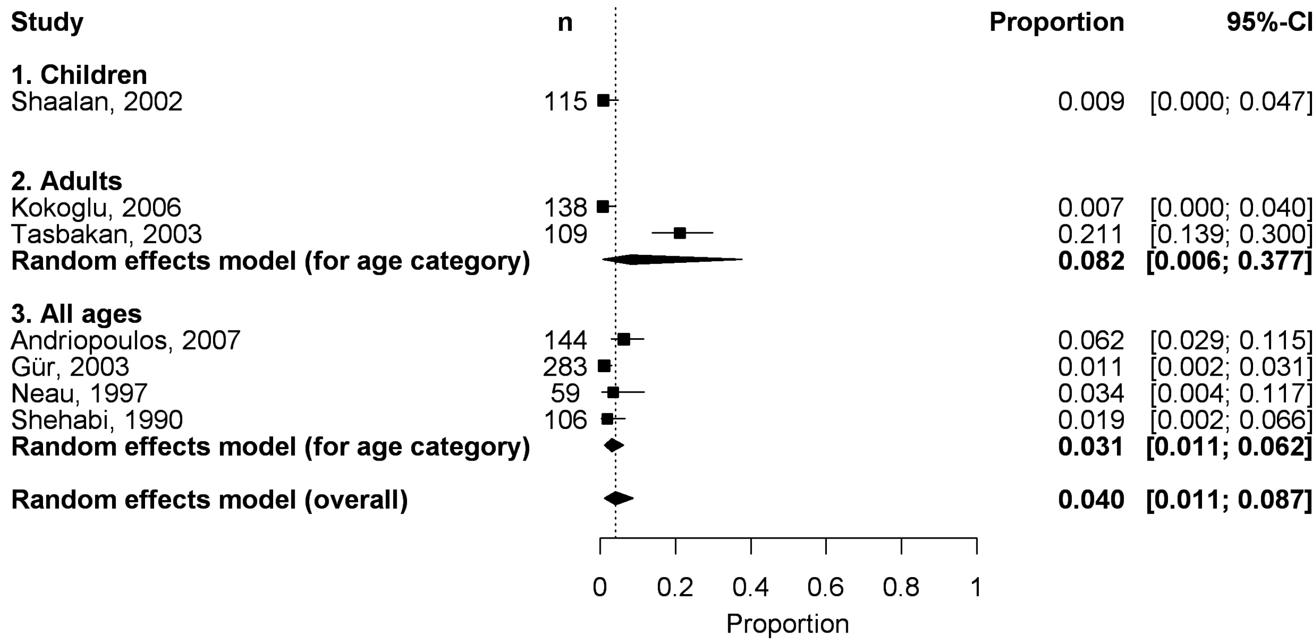


Figure 28: Forest plot for hepatitis

Study	n	Proportion	95%-CI
1. Children			
Afsharpaiman, 2008	44	0.795	[0.647; 0.902]
Galanakis, 1996	52	0.615	[0.470; 0.747]
Giannakopoulos, 2006	52	0.827	[0.697; 0.918]
Gottesman, 1996	88	0.830	[0.734; 0.901]
Issa, 1999	68	0.779	[0.662; 0.871]
Mantur, 2004	93	0.204	[0.128; 0.301]
Shaalan, 2002	115	0.730	[0.640; 0.809]
Tanir, 2009	90	0.856	[0.766; 0.921]
Tsolia, 2002	39	0.692	[0.524; 0.830]
Random effects model (for age category)		**0.707**	**[0.555; 0.838]**
2. Adults			
Abdi-Liae, 2007	85	0.235	[0.150; 0.340]
Akinci, 2006	134	0.522	[0.434; 0.609]
Aygen, 2002	480	0.819	[0.781; 0.852]
Bodur, 2004	86	0.767	[0.664; 0.852]
Celen, 2010	143	0.832	[0.761; 0.889]
Elbeltagy, 2001	131	0.847	[0.774; 0.904]
Kokoglu, 2006	138	0.775	[0.697; 0.842]
Mantur, 2006	495	0.236	[0.200; 0.276]
Memish, 2000	160	0.662	[0.584; 0.735]
Pérez, 1997	241	0.386	[0.324; 0.451]
Tasbakan, 2003	109	0.817	[0.731; 0.884]
Tasova, 1999	238	0.782	[0.724; 0.832]
Random effects model (for age category)		**0.648**	**[0.493; 0.789]**
3. All ages			
Al-Shamahy, 2001	235	0.413	[0.349; 0.479]
Al Dahouk, 2005	30	0.333	[0.173; 0.528]
Alsubaie, 2005	34	0.618	[0.436; 0.778]
Andriopoulos, 2007	144	0.868	[0.802; 0.919]
Barroso, 2002	565	0.485	[0.443; 0.527]
Bukharie, 2009	84	0.643	[0.531; 0.744]
Buzgan, 2010	1028	0.737	[0.709; 0.764]
Colmenero, 1996	530	0.502	[0.458; 0.545]
Fallatah, 2005	159	0.704	[0.627; 0.774]
Gür, 2003	283	0.820	[0.770; 0.863]
Neau, 1997	59	0.153	[0.072; 0.270]
Pourbagher(b), 2006	251	0.845	[0.794; 0.887]
Savas, 2007	140	0.850	[0.780; 0.905]
Shehabi, 1990	106	0.604	[0.504; 0.697]
Troy, 2005	28	0.464	[0.275; 0.661]
Zaks, 1995	90	0.611	[0.503; 0.712]
Random effects model (for age category)		**0.616**	**[0.524; 0.704]**
Random effects model (overall)		**0.648**	**[0.578; 0.715]**

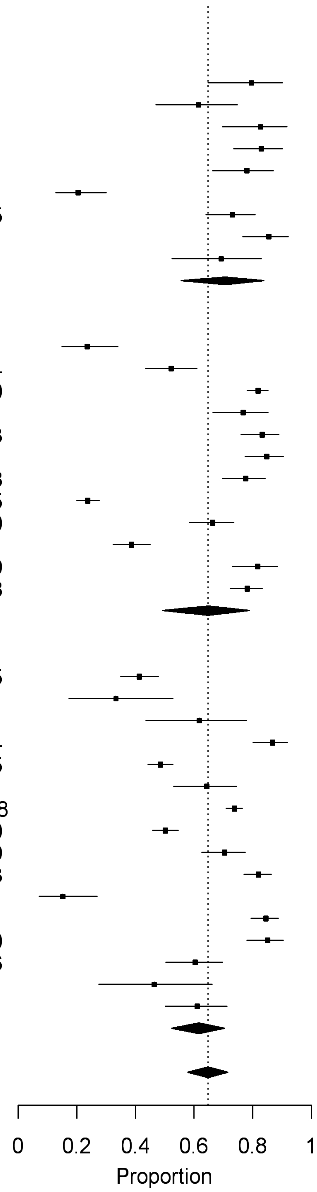


Figure 29: Forest plot for arthralgia

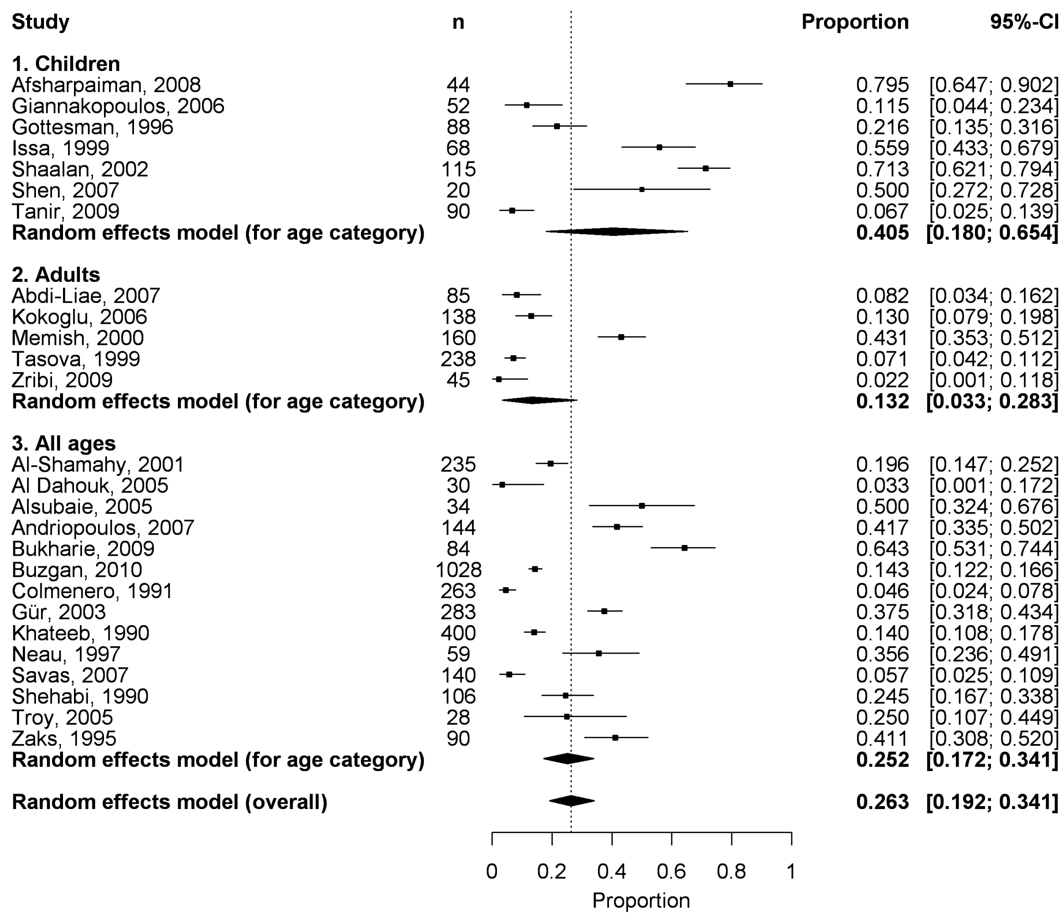


Figure 30: Forest plot for arthritis

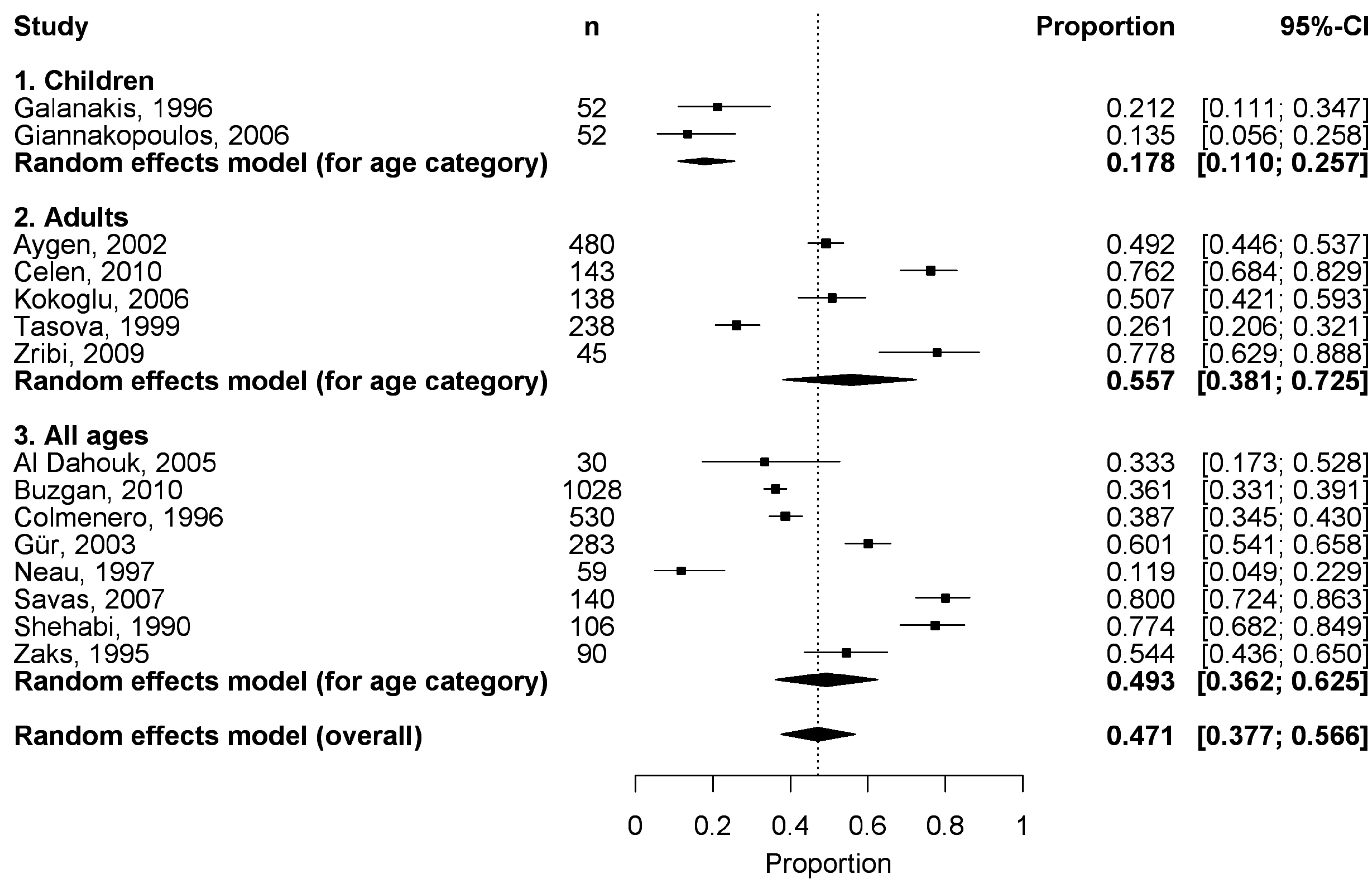


Figure 31: Forest plot for myalgia

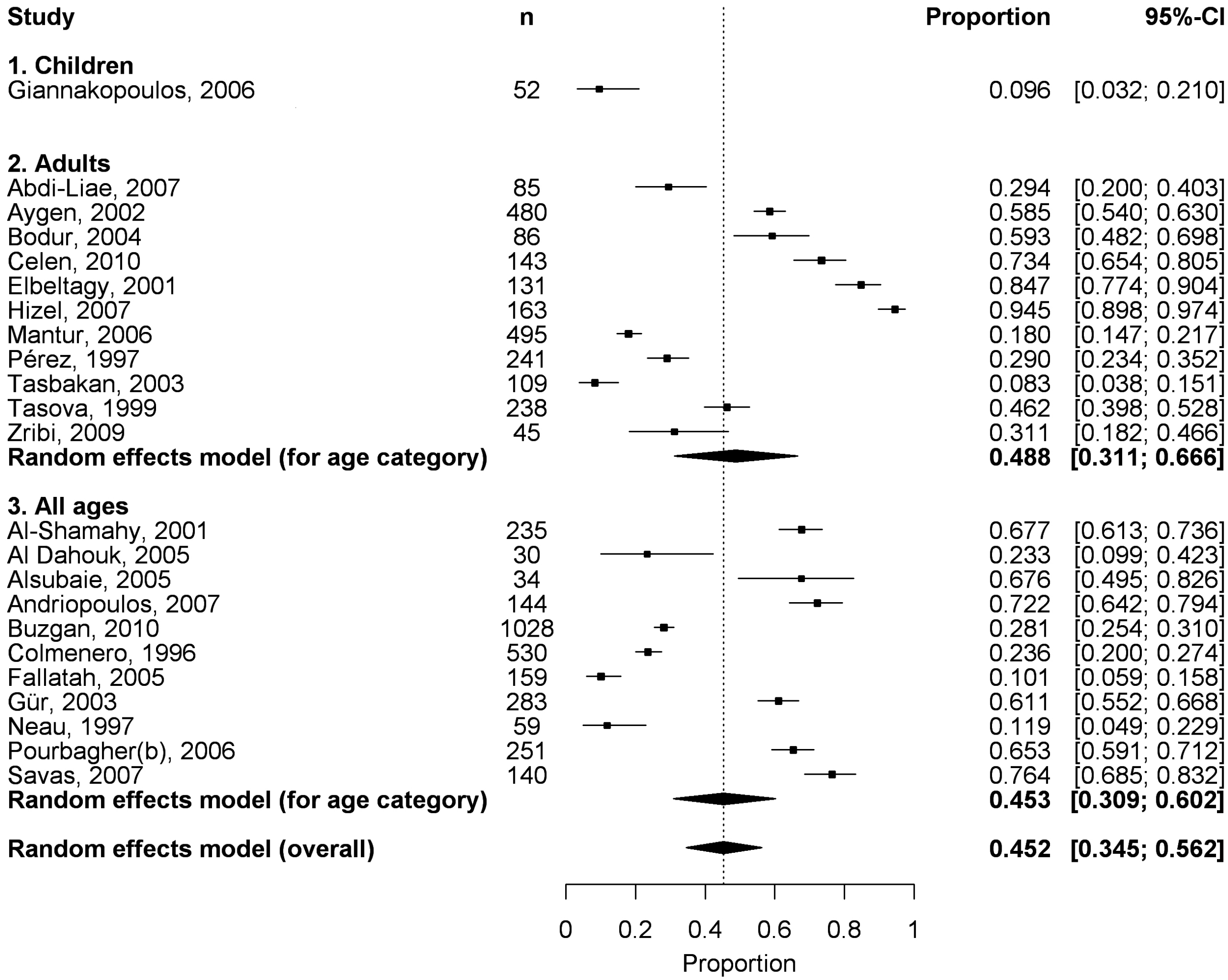


Figure 32: Forest plot for back pain

Study	n	Proportion	95%-CI
1. Children			
Giannakopoulos, 2006	52	0.058	[0.012; 0.159]
Mantur, 2004	93	0.043	[0.012; 0.106]
Shen, 2007	20	0.150	[0.032; 0.379]
Tsolia, 2002	39	0.026	[0.001; 0.135]
Random effects model (for age category)		**0.060**	**[0.030; 0.098]**
2. Adults			
Hizel, 2007	163	0.442	[0.364; 0.521]
Kokoglu, 2006	138	0.304	[0.229; 0.388]
Tasova, 1999	238	0.223	[0.171; 0.281]
Random effects model (for age category)		**0.320**	**[0.198; 0.455]**
3. All ages			
Al Dahouk, 2005	30	0.033	[0.001; 0.172]
Andriopoulos, 2007	144	0.062	[0.029; 0.115]
Buzgan, 2010	1028	0.062	[0.048; 0.079]
Colmenero, 1991	263	0.110	[0.075; 0.155]
Gür, 2003	283	0.382	[0.325; 0.441]
Khateeb, 1990	400	0.062	[0.041; 0.091]
Neau, 1997	59	0.068	[0.019; 0.165]
Pourbagher(a), 2006	251	0.283	[0.228; 0.343]
Savas, 2007	140	0.250	[0.181; 0.330]
Random effects model (for age category)		**0.136**	**[0.068; 0.222]**
Random effects model (overall)		**0.148**	**[0.090; 0.218]**

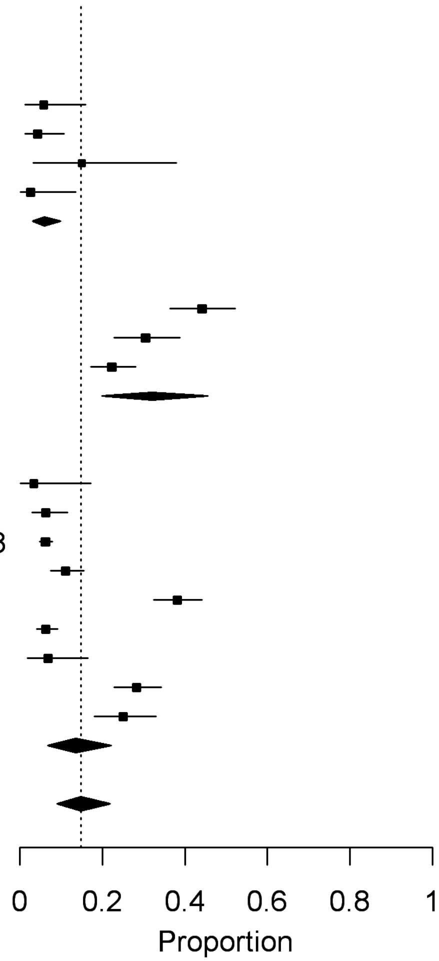


Figure 33: Forest plot for sacroiliitis

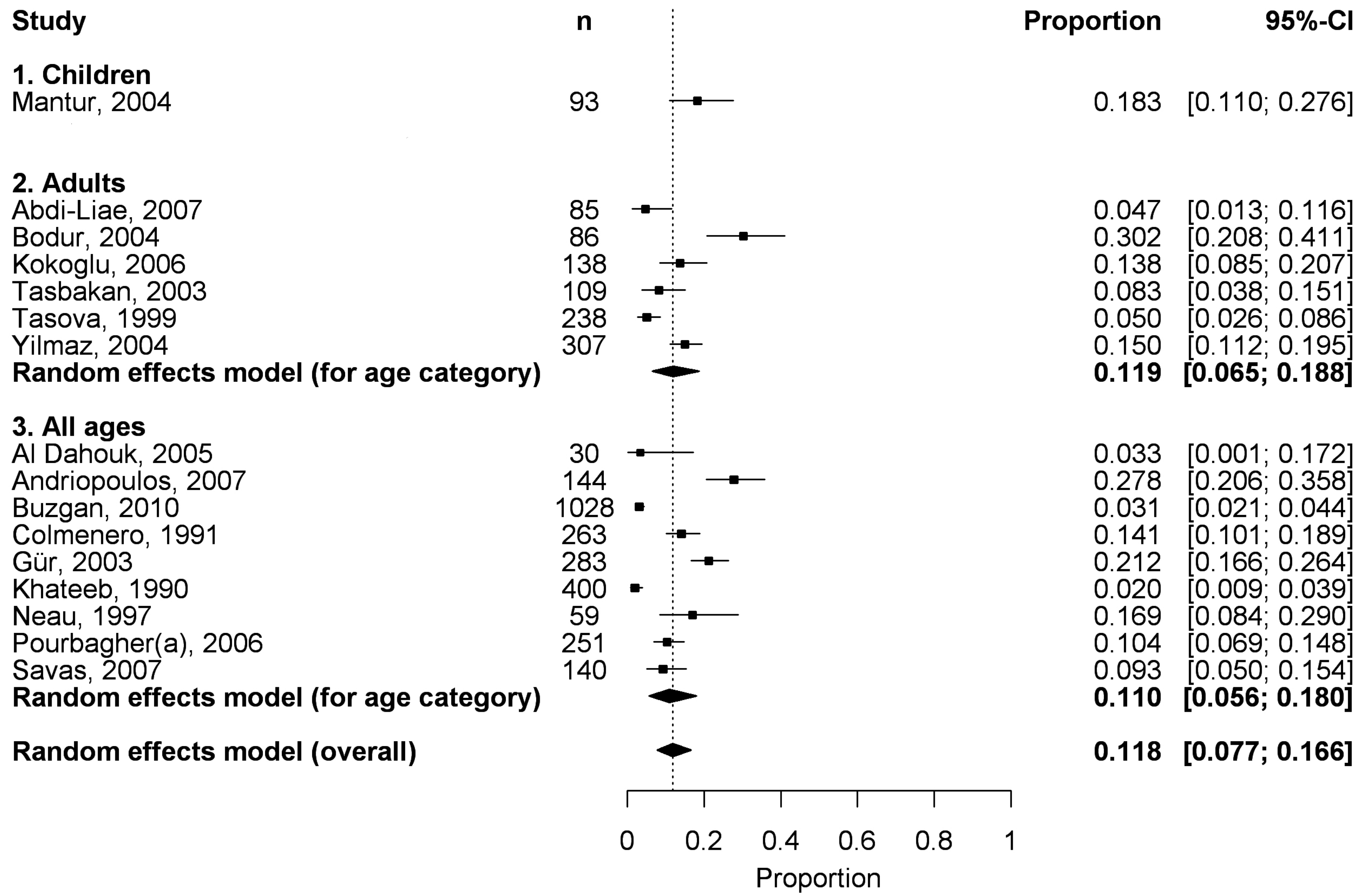


Figure 34: Forest plot for spondylitis

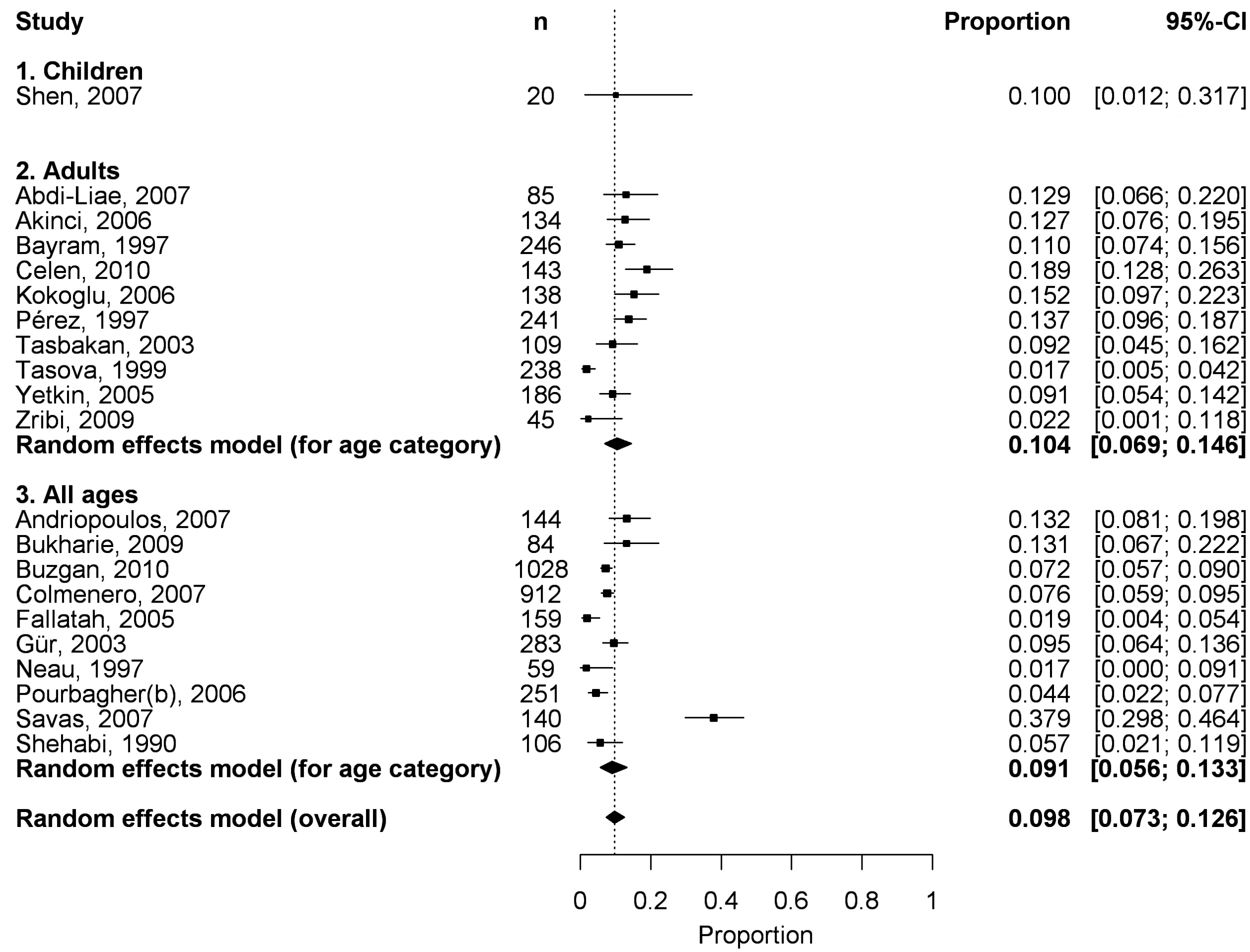


Figure 35: Forest plot for epididymo-orchitis

Study	n	Proportion	95%-CI
1. Children			
Galanakis, 1996	52	0.019	[0.000; 0.103]
Mantur, 2004	93	0.022	[0.003; 0.076]
Random effects model (for age category)		**0.027**	**[0.007; 0.059]**
2. Adults			
Abdi-Liae, 2007	85	0.035	[0.007; 0.100]
Aygen, 2002	480	0.004	[0.001; 0.015]
Celen, 2010	143	0.021	[0.004; 0.060]
Kokoglu, 2006	138	0.014	[0.002; 0.051]
Mantur, 2006	495	0.012	[0.004; 0.026]
Memish, 2000	160	0.019	[0.004; 0.054]
Zribi, 2009	45	0.022	[0.001; 0.118]
Random effects model (for age category)		**0.016**	**[0.009; 0.025]**
3. All ages			
Al Dahouk, 2005	30	0.067	[0.008; 0.221]
Bukharie, 2009	84	0.012	[0.000; 0.065]
Buzgan, 2010	1028	0.007	[0.003; 0.014]
Colmenero, 1996	530	0.015	[0.007; 0.030]
Gür, 2003	283	0.011	[0.002; 0.031]
Neau, 1997	59	0.017	[0.000; 0.091]
Random effects model (for age category)		**0.013**	**[0.007; 0.022]**
Random effects model (overall)		**0.015**	**[0.010; 0.020]**

Figure 36: Forest plot for endocarditis

Study	n	Proportion	95%-CI
1. Children			
Galanakis, 1996	52	0.019	[0.000; 0.103]
Giannakopoulos, 2006	52	0.000	[0.000; 0.068]
Mantur, 2004	93	0.022	[0.003; 0.076]
Shaalan, 2002	115	0.026	[0.005; 0.074]
Tanir, 2009	90	0.022	[0.003; 0.078]
Random effects model (for age category)		**0.024**	**[0.011; 0.041]**
2. Adults			
Abdi-Liae, 2007	85	0.047	[0.013; 0.116]
Aygen, 2002	480	0.065	[0.044; 0.090]
Bodur, 2003	73	0.178	[0.098; 0.285]
Celen, 2010	143	0.056	[0.024; 0.107]
Demiraslan, 2009	917	0.039	[0.028; 0.054]
Kokoglu, 2006	138	0.036	[0.012; 0.083]
Mantur, 2006	495	0.014	[0.006; 0.029]
Memish, 2000	160	0.050	[0.022; 0.096]
Tasbakan, 2003	109	0.009	[0.000; 0.050]
Yetkin, 2006	305	0.066	[0.041; 0.099]
Zribi, 2009	45	0.044	[0.005; 0.151]
Random effects model (for age category)		**0.050**	**[0.033; 0.070]**
3. All ages			
Al Dahouk, 2005	30	0.100	[0.021; 0.265]
Andriopoulos, 2007	144	0.021	[0.004; 0.060]
Bukharie, 2009	84	0.012	[0.000; 0.065]
Buzgan, 2010	1028	0.056	[0.043; 0.072]
Colmenero, 1996	530	0.017	[0.008; 0.032]
Fallatah, 2005	159	0.006	[0.000; 0.035]
Gür, 2003	283	0.071	[0.044; 0.107]
Karaoglan, 2008	300	0.043	[0.023; 0.073]
McLean, 1992	301	0.060	[0.036; 0.093]
Shehabi, 1990	106	0.019	[0.002; 0.066]
Random effects model (for age category)		**0.038**	**[0.023; 0.055]**
Random effects model (overall)		**0.040**	**[0.031; 0.051]**

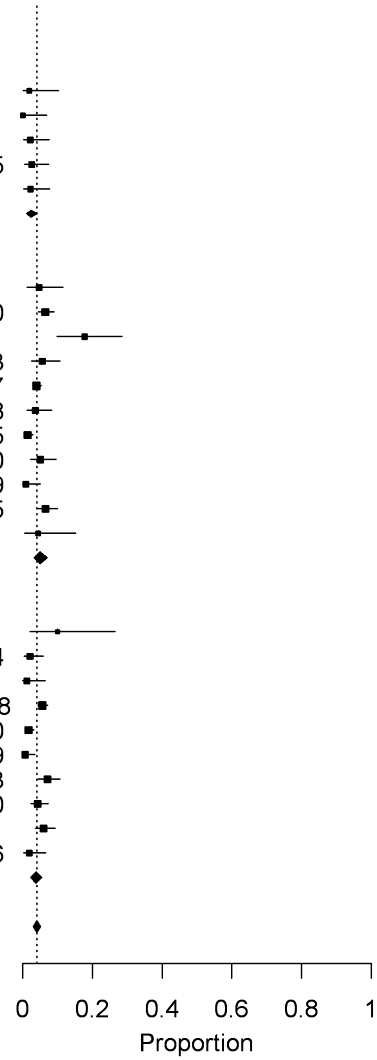


Figure 37: Forest plot for neurological outcomes

Study	n	Proportion	95%-CI
1. Children			
Galanakis, 1996	52	0.115	[0.044; 0.234]
Shaalan, 2002	115	0.009	[0.000; 0.047]
Tsolia, 2002	39	0.051	[0.006; 0.173]
Random effects model (for age category)		**0.054**	**[0.006; 0.144]**
2. Adults			
Aygen, 2002	480	0.002	[0.000; 0.012]
Kokoglu, 2006	138	0.022	[0.005; 0.062]
Mantur, 2006	495	0.036	[0.022; 0.057]
Pérez, 1997	241	0.037	[0.017; 0.070]
Tasbakan, 2003	109	0.018	[0.002; 0.065]
Random effects model (for age category)		**0.022**	**[0.007; 0.046]**
3. All ages			
Al Dahouk, 2005	30	0.300	[0.147; 0.494]
Barroso, 2002	565	0.099	[0.076; 0.127]
Bukharie, 2009	84	0.012	[0.000; 0.065]
Buzgan, 2010	1028	0.007	[0.003; 0.014]
Colmenero, 1996	530	0.023	[0.012; 0.039]
Fallatah, 2005	159	0.013	[0.002; 0.045]
Gür, 2003	283	0.049	[0.027; 0.082]
Hatipoglu, 2005	110	0.100	[0.051; 0.172]
Pourbagher(b), 2006	251	0.112	[0.075; 0.157]
Savas, 2007	140	0.293	[0.219; 0.376]
Shehabi, 1990	106	0.208	[0.135; 0.297]
Random effects model (for age category)		**0.086**	**[0.042; 0.144]**
Random effects model (overall)		**0.060**	**[0.034; 0.092]**

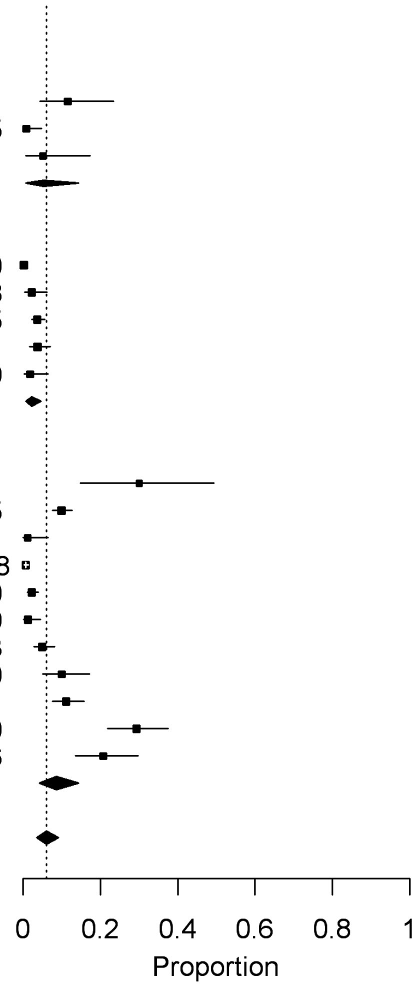


Figure 38: Forest plot for respiratory outcomes

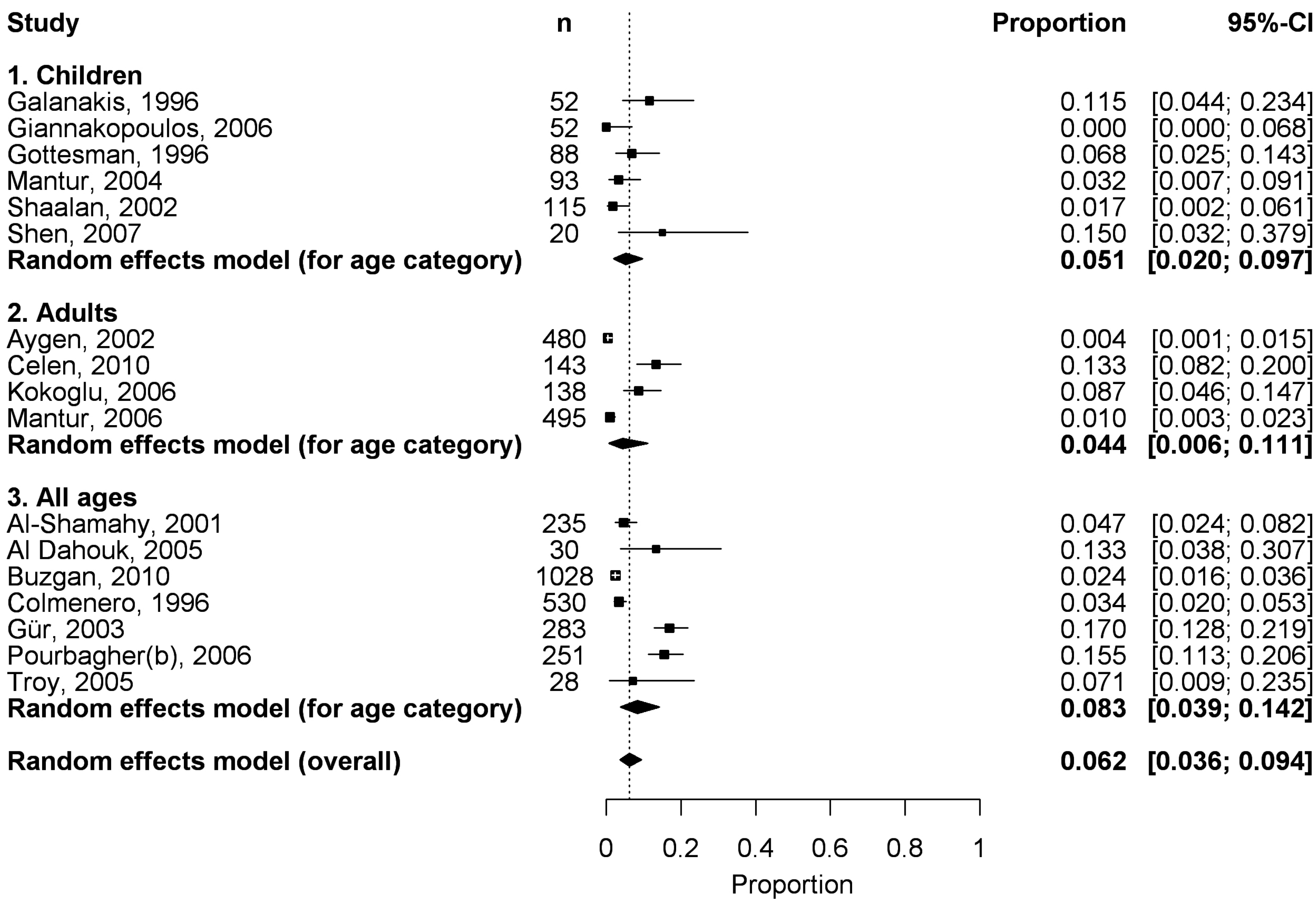


Figure 39: Forest plot for cutaneous outcomes

Appendix 3: Supplementary methods for Chapter 7

Accounting for the order of market visits in the analysis of the Savannah market network

Methods

The Savannah market network was constructed based on the results of the livestock flow simulations. The order in which the markets were visited by each trader was not known but could possibly influence the structure of the network. Potential orders of visits were constructed by running 1,000 stochastic simulations, whilst also ensuring that each scenario was plausible.

This process is best explained using a hypothetical example. Consider a trader who reports purchasing 100 cattle in Market A and 200 in Market B, and selling 10 in Market C and 290 in Market D. The first market visited by this trader must be one where he purchases cattle, i.e. either A or B. If Market B is randomly selected as the first market and 200 cattle are purchased, the second market could theoretically be either a purchase or sale location, i.e. Markets A, C or D. However, Market D is not plausible because the trader does not yet have the 290 cattle that he reports selling there. If Market A is randomly selected as the second visited market, the third market could either be Market C or D. If Market C is randomly selected, the final market visited is D. Therefore, although this trader may sell cattle in Market D which were purchased in the first market that he visited, Market B, these cattle were in fact moved through Markets A and C before reaching their final sale destination.

Results

The results of this ordered analysis are similar to those presented in the manuscript where the market visiting order was not taken into account, except for an increase in the giant strongly connected component (GSCC). As the GSCC is an estimate of the lower bound of the maximum epidemic size for a given disease, an increase in the GSCC reflects a greater potential for spread of the disease to other markets in the network.

Dry season

In the dry season, the market system consisted of 28 markets. They formed a well connected network incorporating all but one of the markets, such that the giant weakly connected component (GWCC) was 27 with most of these markets (median: 20, range: 14-23) forming the GSCC. The majority of markets (17 of 28) received cattle from at least 2 other markets, with a maximum of 10 other markets. Approximately half of the markets (16 of 28) sent cattle to at least two other markets, with a maximum of 11 other markets. However, most cattle movements within the Savannah market network were mediated by a small number of markets: 5 markets accounted for 75.5% and 79.8% of the total weighted in- and out-degrees, respectively.

Wet season

In the wet season, the market system consisted of 26 markets. They formed a well connected network incorporating all but one of the markets, such that the GWCC was 25 with most of these markets (median: 18, range: 13-21) forming the GSCC. The majority of markets (16 of 26) received cattle from at least 2 other markets, with a maximum of 10 other markets. The majority (16 of 26) also sent cattle to at least two other markets, with a maximum of 10 other markets. However, most cattle movements during the wet season within the Savannah market network were mediated by a small number of markets: 5 markets accounted for 77.2% and 79.9% of the total weighted in- and out-degrees, respectively.

Appendix 4: Supplementary results for Chapter 7 – wet season

Wet season empirical data

Of the 226 traders interviewed, 213 also traded in the wet season. Most interviewees (180 of 213) not only acted as traders, but also bought and sold cattle for their own private herds. However, the proportions of purchases and sales that involved their own herds were small. The proportion of purchases that represented cattle taken from their own herds was only 1.5% (IQR: 1.0-2.3%), and the proportion of sales corresponding to cattle being added to their own herds was 1.8% (IQR: 1.1-2.6%).

The distribution of the average number of cattle traded per trader was right-skewed. Compared to the dry season, less cattle were traded with a median of 333 (IQR: 167-660) and a maximum of 2,882. The median numbers of purchase and sale locations of the traders were 4 (IQR:3-5) and 3 (IQR:2-4), respectively, regardless of season, with a maximum of 8. Most of these sites were cattle markets within the Savannah Region. The median number of Savannah markets in which traders operated for purchase or sale was 2 (IQR: 1-4).

Most of the herds (137 of 168) owned by traders from which cattle were being sold were located in the Savannah Region, as well as other areas of Togo (5 of 168), Burkina Faso (22 of 168) and Ghana (4 of 168). Similarly, most of the herds (134 of 167) which received purchased cattle were in the Savannah Region, as well as other areas of Togo (5 of 167), Burkina Faso (22 of 167), Ghana (4 of 167), Benin (1 of 167) and Niger (1 of 167).

Cattle purchases and sales took place in 26 Savannah markets. Nearly three quarters of traders (152 of 213, 71.4%) bought cattle in at least one Savannah market and sold cattle in at least one other Savannah market. Around half of the cattle purchases and sales reported by the traders (53.5% and 51.3%, respectively) took place in Savannah markets, whilst 37.1% and 21.9% of purchases and sales took place in another country. Of the sales and purchases in Savannah markets, 82.4% were conducted in only 4 markets. Outside of Togo, most of the foreign cattle purchases (85.5%) occurred in Burkina Faso and 34.9% of foreign cattle sales took place in Nigeria.

More than half of the traders (116 of 213, 54.5%) operated in at least one other country outside of Togo. Among those traders operating in multiple countries, only one quarter (31 of 116, 26.7%) conducted both purchase and sale activities in at least two countries. Almost two thirds of the traders operating in Burkina Faso (49 of 81, 60.5%) conducted only purchases in Burkina Faso, without any sales. Half (11 of 22, 50.0%) of the traders operating in Ghana, three quarters (25 of 34, 73.5%) of the traders operating in Benin and all (27 of 27, 100%) of the traders operating in Nigeria only sold cattle in these countries, without purchasing. The number of traders operating at different purchase and sale locations as well as the numbers of cattle traded at these sites are summarised in Table A.

Wet season cattle flow simulations

During the wet season, 71.3% (range: 70.0-72.4%) of inflow into the Savannah market system was from Burkina Faso, with 7.9% (range: 7.6-8.3%) from Ghana and 3.1% (range: 2.8-3.5%) from Benin. Half of the cattle leaving the Savannah market system in the dry season (50.7%, range: 48.8-52.8%) were sent to Togolese markets outside of the Savannah Region. In terms of outflow from the Savannah market system, 8.9% (range: 8.3-9.5%) flowed into Ghana, 11.1% (range: 10.1-12.0%) into Benin and 14.1% (range: 13.0-15.2%) into Nigeria. The results of Location Scenario 2 did not demonstrate any major differences, as shown in the supplementary file Appendix 5.

In the wet season, 2,671 (range: 2,543-2,829) cattle flowed into herds in the Savannah Region, equating to 1.6% (range: 1.5-2.1%) of the estimated total cattle population size in the Savannah Region. Given that most herds likely breed their own replacement animals, many more animals flowed in the reverse direction from Savannah herds into the market system. There was a mean of 8,552 cattle (range: 7,899-9,363) leaving herds in the wet season, equating to 6.2% (range: 5.7-6.8%) of the estimated total cattle population size. Location Scenario 2 produced similar results with 2,667 cattle (range: 2,515-2,793) flowing into herds and 8,611 (range: 7,923-9,394) leaving herds. Flows into and out of the Savannah herds followed the same trends as the aforementioned market system flows.

Market network in wet season

The Savannah market system during the wet season consisted of 26 markets. They formed a well connected network incorporating all but one of the markets, such that the GWCC was 25 and nearly half of these markets (13) formed the GSCC. When using the alternative algorithm for reconstructing the order of market visits (see Appendix 3), the GSCC was even higher, with a median of 18 markets (range: 13-22).

The majority of markets (16 of 26) received cattle from at least two other markets, with a maximum of 13 other markets. Approximately half of the markets (14 of 26) sent cattle to at least two other markets, with a maximum of 13. However, most cattle movements within the Savannah market network were mediated by a small number of markets: four markets accounted for 72.8% and 74.4% of the total weighted in- and out-degrees, respectively.

Appendix 5: Supplementary results for Chapter 7 – simulations for wet and dry season

The results of the 10,000 model simulations of livestock flows through the market system in the dry and wet seasons are summarised in the Table 16 below for the independent and non-independent purchase and sale location scenarios, Location Scenario 1 and Location Scenario 2, respectively.

Cattle flow into the Savannah market system: The data represent the mean proportion (%) of the total inflow into the Savannah market system from each of the given locations. The range is given in parentheses as minimum and maximum proportions.

Cattle flow into Savannah herds: The data represent the mean proportion (%) of the total inflow into Savannah herds from each of the given locations. The range is given in parentheses as minimum and maximum proportions.

Cattle flow out of the Savannah market system: The data represent the mean proportion (%) of the total outflow from the Savannah market system going to each of the given locations. The range is given in parentheses as minimum and maximum proportions.

Cattle flow out of Savannah herds: The data represent the mean proportion (%) of the total outflow from Savannah herds going to each of the given locations. The range is given in parentheses as minimum and maximum proportions

Table 16: Results of simulations of livestock flows

Cattle Flows	Origin or Destination								
	Savannah herds	Savannah butchers	Other Togo markets	Other Togo herds	Benin	Burkina Faso	Ghana	Niger	Nigeria
Location Scenario 1 - Dry Season									
Into Savannah market system	14.3 (11.9-17.7)	0 (0-0)	6.2 (5.8-6.9)	0.3 (0.2-0.3)	3.6 (3.1-4.7)	68 (65.2-70.2)	7.6 (7.2-8.0)	0	0
Out of Savannah market system	5.7 (5.2-6.3)	6.2 (5.6-6.9)	49.3 (47.0-51.7)	0.1 (0.0-0.1)	12.3 (11.1-13.8)	3.9 (3.5-4.4)	7.8 (7.3-8.4)	0.0 (0.0-0.1)	14.7 (13.5-15.9)
Into Savannah herds	18.1 (13.3-24.6)	0 (0-0)	6.4 (4.8-8.2)	0.2 (0.0-0.5)	5.7 (4.1-7.9)	64.3 (58.8-69)	5.3 (4.0-7.0)	0	0
Out of Savannah herds	4.0 (2.5-6.4)	4.9 (2.6-8.2)	46.4 (39.4-54.7)	0.0 (0.0-0.1)	21.4 (16.0-25.7)	1.5 (0.9-2.3)	4.0 (2.6-5.8)	0.1 (0.0-0.2)	17.7 (11.4-25.5)
Location Scenario 1 - Wet Season									
Into Savannah market system	11.6 (10.6-13.0)	0	5.7 (5.4-6.0)	0.3 (0.3-0.4)	3.1 (2.8-3.5)	71.3 (70.0-72.4)	7.9 (7.6-8.3)	0	0
Out of Savannah market system	5.9 (5.4-6.4)	6.5 (5.8-7.2)	50.7 (48.8-52.8)	0.1 (0.1-0.2)	11.1 (10.1-12.0)	2.7 (2.2-3.2)	8.9 (8.3-9.5)	0.0 (0.0-0.1)	14.1 (13.0-15.2)
Into Savannah herds	13.7 (11.0-16.8)	0 (0-0)	6.3 (4.7-7.9)	0.2 (0.0-0.6)	5.6 (4.2-7.2)	68.5 (64.9-72.1)	5.7 (4.1-7.6)	0	0
Out of Savannah herds	4.3 (3.5-5.2)	6.2 (5.3-7.3)	48.5 (44.6-52.1)	0.0 (0.0-0.1)	19.1 (16.5-22.1)	1.3 (0.9-1.8)	5.8 (5.0-6.8)	0.1 (0.0-0.2)	14.6 (13.2-16.4)

Location Scenario 2 - Dry Season									
Into Savannah market system	17.6 (14.7-21.8)	0	6.7 (6.0-7.7)	0.2 (0.2-0.3)	2.0 (1.5-3.0)	65.6 (61.9-68.3)	7.9 (7.4-8.4)	0	0
Out of Savannah market system	7.5 (6.5-8.6)	7.4 (5.7-8.8)	47.9 (40.8-54.9)	0	13.1 (9.9-16.8)	1.7 (1.4-2.2)	6.8 (4.4-9.6)	0.0 (0.0-0.1)	15.5 (10.8-20.2)
Into Savannah herds	22.9 (16.1-29.7)	0	5.0 (3.2-6.9)	0.2 (0.0-0.5)	1.6 (0.6-3.0)	64.7 (56.7-70.7)	5.7 (3.8-7.7)	0	0
Out of Savannah herds	5.0 (3.1-7.9)	5.6 (3.4-9.3)	47.0 (39.7-59.0)	0.0 (0.0-0.1)	19.9 (13.0-25.6)	0.6 (0.4-1.1)	3.7 (2.0-6.3)	0.0 (0.0-0.1)	18.1 (10.8-26.3)
Location Scenario 2 - Wet Season									
Into Savannah market system	14.4 (13.1-16.2)	0	6.0 (5.5-6.3)	0.3 (0.3-0.4)	1.4 (1.2-1.6)	69.6 (68.0-71.2)	8.3 (7.8-8.8)	0	0
Out of Savannah market system	8.0 (6.9-9.0)	7.8 (6.5-9.3)	49.9 (43.4-55.5)	0.0 (0.0-0.1)	11.8 (9.0-13.9)	0.5 (0.2-0.9)	7.9 (5.1-10.8)	0.0 (0.0-0.1)	14.1 (9.4-19.1)
Into Savannah herds	18.2 (14.5-23.0)	0	4.6 (3.1-6.0)	0.2 (0.0-0.6)	1.0 (0.4-1.8)	69.7 (64.3-74.6)	6.2 (4.3-8.1)	0	0
Out of Savannah herds	5.7 (4.5-7.1)	7.2 (6.0-8.7)	48.9 (44.0-54.0)	0.0 (0.0-0.1)	17.5 (14.3-21.2)	0.2 (0.0-0.5)	5.4 (3.5-7.5)	0.0 (0.0-0.1)	15.0 (11.6.19-0)

Appendix 6: Newspaper article in Togo-Presse (November 2012)

SANTE ANIMALE

A la traque de la brucellose bovine au Togo

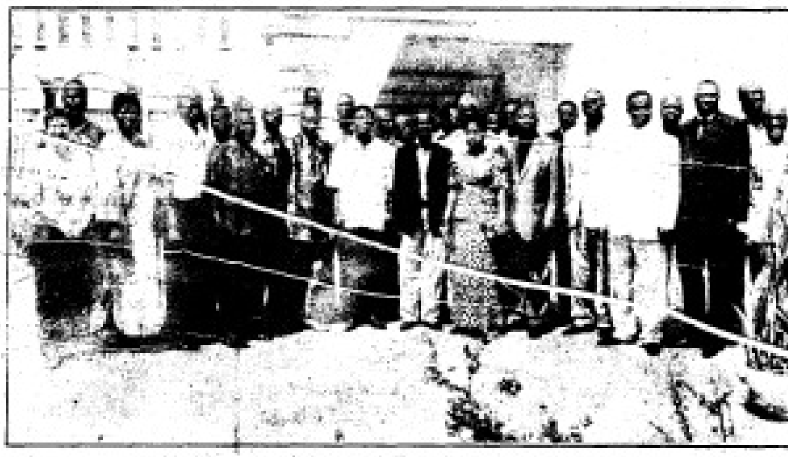
Un atelier de restitution des résultats de l'étude sur la brucellose bovine dans la Région des Savanes s'est tenu, hier, au centre de formation de la FOPADESC à Agoènyivé, Lomé. La rencontre, inclusive, a réuni des acteurs de premier plan impliqués dans cette étude notamment, des chercheurs, des médecins, des vétérinaires, les préfets de la Région et des éleveurs venant de la Suisse, de l'Australie, du Cameroun, de la Côte d'Ivoire et du Togo.

De plus en plus, on note des mouvements de bétail des pays du Sahel, confrontés au problème désertique, vers le Togo qui connaît une très faible capacité d'accueil. Dans ces mouvements, on rencontre la circulation de maladies dont certaines sont communes aux animaux et aux hommes, qui entraînent des pertes économiques.

Sur la base de ces observations, l'étude sur l'épidémiologie des zoonoses comme la brucellose, dont les résultats ont fait l'objet d'un atelier de restitution, hier à Lomé, a orienté ses recherches sur l'existence ou non de ces maladies dans la zone de transhumance circonscrite, notamment la Région des Savanes.

L'autre volet ayant motivé cette étude, a laissé entendre le Prof. Bassirou Bonfoh, directeur général du Centre Suisse de Recherches Scientifiques à Abidjan en Côte d'Ivoire, était de voir ce que représente cette transhumance pour l'économie du Togo et pour sa sécurité alimentaire. « *Les résultats de cette étude vont servir aux scientifiques pour de nouvelles questions de recherche et permettront ensuite de voir, avec le niveau d'infection qu'elle a établi, quels programmes mettre en place pour limiter l'évolution de l'infection* », a-t-il signifié.

La brucellose est une maladie infectieuse (bactérienne) commune aux hommes et aux animaux. Elle peut se transmettre notamment à l'homme par la consommation des produits laitiers crus et par le contact direct avec un animal infecté. La recherche, a expliqué le Dr vétérinaire Anna Dean de l'Institut Tropical et de Santé Publique Suisse, « *apporte une contribution à la compréhension de l'épidémiologie de zoonoses négligées au Togo et en Afrique de l'Ouest, en montrant que des zoonoses majeures (telle que la brucellose) circulent dans la Région des Savanes* ». Elle contribue, a-t-elle en outre souligné, au développement des filières animales et apporte des informations utiles au développement des politiques. « *Nous avons montré et modélisé pour la première fois l'étendue du commerce transfrontalier de bovins au nord du Togo, y compris le risque de transmission de maladies infectieuses* », a précisé Mme Dean.



Vue des participants à l'atelier. (Photo AKOUA/SBI)

L'étude a été menée entre 2011 et 2012 dans 25 villages de la Région des Savanes, choisis au hasard, à partir des prises de sang sur des personnes, des bovins, de petits ruminants, ainsi que des bovins transhumants originaires du Burkina Faso et des commerçants de bovins dans neuf principaux marchés de la région.

Martial Kokou KATAKA

Appendix 7: Photos from the field

Figure 40: The author collecting blood from a cow

Figure 41: The author collecting hygroma fluid from a cow

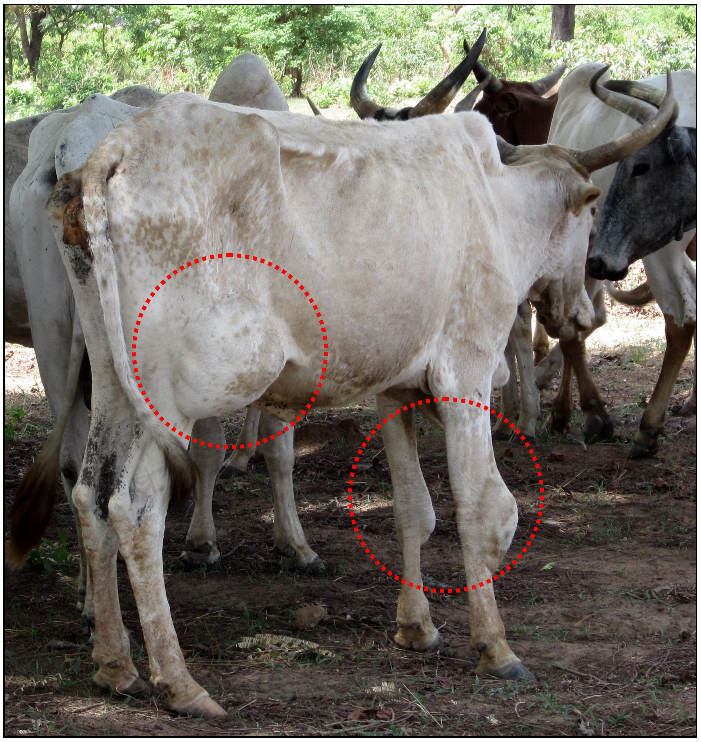


Figure 42: A cow with joint hygromas in right stifle and left and right carpi

Figure 43: The author collecting blood from a goat

Figure 44: Interviewers and nurse visiting households

Figure 45: The author conducting a focus group discussion with Fulani women (Côte d'Ivoire)

Figure 46: Cattle roaming freely around a village

Figure 47: Small ruminants roaming freely between households

Figure 48: Cattle market on the Togo / Burkina Faso border

Figure 49: Cattle market

Figure 50: Temporary housing of transhumant herdsmen during the dry season

Figure 51: ECOWAS International Transhumance Certificate

Figure 52: District health centre

Figure 53: View over the Savannah Region of Togo during dry season

Curriculum Vitae

Anna Dean

Education

2014 **PhD in Epidemiology**
Swiss Tropical and Public Health Institute and University of Basel, Switzerland
Thesis: Epidemiology of brucellosis and Q fever in Togo and the risk of disease spread through cattle trade in West Africa

2009 **Membership of the Australian and New Zealand College of Veterinary Scientists (ANZCVS) - Epidemiology Chapter**
Admitted as a member by way of formal written and oral examination

2008 **Master of International Public Health, Honours**
University of Sydney, Australia
Thesis: Vaccine effectiveness against influenza-like illness during influenza outbreaks in five aged-care facilities in 2007

2003 **Bachelor of Veterinary Science, First Class Honours**
University of Sydney, Australia

Work Experience

2013-present Epidemiologist - Tuberculosis Drug Resistance Surveillance
World Health Organization (WHO), Headquarters
Geneva, Switzerland

2008-2009 Epidemiologist - Zoonotic Diseases
World Health Organization (WHO), Country Office for Vietnam
Hanoi, Vietnam

2008 Epidemiologist - Influenza
World Health Organization (WHO), Regional Office for the Western Pacific
Manila, Philippines

2004-2008 Veterinarian
North Shore Veterinary Hospital
Sydney, Australia

2006 Veterinarian
Vets Beyond Borders
Sikkim, India

2004 University Tutor
The Women's College, University of Sydney
Sydney, Australia

Publications

Journal Articles

Dean AS**,** Zignol M, Falzon D, Getahun H, Floyd K. (2014, *in press*). HIV and multidrug-resistant TB: overlapping epidemics. *European Respiratory Journal*.

Dean AS, Schelling E, Bonfoh B, Kulo AE, Boukaya GA, Pilo P. (2014, *in press*). Deletion in the gene BruAb2_0168 of *Brucella abortus* strains: diagnostic challenges. *Clinical Microbiology and Infection.*

Zignol M, Dara M, Dean AS, Falzon D, Dadu A, Kremer K, Hoffman H, Hoffner S, Floyd K. (2014, *in press*). Drug-resistant tuberculosis in the WHO European Region: An analysis of surveillance data. *Drug Resistance Updates*.

Dean AS, Fournié G, Kulo AE, Boukaya GA, Schelling E, Bonfoh B. (2013). Potential risk of regional disease spread in West Africa through cross-border cattle trade. *PLoS One* 8(10): e75570. doi:10.1371/journal.pone.0075570

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Booy R, Lindley RI, Dwyer DE, Jin JK, Heron LG, Moffatt CRM, Chiu CK, Rosewell AE, Dean AS, et al. (2012). Treating and Preventing Influenza in Aged Care Facilities: A Cluster Randomized Controlled Trial. *PLOS One* 7(10): e46509. doi:10.1371/journal.pone.0046509

Dean AS, Moffat CRM, Rosewell A, Dwyer DE, Lindley RI, Booy R, MacIntyre CR. (2010). Incompletely matched influenza vaccine still provides protection in frail elderly. *Vaccine* 28(3): 864-867. doi: 10.1016/j.vaccine.2009.03.024

Book Chapter

Dean AS, Schelling E, Zinsstag J. (2014, *in press*). 'Brucellosis' in McDowell MA and Rafati S (eds), *Neglected Tropical Diseases – Middle East and North Africa*, Springer.

Conference Presentations

2012 Dean AS, Kulo A, Bonfoh B, Boukaya A, Amidou M, Schelling E (October 2012). *A One Health approach to the ecology of zoonotic diseases in Togo*. Poster presentation, 4th Biennial Conference of International Association for Ecology and Health (EcoHealth). Kunming, China.

2012 Dean AS, Kulo A, Boukaya A, Bonfoh B, Schelling E (August 2012). *Livestock movements and contact patterns in Togo: implications for disease control*. Oral presentation, 13th International Symposium on Veterinary Epidemiology and Economics. Maastricht, Netherlands.

2012 Dean AS, Kulo A, Boukaya A, Bonfoh B, Schelling E (August 2012). *Epidemiology of brucellosis and Q fever in rural, northern Togo.* Poster presentation, 13th International Symposium on Veterinary Epidemiology and Economics. Maastricht, Netherlands.

2011 O'Donnell J, Philips H, Officer K, Dean AS (May 2011). *Sustainable dog management in developing countries*. Oral presentation, Australian Veterinary Association (AVA) Annual Conference. Adelaide, Australia

2008 Dean A, MacIntyre CR, Moffatt CRM, Dwyer DE, Booy R (March 2008). *Vaccine effectiveness against influenza-like illness during influenza outbreaks in five aged-care facilities in 2007.* Poster presentation, 10th International Symposium on Respiratory Viruses. Singapore.

2007 Dwyer D, Moffat C, Heron L, Dean A, Rosewell A, MacIntyre R, Lindley R, Booy R (October 2007). *Active surveillance and point-of-care testing (POCT) allows early intervention for influenza outbreaks in nursing homes.* Poster presentation, 45th Annual Meeting of the Infectious Diseases Society of America. San Diego, USA.

Academic Awards

2002 Australian Small Animal Veterinary Association Prize in Medicine and Surgery, University of Sydney

Stewart Prize in Veterinary Medicine, University of Sydney

2001 Australian College of Veterinary Scientists Prize in Veterinary Pharmacology and Toxicology, University of Sydney

Australian Veterinary Association Prize for Undergraduates in Veterinary Pathology, University of Sydney

C.W. Emmens Prize in Veterinary Physiology, University of Sydney

L.G. Webb Prize for Veterinary Science, The Women's College, University of Sydney

2000	Brenda Mitchell Prize for Veterinary Science, The Women's College, University of Sydney
1999-2001	Dean's List for Academic Excellence, University of Sydney

Leadership

2008-2009	**Australian Youth Ambassador for Development:** Hanoi, Vietnam An international volunteer program of the Australian government's overseas aid agency
2006-present	**Vets Beyond Borders:** Sikkim, India and Sydney, Australia A not-for-profit organisation aiming to build sustainable veterinary services in developing communities President (2009-2010), Board Member (2007-2010), Treasurer (2007-2008), Veterinary Committee Member (2006-present)