Seroprevalence and factors associated with Q fever and Rift Valley fever in goats in Moretele municipality, South Africa

by

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Declaration

I, Rungano Magadu, do hereby declare that the research presented in this dissertation, was conceived and executed by myself, and apart from the necessary guidance from my supervisor, I have received no assistance from any other person or institution. No aspect of the substance, nor any part of this dissertation has been submitted in the past, or is to be submitted for another degree at this or any other University. This dissertation is presented in partial fulfilment of the requirements for the degree MMedVet, Bovine health and Production. I hereby grant the University of Pretoria free license to reproduce this dissertation; in part or as whole, for the purpose of research or continuing education.

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Date_____

Ethics statement

All aspects of this research protocol were approved by the University of Pretoria Animal Ethics committee (Approval number V001-18; appendix 1), The University of Pretoria Faculty of Humanities Research Ethics committee (Approval number GW20170928HS; appendix 2) as well as the Department of Agriculture, Forestry and Fisheries (Approval numbers 12/11/1/1/6 and 12/5/1; appendices 3 and 4).

Dedication

I would like to dedicate this dissertation to my lovely wife Kundai, and my ever-supportive parents Forbes and Evelyn Magadu for always believing in me and supporting me. You are my perpetual source of inspiration.

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Contents

List of abbreviations

AGID	agar gel immunodiffusion
BTM	bulk tank milk
CFT	complement fixation test
CFU	colony forming unit
DIC	disseminated vascular coagulation
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
HAI	haemagglutination inhibition
HGT	haemagglutination
ID	immunodiffusion
i-ELISA	indirect enzyme-linked immunosorbent assay
IFAT	immunofluorescence antibody test
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IP	immunoperoxidase
LCV	large colony variant
LPS	lipopolysaccharide
MIPLD	mouse intra-peritoneal lethal dose
NSS	non-structural protein
ORF	open reading frame
PCR	polymerase chain reaction
Q fever	“query” fever
qPCR	quantitative polymerase chain reaction
recNP	recombinant nucleoprotein
RNA	ribonucleic acid
RT-PCR	reverse transcriptase polymerase chain reaction
RTD-PCR	real-time detection polymerase chain reaction
RT-LAMP	reverse transcriptase loop mediated isothermal amplification
RVF	Rift Valley fever
sAg-ELISA	surface antigen enzyme-linked immunosorbent assay
SCV	small cell variant
SDC/SLP	small dense cell / spore-like particles

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Abstract

SEROPREVALENCE AND FACTORS ASSOCIATED WITH Q FEVER AND RIFT VALLEY FEVER IN GOATS IN MORETELE MUNICIPALITY, SOUTH AFRICA

by

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Q fever is a zoonotic disease caused by *Coxiella burnetii* which infects humans and a wide range of hosts including birds, mammals, ticks, fish and reptiles. Rift Valley fever (RVF) virus (RVFV) is a mosquito-borne pathogen of livestock, wildlife and humans and is found throughout much of sub-Saharan Africa, occasionally causing large scale abortions and mortality. There is little detailed knowledge of the distribution and level of occurrence of these two pathogens in South Africa. The aim of this study was to estimate the prevalence of antibodies against *C. burnetii* and RVFV in goats in Moretele municipality, and to identify factors associated with seropositivity to the two zoonotic pathogens.

Multi-stage random sampling was conducted and sera were collected from 216 goats in 32 goat herds. A questionnaire was completed for each participating goat owner to collect information on potential animal, management and environmental risk factors, as well as potential animal health consequences of the two infections. Rift Valley fever virus antibody testing was done by ID Screen® Rift Valley Fever Competition ELISA test kit (IDVet, Grabels, France) and *C. burnetii* antibody testing was done by LSIVET™ Ruminant Q Fever - Serum/Milk ELISA test kit (Life Technologies, Carlsbad, California, USA) with confirmation using a virus neutralisation test (VNT)**.**

32/216 goats tested positive for *C. burnetii* antibodies and the overall seroprevalence, adjusted for clustering and sampling weights was 16% (95% CI: 10.6-23.5%). The intraclass correlation co-efficient (ICC) was 0.06, indicating low to moderate clustering within herds. Multiple logistic regression revealed age as the only factor that was significantly associated with seropositivity to *C. burnetii*, with a higher seroprevalence in animals ≥2 years of age (26%) than in animals ≤6 months of age (6%) (odds ratio (OR) = 6.6; 95% CI: 1.6-26.7; *P* = 0.010). Regarding potential consequences of infection, females with a history of abortion were more likely to be seropositive (OR = 4.6; 95% CI: 1.1-20.2; *P* = 0.043). Goats in herds that reported >2 abortions in the 12 months prior to sampling tended to have a higher odds of seropositivity than animals in herds with no reported abortions (OR = 2.5; 95% CI: 0.9-6.8; *P* = 0.071). 1/216 goats tested ELISA-positive for RVF virus antibodies and 3 samples were “doubtful”; however, they all tested VNT-negative. The estimated seroprevalence of RVFV was therefore 0% (95% CI: 0.0-1.4%).

In this study, the herd seroprevalence of *C. burnetii* was high at 51% and there was possibility that *C. burnetii* is a likely cause of abortions in goats in Moretele municipality of South Africa. Seropositivity to RVFV could not be demonstrated in this study; but if present, the virus is likely to be circulating at very low levels.

1. Introduction

Diseases in animals and plants can have an impact on human health, and approximately 60% of new human infections in the past 25 years can be attributed to pathogens originating from domestic and wild animals (Brownlie et al. 2006; Cutler et al. 2010). The complex epidemiology of most zoonotic diseases makes them difficult to eradicate and early diagnosis, health education, improved food hygiene and close coordination between medical and veterinary departments will aid in reducing the incidence and prevalence of zoonotic diseases worldwide (Pal 2005). Zoonoses form a large component of the WHO programme on the control of neglected zoonotic diseases (The Control of Neglected Zoonotic Diseases 2014) which comprise over 250 diseases responsible for considerable morbidity and mortality globally and hence the need for a One Health approach in their control (Gayer et al. 2007; Pal 2005).

Zoonotic diseases can be arbitrarily classified into several different categories which include endemic zoonoses, epidemic zoonoses, emerging zoonoses and old zoonoses among others and one disease can be present in more than 1 category. Endemic zoonoses have the greatest impact in developing countries including South Africa (Hatchette et al. 2003; International Livestock Research Institute 2012). Endemic zoonoses are ever present in particular geographic locations and of all the categories of zoonoses, these have the biggest sustained impact on human and animal health and welfare, Furthermore, these zoonoses include diseases such as tuberculosis, brucellosis, Q fever and salmonellosis among others (Gayer et al. 2007)(International Livestock Research Institute 2012). Epidemic zoonoses on the other hand are those diseases which have an “outbreak” type occurrence with the number of human and animal cases spiking periodically before returning to low level prevalence or complete absence after the outbreak control. This class of zoonoses include diseases such as Rift Valley fever (RVF), leishmaniosis, anthrax and rabies, among others (International Livestock Research Institute 2012; Onesmo 2013).

Traditional slaughter of animals in South African communal areas is linked with celebratory functions such as weddings, ancestral appeasement as well as funerals and the slaughter procedures pose a risk to personnel involved in the slaughter process (Qekwana and Oguttu 2014). The provisions of the Meat safety act (Act 40, 2000; Department of Agriculture, Republic of South Africa) allow for the informal slaughter and consumption of livestock wherein cattle, sheep, goats and chickens can be slaughtered for consumption under rural settings. Goats are the domestic species with the highest slaughter statistics and approximately 38% of goat

production in South Africa undergo traditional slaughter in rural areas (Braker et al. 2002; Qekwana and Oguttu 2014). As of 2012, the goat population in South Africa was estimated to be 2.033 million but less than 0.5% of this total figure (less than 10 165) are slaughtered annually at registered abattoirs (Qekwana and Oguttu 2014). Despite the significant informal slaughter statistics of goats in South Africa, there is very little knowledge of the need for pre-slaughter health declaration of stock according to an interview survey conducted by Qekwana and Oguttu (2014). In this survey, 21% of the respondents affirmed that they conduct pre-purchase examinations of their stock, but this is based on traditional indigenous knowledge as opposed to systematic and objective meat safety regulations (Qekwana and Oguttu 2014).

Lifestyle and occupation can greatly amplify the risk of individuals contracting zoonotic diseases, and it is expected that people who interact directly with animals and their products are at increased risk of exposure. Occupations such as being veterinarians, para-veterinarian, abattoir workers and livestock owners and/or handlers are more likely to be infected with zoonoses by virtue of their continued close proximity to animals (Dowd et al. 2013; Kahn 2006). Sporadic outbreaks of RVF in South Africa in 2009-10 and widespread epidemics in 2010-11 resulted in 25 human deaths, and most of the infected individuals were working on farms, in the animal health sector or meat industries. Most of these human cases were infected by direct contact with animal tissues and body fluids or in direct contact with mosquitoes which played a minor role (Archer et al. 2013). A study in Japan found that animal health workers, i.e. veterinarians, had antibody titres for *C. burnetii* that were significantly higher than medical workers and blood donors. Another study in the USA also showed that agricultural workers were six times more likely to have antibodies to *C. burnetii* compared to other professions (Abe et al. 2001; Walsh 2012).

Moretele municipality is a largely communal area that is located about 40 km north-west of the University of Pretoria, Faculty of Veterinary Science. It comprises villages such as Mothle, Ratjiepane, Makapanstad, Mathibestad and Dikebu which are visited weekly by the Production Animal Mobile Outreach Clinic (PAMOC). The PAMOC engages the community in primary animal health care for pets and production animals at low cost as part of its community engagement obligations. Moretele municipality is a peri-urban communal area with many subsistence farmers, with goats being the most populous ruminant species. Moretele municipality is mostly rural and most people live below the poverty line, so there is very little expenditure on primary animal health care, such as supplementary feeding, parasite control and vaccination among others. Also, considering the slaughter practices and minimal to lack

of meat safety procedures, limited knowledge of animal diseases and zoonotic risks as well as limited to lack of personal protective equipment when handling infected animals, zoonotic pathogens may be transmitted at handling and slaughter of infected animals. In this study we investigate the seroprevalence of two zoonotic diseases, an endemic zoonotic disease, Q fever, and an epidemic zoonotic disease, RVF.

2. Literature review: Q fever

Q fever is a febrile zoonotic disease caused by the pleiomorphic Gram-negative bacteria *Coxiella burnetii* (Parker et al. 2006; Woldehiwet 2004). The disease is present throughout the world and was first documented in the 1930s in abattoir workers in Queensland, Australia and at that time, the aetiological agent was unknown. The disease was then named Q fever which was short for “query fever” (Waag 2007). The term “Q fever” for query fever was coined by Edward Derrick in 1937 as the infectious agent had not been demonstrated, and although the nature of the pathogen is now well documented, the name Q fever persists because of the popularity of the name among researchers (Miguel G. Madariaga et al. 2003; Parker et al. 2006).

When the causative bacterium of Q fever was then identified, it was named *Rickettsia diaporica* but eventually changed to *C. burnetii* in honour of Drs Macfarlane Burnet and Herald Rea Cox in recognition of their work in isolation of the Q fever causative agent (Waag 2007). The deadly nature of Q fever is shown by previous and ongoing research into its use as a biological weapon as it causes debility, can be mass propagated, and is stable under conditions of mass propagation, storage and transportation (Miguel G. Madariaga et al. 2003). *C. burnetii* is classed as a category B biological terrorist agent as opposed to class A biological terrorist agent such as anthrax, smallpox, botulism, tularemia and the viral haemorrhagic fevers, despite its potential for large scale propagation and distribution (Miguel G. Madariaga et al. 2003). When aerosolized, a single organism is sufficient to initiate disease in humans and the severity of the disease it causes is mild in most cases. This means by the time an incident is recognised, several individuals will have been affected (Miguel G. Madariaga et al. 2003).

2.1. Aetiology

The aetiological agent for Q fever is the Gram-negative coccobacillus, *C. burnetii*, which is an obligate intracellular pathogen of 0.8 - 1.0 µm length and 0.3 - 0.5 µm width (Angelakis and Raoult 2010; Arricau-Bouvery and Rodolakis 2005; Marrie 1990). *C. burnetii* is non-motile and, although its life cycle is not completely understood, it is believed to be completed in the in the phagosomes of infected eukaryotic cells, primarily macrophages (Waag 2007; Woldehiwet 2004). *C. burnetii* possesses lipopolysaccharide in its cell membrane similar to Gram-negative bacteria but it stains variably with the gram staining technique and better staining results are obtained with the Gimenez staining method (Anastácio et al. 2016; Arricau-Bouvery and Rodolakis 2005). *C. burnetii* was formerly classified in the Family Rickettsiaceae, tribe

Rickettsiae but recent gene sequence analysis classifies *Coxiella* into Order Legionellales, family Coxiellaceae (Arricau-Bouvery and Rodolakis 2005). *C. burnetii* replicates to high numbers but it has a slow doubling time of between 12-20 hours (Woldehiwet 2004). Sequencing of the *C. burnetii* nine-mile strain suggests circular topography of the bacterial chromosome (Arricau-Bouvery and Rodolakis 2005).

C. burnetii can be cultured in chicken embryo yolk sacs and cell cultures while recent lab studies on the physiology of *C. burnetii* have improved its isolation on axenic media (Arricau-Bouvery and Rodolakis 2005; Sanchez et al. 2018). *C. burnetii* grows to high titres in placental tissue of sheep, goats and also in the spleens of experimentally infected mice and guinea pigs (Waag 2007). The shedding of *C. burnetii* by ruminants occurs mainly at parturition or abortion and also in the post-partum period and the organism is found in lochia, vaginal mucus but it has also been demonstrated in urine, milk and faeces (Anastácio et al. 2016). *C. burnetii* possesses lipopolysaccharide in the cell membrane, as do all Gram-negative bacteria, but the endotoxic activity of the of the *C. burnetii* LPS is about 100 – 1000times lower than that of bacteria of the family Enterobacteriaceae (Arricau-Bouvery and Rodolakis 2005). Pleiomorphic forms of *C. burnetii* have been demonstrated in host cells and following bacterial purification, these have been classified by electron microscopy into large cell variants (LCV), small cell variants (SCV) and small dense cells (SDC). Small dense cells are synonymous with spore-like particles (SLP). These cell types show differences in overall structure, antigenicity, physical resistance, protein composition and metabolic capabilities (Heinzen et al. 1999).

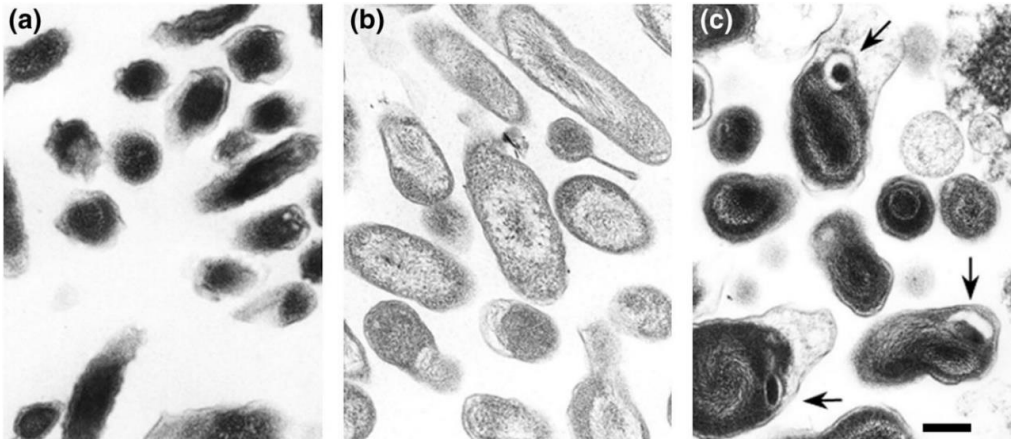

Fig. 1. Electron micrograph of *C. burnetii* showing large cell variants (LCV) [a], small cell variants (SCV) [b] and large cell variants (LCV) with internal small dense particles (SDP) [c]. Reproduced from Heinzen, Hackstadt, and Samuel (1999)

LCVs are similar in structure to most Gram-negative bacteria, which have diffuse chromatin and possess clear distinctions between the outer and inner layers of the cytoplasmic

membrane. They are larger in size; reaching a length exceeding 1.0 µm in length, more pleiomorphic in shape and more metabolically active but less electron dense than SCVs and SDPs (Arricau-Bouvery and Rodolakis 2005; Heinzen et al. 1999). SCVs have condensed chromatin, are smaller in size; 0.2-0.5µm in length and are less metabolically active than LCVs (Arricau-Bouvery and Rodolakis 2005; Heinzen et al. 1999). Both LCVs and SCVs are infectious *in vitro* and *in vivo* and both divide by binary fusion. SDPs are somewhat similar to SCVs but are found within the LCVs and are endospore forms thus, the purified form cannot be isolated, are very resistant to pressure withstanding up to 1400kg/cm and do not possess major outer membrane proteins (Arricau-Bouvery and Rodolakis 2005). Despite evidence of the virulent potential of the LCVs, it is thought they are of little relevance in natural infection because of their fragile nature and inability to survive for long periods extracellularly. The SDPs and SCVs are the persistent forms in the host and these resistant forms can also survive in the environment but not as well as LCVs (Arricau-Bouvery and Rodolakis 2005; Heinzen et al. 1999).

The bacterial cells of *C. burnetii* bacterial cells show the smooth-rough variation just like other Gram-negative bacteria and this is due to variations in LPS (Waag 2007). Phase I bacteria are similar to the smooth variants of some of the Gram-negative bacteria and are found in naturally infected humans, animals and arthropods and these phase I bacteria are very virulent (Arricau-Bouvery and Rodolakis 2005; Parker et al. 2006). Phase II cells of *C. burnetii* correspond to the rough antigenic variants of some Gram-negative bacteria which are less infectious; these phase II cells can be obtained artificially after serial passaging in embryonated eggs and cell cultures (Parker et al. 2006). The LPS in phase II cells of *C. burnetii* is truncated and some of the usual cell surface proteins are absent, and there is a difference in the sugar component of the LPS between phase I and phase II cells of *C. burnetii* (Parker et al. 2006).

C. burnetii has resistance to physical and chemical disruption superior to those of other bacterial vegetative cells (Marrie 2011). Viable *C. burnetii* organisms can be obtained after heating at 63°C for 30 minutes, can be viable after 180 days of exposure to 10% saline, 24hr exposure to formalin or sonication in distilled water for thirty minutes; all these resistance characteristics are attributable to the SCVs (Arricau-Bouvery and Rodolakis 2005; Fournier and Marrie 1998). *C. burnetii* is metabolically inactive at neutral to slightly alkaline pH and this energy conservation tactic contributes to the extracellular resistance of the organism; *C. burnetii* is resistant to UV radiation, pressure of up to 50 000 psi, osmotic and oxidative stress (Raoult et al. 2005; Waag 2007).

2.2 Epidemiology

C. burnetii is the causative organism of Q fever in humans but in the animal kingdom, it has a wide range of hosts which include birds, mammals, ticks, fish and reptiles (Heinzen et al. 1999). Despite the wide range of hosts for *C. burnetii*, domestic ruminants, especially sheep and goats are considered to be the main reservoirs for human infection (Heinzen et al. 1996, 1999). The main mode of human infection is by aerosol transmission from infected animals, hay and fomites rendering people who work directly with animals such as farmers, farm employees, veterinarians and animal health technicians to be at greatest occupational risk (Heinzen et al. 1996, 1999; Woldehiwet 2004).

2.3 Q fever in animals

The disease is present world over, except New Zealand; and unlike in humans, *C. burnetii* in animals is transmitted by ticks as one of the major methods of infection which does not occur in human Q fever (Madariaga et al. 2003). Infected animals shed *C. burnetii* in the faeces, urine, milk and lochia and less commonly in contaminated wool (Heinzen et al. 1996; Miguel G. Madariaga et al. 2003; Marrie 2011). Cattle, sheep, goats, marsupials, wild ungulates, lagomorphs, wild carnivores, apes, bats, chickens, turkeys, pigeons, ducks, geese, reptiles, arthropods and fish have been shown to be susceptible to infection (Kazar 2005; Raoult et al. 2005). Wild animals, despite their susceptibility to infection, do not represent a main direct source of human infection due to infrequent interactions between man and wild animals although recently, wildlife reservoirs have been suggested. Migratory birds can also disseminate *C. burnetii* over long distances via their faeces and any ectoparasites they transport in flight (Kazar 2005; M. G Madariaga 2005).

Although *C. burnetii* is not always a severe threat to the health of animals, it causes occasional abortions sometimes on a scale that is epidemic. The majority of infections are subclinical, and because of this, limited threat of Q fever to animals, the geographical distribution of Q fever in animals is poorly described (Woldehiwet 2004). Research studies have shown that animal and human Q fever occur with higher prevalence in tropical than temperate climates (Kazar 2005; Woldehiwet 2004). Chronically infected cows have been shown to shed *C. burnetii* in milk and birth secretions for several successive years and are very important sources of human infection (Norlander 2000; Woldehiwet 2004). Infected animals do not regularly shed the pathogen and only seem to do so in the post-partum period. While ticks are important in the transmission of animal to animal Q fever, they are not essential in this regard as other modes of infection, such as direct horizontal transmission from infected animals to susceptible ones as well as vertically from infected dam to offspring during pregnancy (Hatchette et al. 2003; Parker et al. 2006).

Ticks of the genera *Ixodes*, *Amblyomma, Dermacentor* and *Rhipicephalus* are considered to be the main ones in tick-transmitted animal Q fever; however, every parasitic tick species in an endemic area can be expected to be a potential vector of *C. burnetii* and the ticks transmit the agent both trans-ovarially and trans-stadially to their larvae and nymphs (Kazar 2005; Waag 2007).

Studies have reported that maximum shedding of *C. burnetii* in cows is at parturition and the maximal shedding continues for up to two weeks thereafter; low-level shedding however, can continue for several months after parturition in cattle (Woldehiwet 2004). In sheep the shedding of *C. burnetii* is short-lived while the shedding of *C. burnetii* by dairy goats is not well-documented, it is suspected to be similar to that of cows (Maurin and Raoult 1999). Experimental infections of sheep with *C. burnetii* showed localization of the pathogen in the kidney, udder and placenta thus showing that animals become persistently infected. Pregnancy has been demonstrated to enhance the multiplication of *C. burnetii* but localization and manner of dormancy in non-pregnant animals is still unclear (Berri et al. 2002; Woldehiwet 2004). A study in South Africa reported a Q fever seroprevalence of 2% in cats and 8% in cattle (Matthewman et al. 1997), while in Zimbabwe the same author described seroprevalences of 13% for cats, 39% for cattle, 10% for dogs and 15% goats (Matthewman et al. 1997). Another study in Egypt reported up to 41% Q fever seroprevalence for camels, 19% for cattle, 11% for buffaloes, 9% of sheep and 7% for goats respectively (Klemmer et al. 2018). A retrospective study by Vanderburg et al. (2014) showed animal Q fever seroprevalence in Africa to be less than 13% on average for cattle while in small ruminants seroprevalence ranged from 11–33% (Vanderburg et al. 2014).

2.4. **Q fever in humans**

In humans, *Coxiella burnetii* causes Q fever or human coxiellosis, which is a disease that is found all over the world except the Antarctica and New Zealand (Fournier and Marrie 1998; Frean and Blumberg 2010; Miguel G. Madariaga et al. 2003). It is classified under the group of emerging and re-emerging diseases, has caused large scale epidemics and while it has a wide range of vertebrate and invertebrate hosts, domestic cattle, sheep and goats are considered the main reservoirs of human infection (Anastácio et al. 2016; Vanderburg et al. 2014). Studies in Tanzania have shown Q fever to be a more common cause of febrile illness than malaria (Vanderburg et al. 2014). Human outbreaks in Europe and Asia had prevalences ranging from 0.15 - 18% and the majority of these were confirmed by immunonofluoresence (Arricau-Bouvery and Rodolakis 2005).

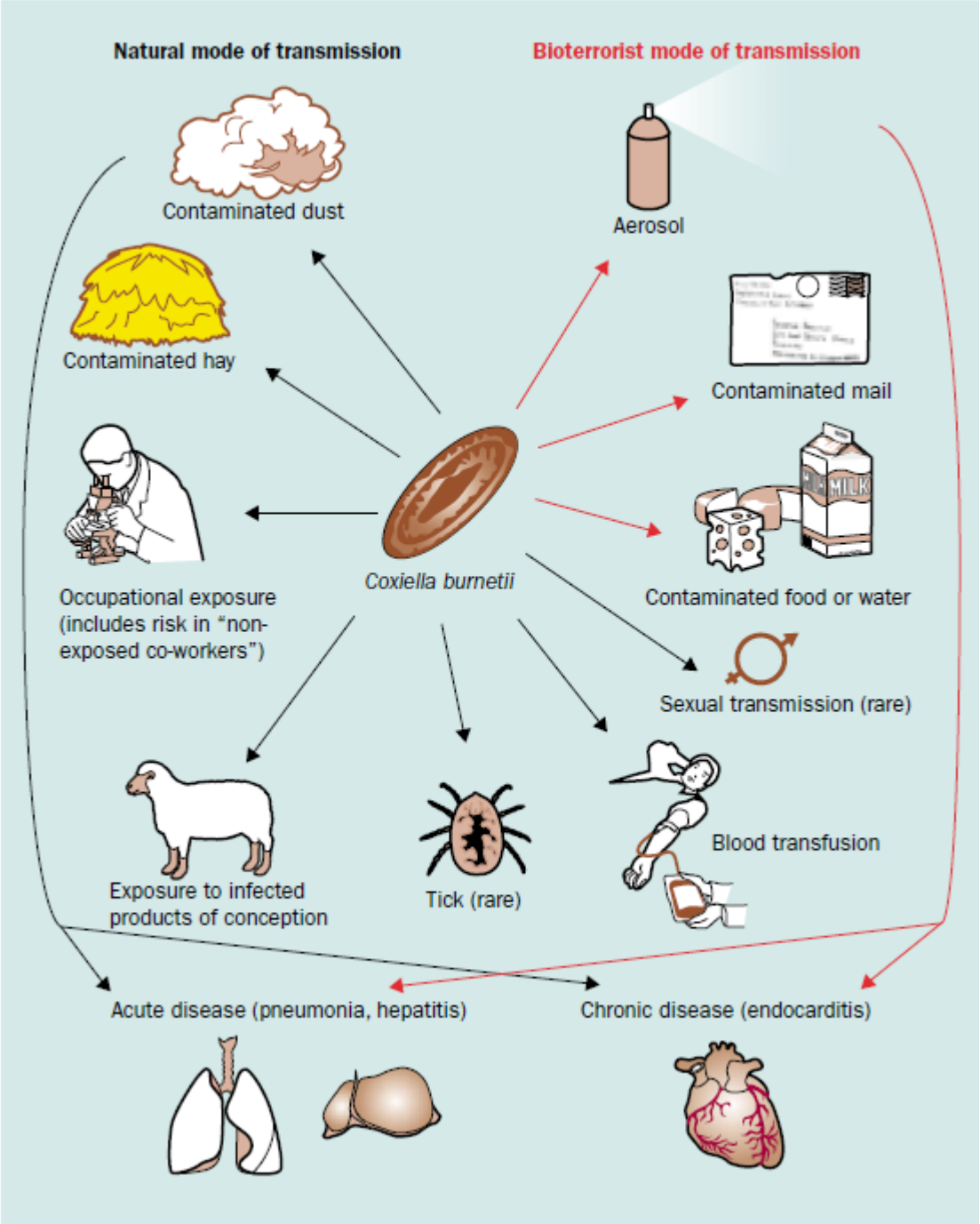

Fig 2. Infection model for *Coxiella burnetii* in human beings. Reproduced from Madariaga et al. (2003)

Human Q fever is mainly an airborne disease, with infection being by inhalation of aerosols generated from infected placentas, lochia, milk and other body fluids, contaminated wool and dust (Arricau-Bouvery and Rodolakis 2005; Fournier and Marrie 1998; Waag 2007) (Fig. 2). Research suggests a seasonal variation with peak incidence of human cases coinciding with early spring and summer when most of pregnant cattle, sheep and goats give birth

(Vanderburg et al. 2014). The majority of infections are in people who are occupationally at risk and these include farmers and farm workers, veterinarians and para-veterinary professionals, abattoir employees and people employed in meat processing plants (Kazar 2005; Waag 2007). Whether or not Q fever can be transmitted by ingestion remains inconclusive based on trials in which consumption of unpasteurized contaminated milk *C. burnetii* did not cause febrile illness in volunteers who ingested contaminated milk (Kazar 2005; Marrie et al. 1996). Unlike animals, tick borne transmission is rare in humans (Fig 2), so is human to human transmission, sexual transmission is unlikely but suspected and nosocomial infection is a possibility (Kazar 2005; Osorio et al. 2003). Lab acquisition of Q fever has been documented where 50 people in the US Institute of Health succumbed to an internal outbreak while working with *C. burnetii* (Kazar 2005). In the USA, human cases reported to the Centers for Disease Control and Prevention (CDC) in the period 2002–2014, more than 50% were hospitalised, with a peak incidence of 71% in 2009 (Kaufman et al. 2018). A study by Anderson et al (2013) revealed that 60% of human cases in outbreaks were asymptomatic seroconversions while 5% of cases progress from acute clinical Q fever to chronic Q fever. Acute Q fever in humans has low mortality of less than 2% but chronic Q fever has mortality approaching 100% (Anderson et al. 2013). Interestingly, according to unpublished CDC data, 79% of the Q fever positive cases in the USA were described in patients who are not typical high-risk individuals and 60% of the human Q fever cases were recorded in people who had no recent prior contact with animals (Anderson et al. 2013).

The range of disease caused by *C. burnetii* in humans varies from acute disease to chronic infections (Fig. 2) which can be fatal, but subclinical forms also do occur (Arricau-Bouvery and Rodolakis 2005; Kazar 2005; Miguel G. Madariaga et al. 2003). The causes of this variability in disease range are not clear, but they are speculated to be virulence of the *C. burnetii* strain, e.g. the nine mile strain is said to be more virulent than the Priscilla strain, route of infection and/or average infective dose (Kazar 2005; Miguel G. Madariaga et al. 2003). Studies in Europe indicate that Q fever affects all ages alike, but it is more common in men than women (Kazar 2005).

2.5. Pathogenesis and clinical signs

2.5.1 Q fever in animals

In domestic ruminants, besides tick transmission, the oropharynx serves as an important portal of entry through direct and indirect contact of infected and uninfected animals. Just like in

humans, the size of the infective dose is very small and one *C. burnetii* organism is sufficient to cause infection in animals (McQuiston et al. 2002; Woldehiwet 2004). Initial multiplication of the bacteria following infection takes place in the regional lymph nodes and then a bacteraemia develops for about 5 to 7 days. Then the *C. burnetii* organisms localize in the mammary gland and placenta of pregnant animals where they cause persistent infections (Woldehiwet 2004).

Most studies on Q fever in animals have been done in cattle, sheep and goats due to their role as the main reservoirs for human infection (McQuiston et al. 2002). In domestic ruminants, most infections are subclinical with infected animals not exhibiting any clinical signs (Marrie 2011; Norlander 2000). Clinical Q fever is more common in sheep and goats than in cattle and is characterized by abortion, stillbirth, delivery of weak offspring and premature partus and some clinical cases of Q fever in ruminants can manifest as pneumonia (Anastácio et al. 2016; Arricau-Bouvery and Rodolakis 2005). *C. burnetii* induced abortions tend to be late stage without any prior indication of infection and this presentation is similar to that of chlamydiosis and brucellosis (Arricau-Bouvery and Rodolakis 2005). Aborted foetuses appear clinically normal but the placentas show inter-cotyledonary fibrous thickening and abnormally tinged exudate which is not pathognomonic for Q fever in animals (Anastácio et al. 2016). Severe inflammation of stroma and myometrium adjacent to the placentome areas is observed in goats (Arricau-Bouvery and Rodolakis 2005). The incidence of *C. burnetii* abortion is usually so low (about 3% of infected pregnant sheep and goats) that it has a mild economic impact on stock owners, but in some on-farm epidemics, *C. burnetii* induced abortion has approached 80% of pregnant animals (Anastácio et al. 2016). High rate abortions are rare in cattle, sheep and goats and when they do occur tend to be limited to goat herds (Anastácio et al. 2016). Clinical Q fever in cattle rarely causes abortions like the small ruminants but rather manifests mainly as a metritis. Ruminants that abort recover quickly and in most cases do not abort in future pregnancies but metritis induced by *C. burnetii* infection can last several months (Anastácio et al. 2016; Arricau-Bouvery and Rodolakis 2005).

2.5.2. Q fever in humans

Following infection, *C. burnetii* invades a variety of cell types that include monocytes, macrophages fibroblasts and endothelial cells (Heinzen et al. 1996). Uptake of the bacteria is believed to be by endocytosis and the bacteria replicates within phagolysosomes at acidic pH of 4.7-5.2 (Heinzen et al. 1996, 1999; Raoult et al. 2005). Entry of the bacteria into eukaryotic cells depends on which phase of the bacteria is involved; phase I *C. burnetii* binds to monocytes via the leukocyte response integrin (LRI) complex and the integrin-associated protein (IAP). Phase II *C. burnetii* invades the monocyte-macrophage system by binding to the

CR3 receptor (Raoult et al. 2005). Phase I *C. burnetii* does not invade monocyte-macrophage cell types very readily but survives well once internalized, whereas phase II *C. burnetii* which readily internalizes into host cells but has poor intracellular survival being rapidly killed off in phagolysozomes (Raoult et al. 2005). It is believed that the difference in receptor affinity for phase I and phase II *C. burnetii* is crucial in survival of the bacteria (Raoult et al. 2005; Waag 2007).

Once *C. burnetii* is internalized in phagosomes, they fuse with lysozomes to form phagolysozomes which subsequently fuse to form unique giant vacuoles of pH 4.7-5.2 within the cell (Raoult et al. 2005). The *C. burnetii* bacteria multiply within these giant vacuoles, demonstrating the acidophilic nature of *C. burnetii* and this is further elaborated by the arrest of *C. burnetii* multiplication by artificial alkalinisation of phagolysozomes with lysozomotropic agents such as chloroquine (Raoult et al. 2005; Waag 2007). The life cycle and cellular structure of *C. burnetii* is complex, involving LCVs which are more stable intracellularly, and SCVs which are less metabolically active and are the extracellular forms of the bacteria which are also the infective forms (Fig 3). (Arricau-Bouvery and Rodolakis 2005; Heinzen et al. 1996). Following phagosomal formation and lysozomal fusion, the SCV develop into LCVs which are more metabolically active and survive better intracellularly in the eukaryotic host cells. Sporogenic differentiation of LCVs leads to the formation of resistant spore like forms of the bacteria called SDCs or SLPs (Heinzen et al. 1996).

C. burnetii infection in humans causes a range of disease in humans from mild acute infections to chronic life-threatening infections. Approximately 60% of human *C. burnetii* infections result in asymptomatic seroconversions (Anderson et al. 2013).

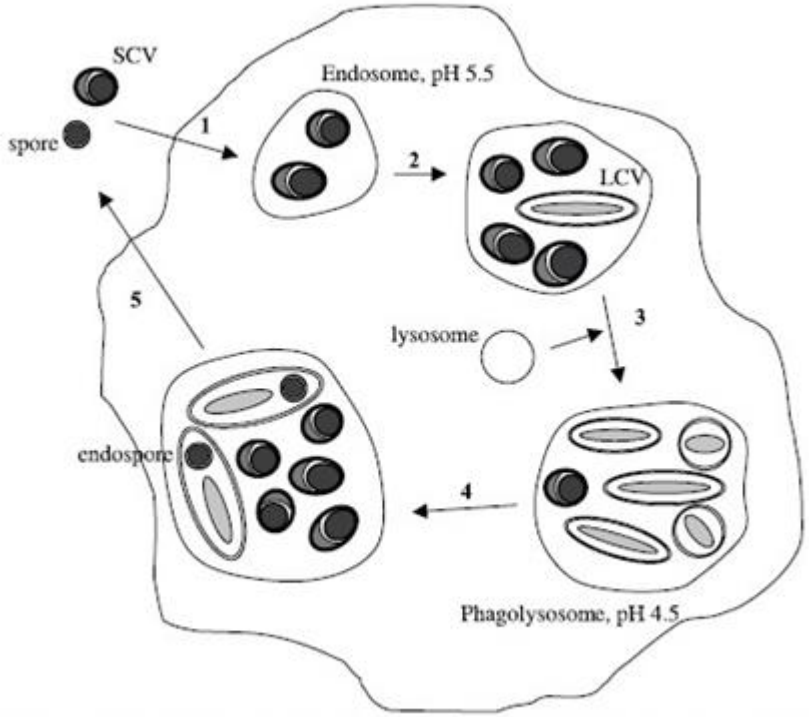

Fig 3. Developmental cycle of *C. burnetii* in the eukaryotic cell showing entry of SCVs into cells, development into LCVs and formation of and release of SDCs. Reproduced from Arricau-Bovery *et al* (2005)

Acute Q fever presents mostly as a mild influenza-like disease, hepatitis or atypical pneumonia. Influenza-like acute Q fever is a febrile disease and self-limiting in most cases and common signs include sudden onset, high fever (up to 40°C, headache, weight loss, myalgia and coughing. Some cases have additional signs including skin rash, nausea, arthralgia, sweating, chills and photophobia. Untreated, the disease lasts for 1 to 3 weeks but in rare cases and persists longer (Miguel G. Madariaga et al. 2003; Marrie 1990). Acute Q fever atypical pneumonia is characterized by fever, headache, myalgia, non-productive coughing which can be absent despite the presence of pneumonia. Pleuritic chest pain, which can be mild to severe, has been reported but generally, there is little discernible pathology on thoracic auscultation. A few cases, however, progress to respiratory distress which can be severe (Kazar 2005; Matthewman et al. 1997). Acute Q fever hepatitis ranges from asymptomatic disease, elevated transaminase enzyme levels all the way through to fever of unknown origin , hepatomegaly with or without jaundice (Miguel G. Madariaga et al. 2003). Less frequently described signs of acute Q fever infection include meningio-encephalitis, pericarditis, pancreatitis and abortion (Maurin and Raoult 1999; Parker et al. 2006).

Chronic Q fever develops in only about 5% of cases resulting in endocarditis, which is often fatal, chronic fatigue syndrome and abortion, which may repeat with future pregnancies. Manifestations include chronic Q fever endocarditis; which is the most common form of chronic Q fever and accounts for between 1.5 to 2% of human cases of endocarditis. Q fever endocarditis manifests several months to years after the acute disease in patients that had pre-existing cardiac valvular disease and those with acquired immunosuppressive conditions such as AIDS but a study in Spain argued that *C. burnetii* is not more common in HIV infected patients (Kosatsky 1984; Meghari et al. 2008). Chronic fatigue syndrome manifests as inexplicable myalgia, arthralgia, night sweating, mood swings and altered sleeping patterns. It usually persists after recovery from acute infection for several months to years. It is believed to be induced by elevated interleukin cytokine dysregulation (McQuiston et al. 2002). Abortion in pregnant women occurs as a result of placentitis and the pathogenesis like that observed in sheep and goats. *C. burnetii* placentitis causes abortions; which are late stage in most cases, and can repeat with subsequent pregnancies, but can also manifest as neonatal death, premature birth and low birth weight. If infection occurs in pregnant women, then the risk of repeat abortions is much higher (Norlander 2000; Woldehiwet 2004).

2.6. Diagnosis

Q fever in humans and animals can be diagnosed by both direct and indirect laboratory methods as clinical signs alone are not reliable. This is because the clinical presentation of Q fever is similar to many other infectious diseases in humans and in animals tend to be mostly subclinical (Kazar 2005). In humans, non-specific laboratory findings for acute Q fever include normal to mild white blood cell count, sedimentation time and erythrocyte count are elevated, platelet counts are elevated during recovery but the acute phase of the disease is characterized by a thrombocytopenia (Miguel G. Madariaga et al. 2003). Liver enzymes and creatine kinase (CK) are generally elevated and several auto-antibodies can be detected. Chronic Q fever also shows a variety of non-specific changes which may include anaemia, raised sedimentation rate, thrombocytopenia, polyclonal hypergammaglobinaemia, elevated liver enzymes and CK, haematuria and cryoglobinaemia which is a condition in which the blood contains large amounts of pathological cold-sensitive antibodies that are insoluble at reduced temperatures (Miguel G. Madariaga et al. 2003).

2.6.1. Direct methods

Demonstration of *C. burnetii* by direct methods is ideal but this is not always possible under ordinary laboratory conditions. *C. burnetii* is highly infectious and direct isolation should only

be done in a biosecurity level 3 laboratory (Arricau-Bouvery and Rodolakis 2005; Miguel G. Madariaga et al. 2003; Waag 2007; Woldehiwet 2004). For direct demonstration of the bacteria, staining with Machiavello or modified Ziehl-Nielsen (MZN) will reveal the presence of large numbers of red cocco-bacilli. This is strongly suggestive of the presence of *C. burnetii;* it is difficult however, to differentiate *C. burnetii* from *Chlamydophila abortus* and *Brucella* spp by microscopy only as causes of abortion (Arricau-Bouvery and Rodolakis 2005; Miguel G. Madariaga et al. 2003). In aborting animals, the best samples for detection of the bacteria are the placenta and amniotic fluid and these should be best transported on ice at 4 - 8°c, however the bacteria is quite stable and will survive even without ice (Woldehiwet 2004). Besides special staining techniques from impression smears, *C. burnetii* can also be demonstrated histologically in prepared sections of placenta, but the exact nature of the bacteria cannot be determined solely by microscopy (Woldehiwet 2004). Histological patterns associated with Q fever vary and may include granulomatous lesions which can be lipid or non-specific with neutrophils and giant cells having been observed in liver and bone marrow biopsies (Miguel G. Madariaga et al. 2003). Doughnut cells which were previously thought to be pathognomonic for acute Q fever seem to be transient and are not as common as previously believed (Miguel G. Madariaga et al. 2003).

C. burnetti was in the past difficult to grow on standard bacteriology media but modern studies into its physiology have allowed for successful culture on axenic media (Sanchez et al. 2018). It can also be propagated in the yolk sacs of embryonated eggs and cell culture lines including monkey kidney cells and vero cells (Arricau-Bouvery and Rodolakis 2005; Woldehiwet 2004). Laboratory animals, although rarely used these days, can also be used with mice and guinea pigs being very susceptible to infection. *C. burnetti*, rapidly multiplies following intra-peritoneal inoculation and the mice and guinea pigs develop a fever about 5 to 8 days post infection (Woldehiwet 2004). The organism is highly concentrated in the spleens of the mice and guinea pigs; and can be further isolated on cell culture lines or yolk sacs of embryonated eggs (Woldehiwet 2004).

The polymerase chain reaction (PCR) has also been used to detect *C. burnetii* DNA directly from clinical samples and can also be used to confirm the identification of isolates of the organism. A study by Vaidya et al (2008) has questioned the reliability of the *C. burnetii* PCR in humans where it detected only 18% of positive cases, a finding which is contrary to the report of Arricau-Bovery and Rodolakis (2005) who highly recommend PCR as a diagnostic tool for Q fever. They empasized its revolutionary impact of PCR speed and accuracy of *C. burnetii* diagnosis due to its high sensitivity and specificity (Arricau-Bouvery and Rodolakis

2005; Kazar 2005; Woldehiwet 2004). PCR can be used directly with clinical samples, cell cultures and milk but also works with frozen or paraffin embedded samples (Kazar 2005; Vaidya et al. 2008). The *C. burnetii* PCR utilizes several specific primers for superoxide dismutase, 16S tRNA or the htpAB repetitive element and these have been successfully used in light cycler nested PCR and it is ideal for early diagnosis of acute Q fever but not chronic Q fever (Arricau-Bouvery and Rodolakis 2005; Miguel G. Madariaga et al. 2003). PCR is the only test that can be used to detect *C. burnetii* induced metritis and the association of PCR and enzyme-linked immunosorbent assay (ELISA) can account partially for the emergence of Q fever due to increased number of positive diagnoses (Arricau-Bouvery and Rodolakis 2005; Fournier and Marrie 1998).

The best PCR target genes are those using insertion sequence IS1111 which is located in every strain of *C. burnetii* isolated so far and the nine mile strain initially isolated in Montana has 19 copies of this sequence(Waag 2007). Human samples for testing can be collected in EDTA and citrate tubes (Waag 2007). *C. burnetii* adapted nested PCR has been used successfully for the diagnosis of chronic Q fever but its sensitivity in acute Q fever cases has been questioned and it is suggested that it be used with serology (Kazar 2005). Real time PCR has also been used successfully to determine the antibiotic sensitivity profile of *C. burnetii* isolates in humans (Kazar 2005). Modern day PCR probes have been developed and these can identify the organism in blood, urine and tissue samples while posing minimal hazard to diagnostic and clinical personnel (M. G Madariaga 2005).

2.6.2. Indirect methods of *C.burnetii* diagnosis

Due to the high infectious risk of the organism and the complexity of its laboratory isolation, serological techniques are routinely used to confirm human and animal Q fever (Arricau-Bouvery and Rodolakis 2005; Waag 2007). Serological tests that have been successfully used to detect *C. burnetii* are ELISA, complement fixation test (CFT), microagglutination, indirect haemolysis, western blot, dot blotting, slide agglutination, radio-immunoassay, cross adsorption and micro-immunofluorescence with ELISA being the most sensitive and the immunofluorescence test (IFT) being the easiest to perform (Kazar 2005; Waag 2007). Acute Q fever in humans is characterized by an initial rise in IgM antibody to phase II antigen and then subsequently a rise in IgG level to phase II antigen (Miguel G. Madariaga et al. 2003). IgM persists for several months at low concentrations after acute infection and is not an indicator of current disease activity (Miguel G. Madariaga et al. 2003). Chronic infections are characterized by high titres of IgA and IgM to phase I and phase II *C. burnetii* antigens (Heinzen et al. 1999; Miguel G. Madariaga et al. 2003; Tissot-Dupont et al. 2007).

The IFT is one of the three most widely used serological test for human samples, the other two being CFT and ELISA, because it is accurate, readily available in most countries and simple to perform (OIE 2018) . The *C. burnetii* IFT allows the distinction between phase I and phase II antibodies with acute Q fever being characterized by higher levels of phase II antigens than phase I antigens and the opposite being true for chronic Q fever (Arricau-Bouvery and Rodolakis 2005; Waag 2007). IFT which is used widely in Q fever diagnosis is not routinely used for diagnosis of Q fever in animals because it cannot be automated, is not convenient for large scale screening in cases of epidemics and could be subjective, possibly leading to bias in reading the test result (Arricau-Bouvery and Rodolakis 2005). No commercial *C. burnetii* IFT kits are available for diagnosis of Q fever in animals despite the test being more sensitive than *C. burnetii* CFT, additionally, the *C. burnetii* CFT is capable of detecting acute phase antibodies before CFT (Arricau-Bouvery and Rodolakis 2005; OIE 2018; Waag 2007). *C. burnetii* IFAT carries with it the advantage of working well with unpurified diagnostic antigen but has a disadvantage of inconvenience when working with large numbers of samples (Waag 2007).

CFT is one of the three major tests for serological *C. burnetii* detection but its sensitivity is poor, since the antigen used in the test sometimes fails to detect antibodies in samples (Arricau-Bouvery and Rodolakis 2005; OIE 2018). Compared to IFT, CFT is less specific, less sensitive, is more time consuming and has a prozone effect associated with the test results (Miguel G. Madariaga et al. 2003). The prozone or hook effect is an immunologic phenomenon where very high concentration of antibodies or antigen physically hinder the formation of immune complexes, thereby causing false-negative serological test results (Butch et al. 2000). CFT requires acute and convalescent serum samples and this is not ideal for purposes of disease identification and control (Miguel G. Madariaga et al. 2003). A fourfold increase in antibody titres between acute disease and convalescence is diagnostic for Q fever and generally, antibody titres tend to be much higher with chronic Q fever compared to acute Q fever (Waag 2007). CFT is less sensitive than ELISA and IFT for animals samples and as such, its routine use in veterinary practice has declined significantly in the past 10 years (OIE 2018).

ELISA is widely used for the diagnosis of Q fever in animals because it allows for testing of large numbers of samples simultaneously. Herds can be screened by bulk tank milk (BTM) samples or pooled serum samples, but this pooled testing has the drawback of not identifying the specific animals that produce *C. burnetii* antibodies. Also, there is no true correlation

between sero-response and bacterial shedding so the detection of antibodies is not an indication of active infection (Arricau-Bouvery and Rodolakis 2005; Waag 2007). The *C. burnetii* ELISA, carries the disadvantage of requiring highly purified diagnostic antigens (Waag 2007).

In dairy herds BTM is suitable for screening *C. burnetii* in lactating animals and ELISA can demonstrate herd exposure to *C. burnetii* and the result of milk testing are comparable to serum samples due to the transfer of antibodies from blood to milk during lactation (Anastácio et al. 2016; Arricau-Bouvery and Rodolakis 2005; Hatchette et al. 2003). Most animals that shed *C. burnetii* in the faeces and milk are seropositive but some can shed *C. burnetii* in the faeces and milk despite being seronegative while others are seropositive but do not shed the organism in faeces (Arricau-Bouvery and Rodolakis 2005). Infected animals shedding the organism and appearing sero-negative on testing pose the greatest risk to animal and public health; therefore, PCR is one of the quickest and most sensitive tests to pick up such shedders (Arricau-Bouvery and Rodolakis 2005; McQuiston et al. 2002; Waag 2007).

Kazar (2005) has suggested microagglutination as the method of choice for detecting *C. burnetii* in human cases. The antibody titre cut off values for positive diagnosis are recommended as 50 for IgM and 200 for IgG for phase II antibodies in acute Q fever and 800 for phase I antibodies in chronic Q fever when testing a single serum sample using microagglutination (Kazar 2005). ELISA is superior to microimmunofluoresence both in sensitivity and specificity. In acute disease, the sensitivities are 58.4% and 80% for the microimmunofluoresence and ELISA respectively (Arricau-Bouvery and Rodolakis 2005). In chronic Q fever however the diagnostic sensitivity of microimmunofluoresence is 100% as it detects anti-phase 1 antibodies to *C. burnetii.*

The LSIVet™ Ruminant Q Fever – Serum/Milk ELISA (Life technologies, Carlsbad, California) recently changed name and is now marketed as Priocheck®, (Thermofischer, Lelystad, Netherlands) is an indirect ELISA for the detection of antibodies directed against *C. burnetii* in serum, plasma or milk. It detects the presence of phase 1 and phase 2 antibodies to *C. burnetii. C. burnetii* occurs in two forms; phase I which is pathogenic, and is isolated from animals and humans following natural infection. The less pathogenic phase II *C. burnetii* is obtained by repeated passages in embryonated eggs or tissue cultures. This antigenic difference between phase I and phase II *C. burnetii* is based on cell surface lipopolysaccharide (LPS) antigens. This classification into phase I and II *C. burnetii* is very similar to the smooth

and rough classification for several Gram-negative bacteria with the naturally occurring smooth variants being pathogenic and the rough variants obtained after attenuation by serial passages in unnatural hosts cell types.

Validations performed by INRA and Life Technologies show that the LSIVetT™ Ruminant Q Fever - Serum/Milk kit exhibits greater sensitivity for detecting *C. burnetii* shedding animals than the Nine Mile ELISA kits and the documented diagnostic sensitivity and specificity for this test is 87% and of 100% respectively (de Oliveira et al. 2018). Another indirect ELISA, CHEKIT® Q Fever Antibody ELISA, (IDEXX Laboratories, Maine, USA) is based on *C. burnetii* purified antigen of the Nine-mile strain of Q fever. Based on 81 samples, the manufacturer claims sensitivity and specificity of 100% for both. The CHEKIT® can also be used in serum and milk samples, but theoretically, it is superior to the LSIVet Ruminant Q fever ELISA in terms of diagnostic accuracy. The sample size, however, was too small for this assertion to be reliable and this must still be evaluated further (Plummer et al. 2018). According to *Plummer et al* (2018), the LSIVet Ruminant Q fever - Milk/Serum (Life technologies, Carlsbad, California) has a diagnostic sensitivity estimated to be 85% and specificity of 95%.

2.7. Treatment

2.7.1 Animals

Most cases are subclinical and even when clinical signs do occur, they are rarely on a scale that is economically significant to the farmer. As such, many owners will be unaware of the infection status of their herds and herds unless herd serology is done (Roest et al. 2011). The treatment of cases of Q fever is rarely done in animals, although, under experimental conditions tetracyclines have been used to reduce bacterial shedding by infected animals (Arricau-Bouvery and Rodolakis 2005). Cure rates of up to 96% following 2 injections of long acting oxytetracycline at 20 mg/kg 15 days apart have been reported in cattle with vaginal mucus being the sample tested for *C. burnetii* by PCR (Arricau-Bouvery and Rodolakis 2005).

In *C. burnetii* abortion epidemics, tetracyclines have been used with success in reducing the occurrence of abortions and reducing shedding. However, for effectiveness of therapy, real time PCR should be performed to assess efficacy of antibiotic as resistance is a possibility (Waag 2007). PCR of whole blood is the recommended diagnostic tool as the presence of *C. burnetii* DNA is indicative of a bacteraemia; which happens in infected states or failed treatment (Anderson et al. 2013).

2.7.2. Humans

Doxycycline and hydrochloroquine have been used together daily for 18 - 36 months post-acute disease or Oxfloxacin can be taken daily for 36 months as therapies for chronic Q fever (Maurin and Raoult 1999). Acute cases of Q fever are rarely treated as they are usually self-limiting and by the time serological positive diagnoses are made, patients will be convalescent. If diagnosis is made early for cases of acute fever, these can be managed with tetracyclines with good results but treatment is more effective with rifampin and pefloxacin (Arricau-Bouvery and Rodolakis 2005). Cases of Q fever meningioencephalitis have been successfully treated with fluoroquinolones due to their blood-brain barrier penetrating ability but they are contraindicated in children and pregnant women (Anastácio et al. 2016). Macrolides present a good alternative but antibiotic sensitivity testing should always be performed because resistance is always a possibility. Long term therapy with chloroquine, doxycycline or ofloxacin have been said to lower chronic Q fever mortality to less than 5% (Raoult et al. 2005; Waag 2007).

2.8. Control of Q fever in animals

Due to the highly infectious nature of the organism, the multitude of potential reservoirs and resistance of the organism under environmental conditions, the control of Q fever is complex and multi-faceted and involves mostly measures that prevent new infections in susceptible humans and animals. One of the most important control measures that have been suggested is precaution when introducing a new animal or animals into a herd that is *C. burnetii* free. Strict testing of the sellers' herd should be requested before animal movement (Arricau-Bouvery and Rodolakis 2005). Parturition of ruminant animals should ideally take place in designated areas which must be routinely disinfected and placentas disposed of before wild and domestic carnivores ingest them. Strict biosecurity must be employed including the use of gloves, masks and other protective equipment in cases where *C. burnetii* infection is suspected or confirmed. Manure should not be spread in windy weather due to the aerosol risk associated with such practice.

In epidemic abortions due to *C. burnetii*, antibiotic therapy should be instituted and efficacy of treatment confirmed by PCR. Even though ingestion is not a main mode of transmission, pasteurization of milk at 72°C for 15 seconds or complete sterilization will reduce the risk of oral infections with *C. burnetii* for humans. Public awareness campaigns for people who are occupationally and geographically at risk of infection should be routinely practised. Individuals with the highest geographical risk are those who live in premises that are in proximity with large

and intensive ruminant farms, especially small ruminants, and these should be the main targets of these public awareness campaigns (Hatchette et al. 2003). In some countries, vaccination has been used with variable to poor success rates. In the 1970s and 1980s, Slovakia reduced the seroprevalence and clinical incidence of Q fever in animals using vaccines. Vaccines for animals are humans are available in many countries and include formalin killed whole cell vaccines and chloroform methanol-extracted bacterial residue (Anastácio et al. 2016; Arricau-Bouvery and Rodolakis 2005; Raoult et al. 2005; Waag 2007).

3. Literature review: Rift Valley fever

Rift Valley fever (RVF) is a mosquito-borne disease of livestock and humans caused by the Rift Valley fever virus (RVFV) of genus *Phlebovirus*, family Phenuiviridae and causes cyclic devastating outbreaks of severe disease in Africa and recently in the Arabian peninsula (Fafetine et al. 2013; Ly et al. 2017). RVF was first described in 1931 near Lake Navasha in the Rift Valley in Kenya (Daubney and Hudson 1931) .

3.1. Aetiology

Rift Valley fever virus is a member of the genus *Phlebovirus* within the family Phenuiviridae which comprises single stranded RNA viruses (Abudurexiti et al. 2019). The viral Order Bunyavirales was amended at the recent taxon ratification vote with the changes being adopted by International Committee on Taxonomy of Viruses. RVF virus is an enveloped spherical virus of 80-100nm diameter and possesses a tripartite genome of approximately 11.9 kilo base-pairs (B. H Bird et al. 2009; Flick and Bouloy 2005). The tripartite genome comprises three segments (Fig. 4), namely the large (L) segment, medium (M) segment and small (S) segment (Flick and Bouloy 2005; Pepin et al. 2010). The family Bunyaviridae includes other important viruses such as Nairobi sheep disease virus, Akabane virus, Crimean Congo hemorrhagic fever virus, La Crosse virus, the Hantaviruses and Sandfly fever Sicilian virus (B. H Bird et al. 2009). Most of these viruses are transmitted by phlebotomine sandflies hence the name of the genus *Phlebovirus* however, there are exceptions to this rule, such as RVF virus which is transmitted by mosquitoes and Uukuniemi related viruses which are transmitted by ticks (Pepin et al. 2010). RVF virus in serum remains infective at 4°C for several weeks and this naturally presents a biohazard for personnel working with diagnostic specimens (B. H Bird et al. 2009).

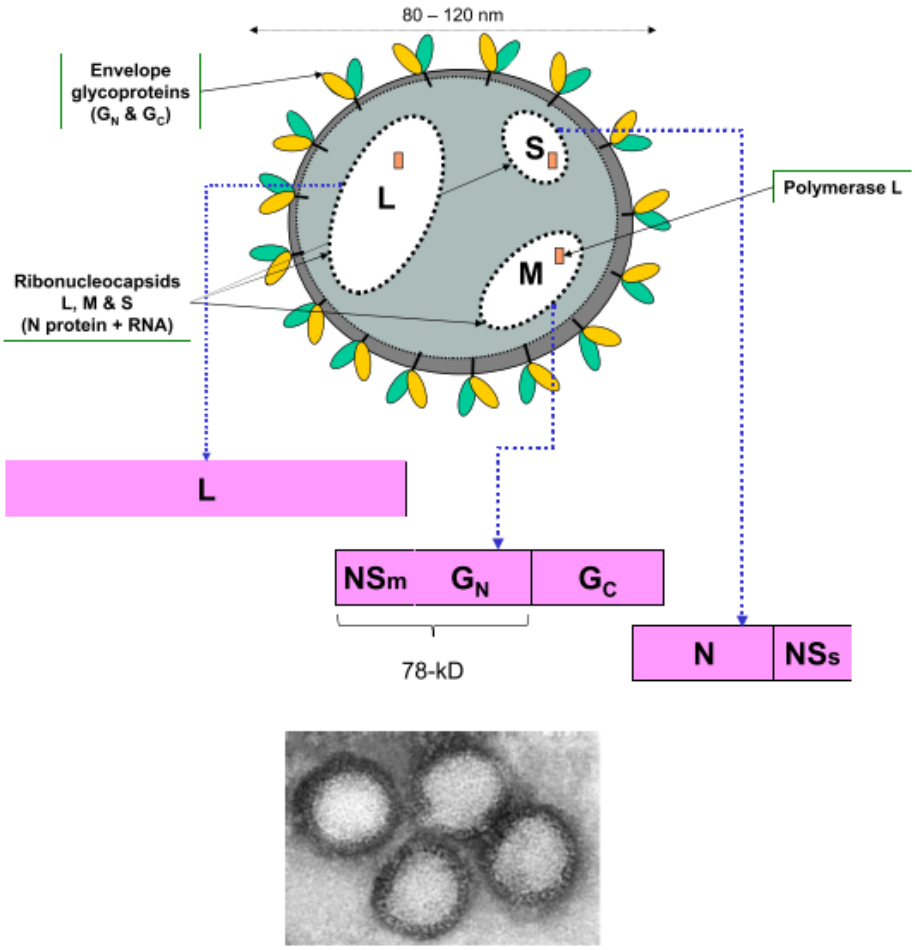

Fig 4. Schematic (top) and electron micrograph (bottom) of Rift Valley fever virus**.** Reproduced from Pepin (2010)

According to Pepin 2010, the RVF virus RNA is made up of segments which are called small (S segment), medium (M segment) and large (L segment). The S segment is ambisense and codes for nucleoprotein in the antigenic strand and codes for non-structural protein in the genomic strand. The M segment codes for at least four viral proteins, two of which are major envelope surface glycoproteins. The L segment codes for a 237kDa viral polymerase enzyme and a 6.4kDa open reading frame (ORF).

RVF virus possesses an envelope which comprises a lipid bilayer with embedded Gn and Gc glycoproteins forming subunits of 5-8nm in length (Pepin et al. 2010). Like all other viruses, RVF virus does not have a matrix (M) protein to link viral surface proteins with the ribonucleoproteins (RNPs) (Flick and Bouloy 2005). Recent cryo-electron microscopy of Uukuniemi virus and RVF virus has demonstrated that phleboviruses are icosahedral in nature rather than pleomorphic (Pepin et al. 2010). Three dimensional reconstruction has shown that

at 22Á isolation that the capsomeres of the RVF virion resemble hollow cylinders and these are situated at five and six coordinated positions (Pepin et al. 2010). In the envelope is a layer of RNP which is located proximal to the inner leaflet of the membrane and this is strongly suggestive of an interaction between cytolic tails of the glycoproteins and the RNPs and this compensates for the absence of M protein (Pepin et al. 2010). Sequencing has shown that the 3' and 5' terminal sequences are complementary for each other and have a panhandle structure and this explains the circular morphology of RNPs when observed under the electron microscope (Pepin et al. 2010).

RVFV is readily inactivated by strong detergents and 1% sodium hypochlorite or by formalin fixation. Due to the ease of horizontal transmission of the virus, it is strongly recommended that laboratory based investigations should be performed in biosecurity level 3 facilities (Flick and Bouloy 2005; Pepin et al. 2010).

3.2. Epidemiology

RVFV is a mosquito-borne pathogen of livestock, wildlife and humans and is found throughout Africa, and now in the Arab peninsula (B. H Bird et al. 2008). Case reports of an illness similar to what is now known as Rift Valley fever were first reported in 1910 in western Kenya (Brian H Bird et al. 2007). RVFV was first isolated in western Kenya in 1931 as the aetiology behind enzootic hepatitis in sheep (Daubney and Hudson 1931). RVFV was first identified outside of continental Africa in 1979 when it was reported in Madagascar and it has since become endemic there (Hunter et al. 2002).

Young lambs, goat kids and calves are very susceptible to extremely severe infection and mortalities can approach 100%. Adult sheep, goats, cattle, water buffalo and humans also acquire severe clinical illness and abortion but mortalities are usually low. Dogs, cats, and horses suffer transient viremia but they do not show clinical signs while pigs are resistant to infection (Swanepoel and Coetzer 2004).

RVFV is an important veterinary pathogen that is linked to large scale abortion and mortality of young animals, especially sheep and goats (Miller et al. 2002). RVF is classified as an emerging disease of livestock and humans and important endemic disease in sub-Saharan Africa (Shoemaker et al. 2002). The virus is transmitted by the bite of an infected mosquito or exposure to tissues and blood of infected animals (Shoemaker et al. 2002). Large outbreaks are typically seen in livestock during periods of unusually high and sustained periods of rainfall

and this flooding increases breeding sites by causing flooding of low lying grasslands called dambos and over-abundance of adult competent *Aedes* mosquito vectors (Sang et al. 2010; Shoemaker et al. 2002). Human infections typically result from the bite of infected mosquitoes or aerosol infection when slaughtering infected animals, conducting post mortems or from contact with aborted foetal materials (B. H Bird et al. 2008). Vector competence is the ability of a blood sucking insect to become infected after ingesting an infected blood meal and then transmitting the virus to other animals at subsequent feedings on vertebrate hosts (Moutailler et al. 2008).

In endemic areas in Africa, floodwater-breeding mosquitoes such as *Aedes vexans* and *Aedes mcintoshi* likely serve as the primary vectors and can transmit the virus transovarially. (Moutailler et al. 2008). RVFV has been isolated from more than 40 species of mosquitoes in 8 different genera and laboratory studies have shown that several species of mosquitoes and sandflies can be orally infected when feeding, although only some of them can transmit RVFV at subsequent feedings Eggs are laid in dambos and when these eggs hatch, transovarially infected adults emerge. These adults transmit RVFV to domestic animals i.e. cattle, sheep, goats and camels (Sang et al. 2010). The resultant high viraemia in these animals then leads to infection of secondary mosquito vectors, particularly in the genus *Culex*, resulting in epizootics and infection of large numbers of animals and humans (Sang et al. 2010).

Before the 1977 Egyptian outbreak, the virus was primarily thought to be a disease of animals with little impact on humans. However, since then, acute febrile illness with haemorrhagic syndrome have been reported in humans in Africa, Saudi Arabia and Yemen (Sang et al. 2010). Infections typically cause severe disease and abortion in livestock, especially sheep and cattle (Shoemaker et al. 2002). The 1974-76 major outbreak in South Africa was much more widespread than the 1950-51 but no estimates of the total losses attributed to the outbreak were ever tabulated (Swanepoel and Coetzer 2004).

People in an outbreak area are at an increased risk, particularly those who work directly with animals and their products and this poses an occupational risk (Shoemaker et al. 2002). The first documented outbreak of RVF outside of Africa was in the year 2000 and this devastated Saudi Arabia and Yemen (Shoemaker et al. 2002). A large scale epidemic in Kenya, Tanzania and Somalia in 1997-98 had an estimated human case count of more than 27 500 cases and more than 170 human deaths (Sang et al. 2010). In 1987, there was a massive outbreak reported along the Senegal-Mauritania border which affected 89 000 humans while the

outbreak in Saudi Arabia and Yemen in 2000 had an estimated 2000 human cases and 245 deaths (B. H Bird et al. 2008). In 2006-7, following heavy rainfall in East Africa, a heavy outbreak of RVF was recorded in Somalia, Tanzania and Kenya resulting in 1 062 human cases and 315 deaths (B. H Bird et al. 2008).

RVF occurs in South Africa as outbreaks interspersed with periods of absence and the inter-epidemic survival mechanism of the virus is not well understood but it is thought to include trans-ovarial transmission in the vector and low level transmission between the hosts and the vector (Pienaar and Thompson 2013). Outbreaks are precipitated by an explosion in vector numbers due to the abundant water as is the case with flooding, higher than normal rainfall or man activities like building dams (Al-Afaleq and Hussein 2011). Research has shown that RVF is likely to recur in areas where it has occurred before (Pienaar and Thompson 2013). The first documented occurrence of RVF in South Africa was in 1950 and several outbreaks have occurred since; however, a comprehensive record has not been compiled as the majority of the smaller outbreaks were never recorded (Pienaar and Thompson 2013).

From 1950 to 2011, one or more outbreaks of RVF have been recorded for 27 seasons. The major RVF outbreaks recorded in the 61 year period from 1950 to 2011 were in the years 1950-51, 1974-76 and 2010-2011. Some lesser epidemics of RVF that have been documented in South Africa were in the periods: 1952-53, 1955-59, 1969-71, 1981 and 1996 (Swanepoel and Coetzer 2004). In 2008-2011, in South Africa, 690 farms were confirmed positive for RVF and about 95% of these had the most susceptible hosts to RVF which were small stock (sheep and goats) and/or cattle with the remaining farms keeping wildlife and wild camelids (Métras et al. 2015). In 2008 from January to June, 13 outbreaks were recorded by the Department of Agriculture, Forestry and Fisheries (DAFF) of South Africa and these affected 4 provinces namely Gauteng, Mpumalanga, Limpopo and North-West, including an outbreak on a farm in the southern part of Limpopo, close to the border with North West and Gauteng provinces (Mapaco et al. 2012). The 2010-11 outbreaks were considerably larger and more devastating than the 2008-09; with more than 9000 head of livestock lost and 13 human fatalities recorded. The human case fatality was 13/302 (8%) (van den Bergh et al. 2019). In this outbreak, the eastern Free State province, areas of Northern Cape, Eastern Cape, Gauteng, and Mpumalanga provinces and the Western Cape were affected by the outbreak as shown in Fig 1 (Mapaco et al. 2012). Domestic livestock ruminants i.e. sheep, cattle and goats were affected as were different wildlife species were affected (Mapaco et al. 2012). The most recent outbreak recorded in South Africa was in 2018, affecting a single farm in the north-western Free State (van Vuren et al. 2019).

The inter-epidemic survival of RVFV has been the subject of much discussion and there have been several theories postulated. One of them is that it survives in the eggs of *Aedes* spp. mosquitoes but how widely this occurs in nature has not been determined (van den Bergh et al. 2019; Linthicum et al. 1985a). Alternatively, it has been suggested that there is low-level circulation of the virus between the mosquitoes and a yet to be identified reservoir of infection. Another theory is that there are areas that are suspected to be endemic for the virus which serve as sources of RVF outbreaks when infected hosts or vectors move from there (van den Bergh et al. 2019). Some of these areas are suspected to be in the low-lying parts of southern Africa, which include Mozambique as well as the north-eastern parts of South Africa. Seroprevalence of RVF has been shown to be 37% in cattle, 30% in African buffalo and 29% in domestic ruminants in the 7 provinces of Mozambique but despite the high seroprevalence, there have been very few outbreaks reported in the country (Moiane et al. 2017). In northern KwaZulu-Natal, adjacent to the Mozambique border, a high rate of seroconversion has been reported in domestic livestock (van den Bergh et al. 2019).

3.3. Pathogenesis and clinical signs

Infection with RVFV in animals is usually due to the bite of an infected mosquito, and in man, due to aerosol infection when handling RVFV-infected tissues. Transmission of the virus from the site of infection is thought to be via the lymph node with subsequent localization of the virus in regional lymph nodes, as for many other arboviruses (Flick and Bouloy 2005). Following replication in the regional lymph nodes, the virus spreads hematogenously to the target organs, mainly the liver, spleen and sometimes the brain (some animals and humans die of encephalitis) (Flick and Bouloy 2005). RVFV also replicates in the walls of the small blood vessels, adrenocortical cells and the renal glomeruli. In pregnant animals, the virus can also be found in the viscera and brain of the aborted foetus, as well as in the placenta (Flick and Bouloy 2005). Sero-sanguineous fluid found in the thorax of aborted foetuses has high concentrations of RVFV, up to 10^9 CFU/ml, but the low pH associated with advanced autolysis rapidly deactivates the virus and hinders viral isolation from many organs for diagnostic purposes (Flick and Bouloy 2005).

3.3.1. Rift Valley fever in humans

The incubation period of RVF is about 4 – 6 days in humans and is, in most cases, a self-limiting acute febrile illness that in only 1-2% of cases can advance to more serious and

potentially life threatening complications. These complications include hepatitis, retinitis, delayed onset encephalitis, blindness or a haemorrhagic syndrome that has a case fatality rate of 10-20% (Fig. 6) (B. H Bird et al. 2008; Ikegami and Makino 2011). Following incubation, the signs and symptoms of acute RVF in humans appear abruptly and include chills, dizziness malaise, weakness, severe headache, nausea and a feeling of “fullness” around the area of the liver (Ikegami and Makino 2011). These signs then progress to a fever of between 38.8 – 39.5°C, decreased blood pressure, pain in the back, neck shoulders or legs, shivering, rigor, constipation, insomnia and/or photophobia. Other signs and symptoms that are noted with less frequency are epistaxis, abdominal pain, vomiting and/or diarrhoea. Generally symptoms start to subside on the third day and usually by day 4, temperature will have dropped to normal (Ikegami and Makino 2011).

Some patients experience a relapse of fever 1-3 days after the initial drop in temperature and accompanying severe headache for another few days. Conversely, after the initial drop in body temperature and persistent leg aches for about fourteen days and persistent abdominal discomfort for several weeks . During the convalescence period, patients are often weak, have frequent headaches, are sweaty and experience severe ocular pain when they try to move their eyes and some degree of imbalance (Ikegami and Makino 2011; Pepin et al. 2010). A summary of clinical RVF in humans is shown in Fig. 5.

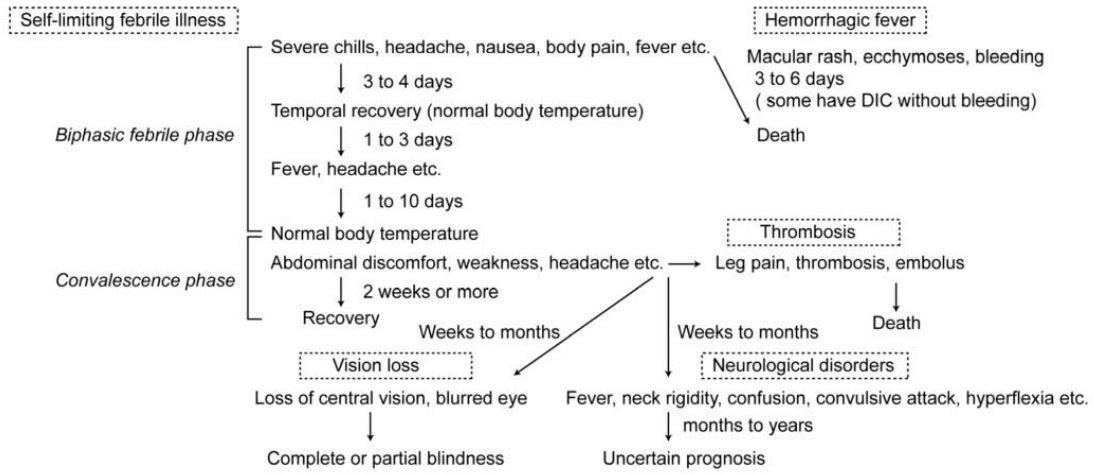

Fig 5. Summary of pathological forms of Rift Valley Fever in humans. Reproduced from Ikegami and Makino (2011).

3.3.2. Rift Valley fever in animals

Of the domestic animals, ruminants are especially susceptible to severe RVF clinical disease. Young lambs and goat kids less than 14 days of age are particularly vulnerable and mortalities

can approach 100% in outbreaks. Young calves also show severe clinical signs and mortalities can be in the region of 20 - 70%, which is similar to the mortality expected in adult sheep. Of the adult ruminants, cattle show the least severe clinical disease and in most outbreaks, adult cow mortalities are below 10%. RVF in animals is a disease that affects mainly the liver with rapid hepatocellular changes and subsequent extensive necrosis. This hepatic necrosis occurs in all species which are susceptible to RVF, including humans, and in many cases the extent of the hepatic damage can be so extensive that the liver's normal architecture is lost (Pepin et al. 2010).

Infections with RVFV lead to characteristic serum and haematological values and thrombocytopenia (Flick and Bouloy 2005). The primary pathology is hepatic necrosis which can be focal coagulative necrosis and the pattern of hepatocyte damage is centrilobular or midzonal. Extreme liver pathology is similar in new-born lambs, mice and hamsters which are very susceptible to infection with RVF (Ikegami and Makino 2011). The severe hepatic necrosis reduces production of coagulation proteins leading to disseminated intravascular coagulation (DIC) and impaired blood flow and this combined with the thrombocytopenia leads to development of the haemorrhagic syndrome (Flick and Bouloy 2005). Other pathology that has been noted is necrosis of the intestinal villi particularly in the jejunum and ileum and depletion of splenic lymphocytes (Ikegami and Makino 2011).

RVF causes infection that is acute and often fatal in young lambs and the associated clinical signs are severe in the young lambs and these appear quite abruptly. Viraemia which appears within about 16 hrs of peripheral infections and include fever of between 40 and 41°C, inappetence, lethargy with death following in 12 - 18 hrs after onset of clinical signs (Ikegami and Makino 2011; Musser et al. 2006). Mortality of lambs of age new-born to about 2 weeks of age can be between 95-100% (Ikegami and Makino 2011). The viraemia in older ruminant animals is shown to appear about 24 – 48 hrs post infection and lasts about 7 days (Coetzer 1982).

Mortality in sheep older than two weeks approaches 30% and abortion rates in pregnant ewes approaches 100% (Musser et al. 2006). Cattle are less susceptible than sheep and mortality is usually about 5% with some abortions. Older goats and buffalo can be affected, as can be dogs and cats but they tend to get an asymptomatic viraemia. Pigs and horses are resistant to infection but horses do get a transient while birds are refractory to (Musser et al. 2006). Camels get inapparent disease with occasional abortions. According to Coetzer and

Swanepoel (2004), studies have reported maximum viral titres up to $10^{10.1}$ Mouse intraperitoneal lethal dose 50 ($MIPLD_{50}$) for lambs, $10^{7.6}$ $MIPLD_{50}$ for adult sheep,$10^{7.6}$ $MIPLD_{50}$ for young calves, $10^{8.2}$ $MIPLD_{50}$ for goat kids, $10^{5.6}$ $MIPLD_{50}$ for adult goats and $10^{8.6}$ $MIPLD_{50}$ for humans.

The incubation period of RVF in animals is between 1 - 6 days depending on species and age, but new-born lambs are usually dead within 12 - 36 hours especially if they have no passive immunity from colostrum (Musser et al. 2006). Adult sheep and goats have an incubation period that is 3 days minimum and high rates of abortion at any stage of pregnancy while others do not exhibit any clinical signs at (Ikegami and Makino 2011; Moiane et al. 2017; van Vuren et al. 2019). Abortion rates in ewes are in the range of 40 – 100% and the aborted foetus is usually autolyzed and some cases have retained placentas which then leads to endometritis (Musser et al. 2006). Clinical signs in older sheep include a fever of between 40 - 41°C, loss of appetite, jaundice, weakness and some of them have a bloody nasal discharge. Between 20-30% of adults can die acutely and this represents a huge loss during periods of outbreak and the cause of death is severe hepatic necrosis and vascular collapse (Musser et al. 2006). Clinical signs in cattle are more or less similar but the morbidity and mortality are much lower and often the only discernible clinical sign is the fall of calving rate (Musser et al. 2006). In calves, clinical signs include a fever of between 40 and 41°C and death occurs about two to eight days after the onset of clinical signs with a mortality rate of about 10 – 70% (Swanepoel and Coetzer 2004). Adult cattle have a mortality rate of about 10% and again clinical signs include a fever of between 40 and 41°c, excessive salivation and anorexia, foetid diarrhoea, weakness and a sharp drop in milk production with abortion rates in pregnant cows approaching 85% (Swanepoel and Coetzer 2004). The duration of illness is much longer in calves compared to adults and many of these calves develop icterus secondary to the hepatic damage (Swanepoel and Coetzer 2004). The factors involved in the fatal outcome of RVF in animals are anaemia, hypovolaemic shock and hepatic failure (Coetzer 1982).

3.4. Diagnosis

Considering the association between RVF and heavy rainfall patterns, high mosquito density as well as an outbreak of abortions, mortality of young animals and illness of farm workers, RVF should always be considered under these circumstances especially in sub-Saharan Africa where the disease is endemic (Flick and Bouloy 2005; Swanepoel and Coetzer 2004). A variety of techniques are available for the diagnosis of RVF and these can be broadly classified into virus isolation, antibody demonstration, histopathology and nucleic acid amplification and detection (Pepin et al. 2010). Under outbreak conditions, RVF should be demonstrated by

virology and serological techniques in tandem (Arricau-Bouvery and Rodolakis 2005).

3.4.1. Direct methods of RVF diagnosis

Historical diagnosis of RVF used to be done by inoculating live lambs with infectious sera and then this technique was modified to use mice as they were shown to be just as susceptible to infection with RVFV (Flick and Bouloy 2005). Modern day RVF virology isolation techniques have done away with animal testing completely in most cases and use tissue cultures instead which include green monkey kidney and mosquito cell cultures (Flick and Bouloy 2005). Virus isolation by tissue culture cultivation and virus neutralization tests is the method of choice for diagnosing RVF in suspect diagnostics samples (Flick and Bouloy 2005). Virus isolation on its own is expensive to perform and is quite lengthy in terms of time required to complete the process and this is a constraint on regulatory officials in epidemic conditions where a prompt diagnosis is required (Pepin et al. 2010). Virus neutralization test (VNT) can only be performed when standard stocks of live virus and tissue culture are available, and because of this, it is rarely used, and even when considered, it requires highly specialized laboratories; VNT is very specific and has very little cross reaction with other phleboviruses (Pepin et al. 2010).

For diagnostic purposes, a good indication for the presence of RVFV is always provided at histopathology using electron microscopy and indirect IFT. The hepatocytes appear “disorganized” with rod-like structures in the nuclei and this comprise the non-specific structural (NSs) protein (Flick and Bouloy 2005; Ikegami and Makino 2011).The filamentous appearance of these intra-nuclear inclusion bodies is attributed to the presence of terminal 10 to 17 amino acids at the carboxyl end of the chain. These carboxyl ends self-associate to give the characteristic filamentous appearance which are pathognomonic, especially in the livers of baby lambs (Ikegami and Makino 2011; Swanepoel and Coetzer 2004).

3.4.2. Indirect techniques for RVF diagnosis

Evidence of infection can also be demonstrated by serological techniques which specifically detect RVFV-specific IgG and IgM for serum samples derived from humans and animals (Flick and Bouloy 2005). ELISAs are used in many reference laboratories and are preferred over many other serological techniques such as CFT, HAI and plaque-reduction neutralization (Flick and Bouloy 2005).

For laboratory diagnosis of RVF, serum or heparinized blood should be collected from live animals or tissues such as the liver, spleen, kidney, lymph nodes, heart blood or brain of aborted foetuses (because it is usually less autolyzed than the other tissues) should be collected in dead animals (Swanepoel and Coetzer 2004). Specimens must be securely packaged at transported under fridge conditions at 4°C, or if fridge conditions are not available, tissues can be transported in glycerol-saline solution at atmospheric temperature, while tissues for histopathology can be transported in 10% formalin (Swanepoel and Coetzer 2004). In animals that survive the disease, paired serum samples collected three weeks (at disease onset and then 3 weeks later)apart can be collected for serology and these should demonstrate a four-fold increase in antibody titres (Swanepoel and Coetzer 2004). IFT can be used to rapidly detect viral antigen in impression smears of infected tissues while RVF CFT can be used to detect viral antigens in tissue suspensions as can RVF immunodiffusion (Swanepoel and Coetzer 2004).

Immuno-peroxidase (IP) staining can be used for tissue sections while ELISA and haemagluttination (HGT) can be used to detect antigens in serum samples (Swanepoel and Coetzer 2004). RVF RNA detection by hybridization with radio-labelled DNA is not as sensitive as virus isolation or antigen ELISA (Swanepoel and Coetzer 2004). Viral RNA can be readily detected in serum and body tissues using vero cells, baby hamster kidney 21 (BHK21), CER cells, mosquito cell lines, calf/ lamb/goat kidney or testes cell lines (Swanepoel and Coetzer 2004). Although modern day diagnostics are moving away from laboratory animal testing, suckling or weaned mice and hamsters can be infected intra-peritoneally or intracerebrally to amplify the virus and the intracerebral route is the more preferred with infected mice and hamsters dying with two to five days of infection (Swanepoel and Coetzer 2004). In cell cultures, cytopathic effect is observed about one to three days post inoculation with virus but virus identification can be done much earlier at about 24 hrs post-inoculation thereby accelerating diagnosis using immunofluorescence (Swanepoel and Coetzer 2004). If mosquito cell lines are being used, cytopathic effect is not always present and as such IFAT needs to be run on the samples to confirm or deny the presence of RVF virus post inoculation (Swanepoel and Coetzer 2004).

Most serological tests for RVF can be done using inactivated antigen except the virus neutralization tests and these are safer to conduct if the required biosecurity facility is not

available (Swanepoel and Coetzer 2004). RVF serology, just like that of other diseases, always has the draw-back of distinguishing if seroconversion is due to current, recent or past infection. As such, paired serum samples separated by a period of three weeks with the first one being collected in the acute stage of the disease and the subsequent sample being collected in the convalescent period and there should be at least a fourfold increase in RVFV immunoglobulin between the two samples (Swanepoel and Coetzer 2004). IgM capture ELISA allows detection of recent infection to be done on a single serum simple for both human and animal samples (Swanepoel and Coetzer 2004). A RVF antigen capture ELISA has been developed, but it poses a biosafety risk for personnel since it uses live antigen (Pepin et al. 2010).

The ever present risk of laboratory infection of personnel working with RVF samples has necessitated development of the sandwich ELISA (sAG-ELISA) which is entirely safe and it can be routinely used for surveillance and diagnosis in non-endemic areas (Pepin et al. 2010). The sAg-ELISA is used for the detection of nucleocapsid (N) protein in specimens that have been deactivated by heat of 56°c for 60 minutes in the presence of Tween 20 (Pepin et al. 2010). The detection limits for the sAg-ELISA range from $\log_{10}10^{2.2} – 10^{3.2}$ $TCID_{50}$ per reaction volume (Pepin et al. 2010). The diagnostic sensitivity and specificity of sAg-ELISA when compared to virus isolation is 67.7 – 70% and 97.9 – 100% respectively (Pepin et al. 2010).

Several other RVF-ELISAs have been developed for the detection of RVFV IgG and IgM based on inactivated antigens from tissue culture (Pepin et al. 2010). This added advantage of safety for personnel strongly motivates for their replacement of traditional diagnostic methods which use live antigen and pose a risk to personnel working with diagnostic samples (Pepin et al. 2010). Production of antigen for these modern ELISAs requires biosecurity level 3 laboratories because of the use of live organism but recently, an ELISA based on recombinant nucleoprotein (recNP) has been developed for safe use in humans and animals (Pepin et al. 2010). The nucleocapsid is quite specific for the family Bunyaviridae and no other pathogens except African phleboviruses can obscure RVF diagnosis by this technique (Pepin et al. 2010). Nucleoprotein is the most abundant and immunogenic component of the RVF virion and it can now be safely mass produced in a highly purified soluble form. This allows for cheaper, automated mass screening of sera without the risks of using live antigen and thus allowing routine use of the ELISA in areas that are traditionally RVF free (Pepin et al. 2010).

Most ELISAs utilize only inactivated virus as antigen for the detection of RVF antibodies in

ELISA and recombinant techniques have been used to detect antibodies to various members of the family Bunyaviridae which includes the RVF virus (Jansen van Vuren et al. 2007). The RFV i-ELISA is one of the simplest available tests for the detection of RFV antibodies (Jansen van Vuren et al. 2007). The wider use of the i-ELISA in routine diagnostics has been hampered by use of semi-purified or unpurified antigen causing an unspecific signal in the test reading (Jansen van Vuren et al. 2007). The cost and effort of producing highly purified viral antigen is outweighed by producing equal amounts of recombinant antigen which is faster and cheaper (Jansen van Vuren et al. 2007). Recombinant antigens are much safer as they are not infective and are also very stable (Jansen van Vuren et al. 2007). The working dilutions of IgM i-ELISA and IgG i-ELISA for RVF are 1 : 500 and 1 : 2000 respectively and as such, a 2.5mg batch of pure antigen is sufficient to test 9000 and 36 000 serum samples for IgM and IgG i-ELISAs respectively (Jansen van Vuren et al. 2007). The RVF i-ELISA has strong correlation of results with the RVF VNT which is the reference test.

According to Paweska *et al* (2005), RVF i-ELISA based on N protein is more sensitive than classical serological tests in sheep sera (Jansen van Vuren et al. 2007). Van Vuren *et al* (2007) have argued the potential of RVF IgM and IgG i-ELISA based on N protein as replacements for classic diagnostic methods which cannot differentiate classes of immunoglobulins and always carry the biosafety risk for the diagnostic personnel. The IgG i-ELISA has a lower diagnostic specificity compared to the VNT because the ELISA detects antibodies against all components of the virion. VNT, on the other hand, only detects antibodies against the viral neutralizing epitopes and this may account for the lower diagnostic specificity of the IgG i-ELISA in the study by Paweska et al (2008). According to Van Vuren *et al* (2007), the igG i-ELISA based on RNP was more sensitive than VNT in detection of early immune response in sheep infected with live attenuated Smithburn strain of the virus (Paweska et al. 2008).

Neutralizing antibody in RVF infection is detectable as early as three days post infection and by day four to six, the antibody is detectable by haemagglutination inhibition (HAI), immunodiffusion, ELISA and indirect IFT while the earliest the CFT is of any diagnostic value is day nine post infection (Swanepoel and Coetzer 2004). CFT and immunodiffusion (ID) produce the lowest antibody titres of all the serological tests and are maximally effective at two to six weeks post infection and then barely useful after six months (Swanepoel and Coetzer 2004). CFT for RVF is not recommended for routine field diagnostics due to the lability of the CFT test, but however, high antibody titres are useful at indicating recent infection (Swanepoel and Coetzer 2004). HAI, ELISA, indirect IFT and virus neutralization tests are effective from about two weeks to about six months post infection and then the antibody titres steadily decline

over several years but neutralizing antibody is most likely demonstrable for the rest of the individual's life (Swanepoel and Coetzer 2004). A study on 195 cattle in Madagascar in 1991 suggested that within two months, less than 30% had demonstrable IgM antibody and this had completely disappeared by six months (Swanepoel and Coetzer 2004).

RVF serology in theory has the risk of cross-reaction with other phleboviruses also known to occur in Africa such as the Sandfly fever virus, St. Floris viruses, Gabek-forest virus and Arumowot viruses among others and thus the virus neutralization test which have the lowest cross reactivity would seem more suitable for diagnostic use (Swanepoel and Coetzer 2004). Establishing definite diagnosis of RVF induced teratology is difficult as there are various other teratogenic viruses in domestic animals, and furthermore, foetuses that are not immunocompetent at the time of teratogenic infection and as such when tested will be negative for both antibody and virus and presently the suspicion is based on circumstantial evidence (Swanepoel and Coetzer 2004).

In cases of epidemics, RVF nucleic acid assays allow for the rapid detection of RVF and these are highly sensitive reverse transcriptase polymerase chain reaction (RT-PCR) and real-time detection polymerase chain reaction (RTD-PCR) using Taqman probes (Pepin et al. 2010). Recent developments have given birth to real-time reverse-transcription loop mediated isothermal amplification (RT-LAMP) which targets the L segment of the RVF RNA and the RT-LAMP technique diagnostic limit was 0.065$TCID_{50}$ per reaction volume and approximately ten copies of RNA per assay (Pepin et al. 2010). Comparative studies of RVF RT-LAMP Taqman - based RTD-PCR and virus isolation techniques showed 100% agreement (Pepin et al. 2010). The assay is very sensitive and specific for the detection of RVF RNA in clinical samples suspected to contain RVF virus for both humans and animals (Pepin et al. 2010). The RT-PCR has an added advantage of being cheap and convenient to use as it presents as a portable device and can be easily used in resource constrained countries and communities (Pepin et al. 2010). The first time that quantitative RT-PCR was used for case confirmation was in the 2006 RVF outbreak in Kenya. It should be noted however, that the inclusion or exclusion of RVF virus, just like with all viral haemorrhagic fevers, as causative agent of disease should not rely solely on nucleic acid detection, but should be run in conjunction with type specific antibodies in neutralization tests (Pepin et al. 2010).

The viraemia associated with RVF is of short duration in adult ruminants and as such nucleic acid detection is not reliable after the fever stage but IgG and Ig M start to elevate a few days post infection (Pepin et al. 2010). Nucleic acid detection assays require sophisticated laboratories and highly trained personnel and these are not always available in remote areas during periods of outbreak (Pepin et al. 2010). A variety of immunological techniques are available for rapid detection including agar gel immunodiffusion (AGID) with homogenized tissue, immunostaining of impression smears and cryostat hepatic samples, spleen or brain (Pepin et al. 2010). IFT assays have been developed, utilizing IgG monoclonal antibodies for virus specific antigens, but these tests carry with them the disadvantage of requiring tissue culture of virus for amplification. This poses a biosecurity risk for personnel as live antigen is a requirement. However, despite the risk, RVF IFT has been said to be very reliable (Sissoko et al. 2009).

The ID Screen® Rift Valley Fever Competition ELISA is an indirect serological test which may be used on ruminants, horses, dogs and other species. It contains no live pathogen but rather the RVFV nucleoprotein (NP). This ELISA test kit was found to have sensitivity and specificity both of 100% (Comtet et al. 2000). The ELISA kit is particularly easy and ready-to-use components and results can be obtained in as little as 60 minutes (Comtet et al. 2000).

3.5. Prevention and control

The association of RVF outbreaks and abnormally high rainfall offers epidemiologists some ability to predict anticipated periods and regions with increased disease risk. Identification of these presents an opportunity to mitigate losses by targeted vaccination in these areas with effective vaccination (B. H Bird et al. 2008). Vaccination remains the only real practical and effective way to prevent RVF in domestic ruminants (Pepin et al. 2010). The first RVF vaccine was developed by Smithburn and the vaccine is still in use today. The Smithburn vaccine was developed initially in Uganda by 82 intracerebral passages in mice to attenuate virulence and then further passages were done in South Africa in mice and embryonated eggs before being registered as an experimental vaccine in 1951 (Pepin et al. 2010; Swanepoel and Coetzer 2004). However, the attenuation was not complete and it has been shown to cause abortions in domestic animals and foetal teratogenicity when used in pregnant animals. It is suggested that the Smithburn strain of the RVF vaccine only be used in the face of devastating outbreaks and only in non-pregnant females (Pepin et al. 2010).

Due to the limitations of the modified Smithburn vaccine, an inactivated vaccine has been

developed to allow for safer vaccination but the inactivated vaccine does not confer long lasting immunity as seroconversion produces lower titres than the live attenuated vaccine and boosters will be required (Musser et al. 2006; Pepin et al. 2010). The Smithburn vaccine is cheap, can be mass-produced and induces long-term immunity in sheep six to seven days post vaccination. Considering that the Smithburn vaccine is not completely attenuated, there is a theoretical risk that it could revert to virulence and precipitate outbreaks if mosquitoes feed off vaccinated animals in countries where RVF is not endemic (Swanepoel and Coetzer 2004).

The United States Army Research Institute of Infectious Diseases (USAMRIID) developed a vaccine that was attenuated with florouracil called the MP12 using the ZH548 strain of the virus which was responsible for the Egyptian outbreak. The resultant immunity was good but field trials of the vaccine conducted in South Africa again caused abortions and teratogenicity with a prevalence of up to 14% in ewes vaccinated in the first trimester of pregnancy (Pepin et al. 2010; Swanepoel and Coetzer 2004). Ruminant foetuses in advanced pregnancies do not develop teratogenic effects because the process of organogenesis would have already have been completed (Swanepoel and Coetzer 2004). The MP12 is still being considered as a human and animal vaccine for RVF despite being shown to be neuro-virulent in hamsters (Pepin et al. 2010). The Clone 13 has been shown to be avirulent and is an alternative based on another isolate of RVF and the avirulence is attributable to a NSs protein depletion (Coetzer 1982; Pepin et al. 2010). The vaccine imparts good immunity through high antibody titres, is non-abortifacient and non-teratogenic in pregnant ruminant animals and does not revert back to virulence due to the deleted code for the virulence factor (Pepin et al. 2010). The Clone 13 vaccine has been documented as conferring up to 87.5% protection against abortion in pregnant animals challenged with field strains of the virus. Clone 13 is a natural virus originating from a mild RVF genetic strain and genetic manipulation has allowed for production of similar or different viruses through reverse genetics to shut down its pathogenicity. Another modified vaccine developed is the rMP12 which was developed by inserting a Clone 13 mutation into the S segment or splicing out the NSs sequence all together from /mp12 or removal of the virulent backbone from ZH501 (Pepin et al. 2010). Interestingly, with all attenuated vaccines, there are missing genes when compared to field strains and this has led to postulations about the laboratory ability to differentiate infection immunogenicity versus vaccine immunogenicity (Pepin et al. 2010). This has naturally led to the development of the NSs ELISA whose reliability is still under investigation (Pepin et al. 2010).

In outbreak-prone areas in sub-Saharan Africa, it is advised to vaccinate offspring of immunized ewes at six months of age when passive immunity has lapsed with a single dose of modified live Smithburn vaccine and this affords life-long protection. Lambs and kids from susceptible dams can be immunized at any age (Swanepoel and Coetzer 2004). The modified Smithburn vaccine is said to induce poor immunity in bovines and thus it is recommended that they are vaccinated with formalin inactivated vaccine, then a booster three to six months later and then annual boosters (Swanepoel and Coetzer 2004). According to Coetzer and Swanepoel (2004), in cases of outbreaks, control measures over and above the vaccination which can be considered to minimize mosquito bites include burning of grass in dambos to reduce mosquito egg viability, strategically placement larvicides in water to kill mosquito larvae. Chemical control of adult mosquitoes with insecticides can also be instituted and the moving of livestock from low lying areas to higher altitudes to reduce mosquito populations. Confinement of animals in mosquito-proof housing where possible is recommended but not practical in most African settings.

3.6 Problem statement

Q fever is an important zoonotic disease, outbreaks in humans can have significant economic impact through lost hours of work due to illness, and mortalities can also result especially in people with pre-existing heart disease. Aerosol from infected domestic livestock is the biggest route of infection and establishing the seroprevalence in animals will feed into establishing the risk for humans who interact closely with infected livestock. Rift Valley fever continues to cause massive livestock losses during outbreaks and human fatalities are also incurred. The inter-epidemic period is of particular interest and evidence of exposure in different parts of the country in between outbreaks might shed more light on this critical component of epidemiology.

Our study area in Moretele municipality which is in the central to part of South Africa, has very little information on the occurrence of RVF even though suitable floodplain habitats exist for the *Aedes* spp. mosquitoes. The Moretele municipality is in close proximity to the floodplains of the Apies, Moretele and Tshwane rivers and this may be a risk area for exposure to RVFV. In 2008 an outbreak occurred at Bela Bela, close to the study area. Whether or not *C. burnetii* or RVFV occur in the study area, and their respective seroprevalences, are unknown.

4. Aims and objectives

4.1. Aim

To determine if there is any serological evidence of *Coxiella burnetii* and Rift Valley fever virus in goats in Moretele district and to identify factors associated with seropositivity.

4.2. Main objectives

Objective 1: To determine the seroprevalence of *C. burnetii* in goats in the Hammanskraal communal area.

Objective 2: To determine the factors associated with presence of antibodies to *C. burnetii* in goats in Hammanskraal.

Objective 3: To determine the seroprevalence of Rift Valley fever virus in goats in the Hammanskraal communal area.

Objective 4: To determine the factors associated with presence of antibodies to Rift Valley fever virus in goats in Hammanskraal.

5. Materials and methods

5.1. Study area

Eight villages were selected for sampling from two state veterinary areas in the Moretele municipality. Goat population statistics obtained from two animal health technicians responsible for animal health in the selected state veterinary areas. Only villages where several goat owners owned five goats or more were included and the goat owners were put on a list of premises for sampling. The selected villages were Dertig, Thulwe, Kontant, One and ten, Makapanstad, Tladistad, Dikebu and Moretele; these selected villages were all in the Moretele municipality in North West area.

The villages in the study area is shown in the image below:

Fig 6. Map of study area showing the different villages

5.2. Study design

A cross-sectional study was designed to estimate the seroprevalence of both Q fever and RVF using multi-stage random sampling. Random sampling of households followed by convenience sampling of animals within households was conducted.

5.3. Sample size

Sample size (n) was calculated based on the equation for a sample size to estimate prevalence in an infinite population, assuming simple random sampling (Thrusfield 2007):

$$n = \frac{1.96^2 \mathrm{P}_{\mathrm{exp}}(1 - \mathrm{P}_{\mathrm{exp}})}{\mathrm{d}^2}$$

where:

- *n* = required sample size
- P_{exp} = expected prevalence
- d = desired absolute precision

At level of confidence of 95%, expected prevalence of 20% (P_{exp} = 0.2) and desired absolute precision of 7.5% (d = 0.075), the minimum sample size for *C. burnetii* testing was calculated as 110 goats, assuming simple random sampling.

At level of confidence of 95%, expected prevalence of 10% and desired absolute precision of 5%, the sample size for Rift Valley fever is 139 goats, assuming simple random sampling.

For the multistage survey design, assuming an average cluster size (*m*) of 5 animals per farm, and an intraclass correlation coefficient (ρ) of 0.1 for RVF and 0.2 for *C. burnetii*, the sample size is multiplied by the design effect (*D*) as follows (Bennett et al. 1991):

For *C. burnetii*: $D = 1 + \rho(m - 1) = 1 + 0.2(5 - 1) = 1.8$, therefore sample size = 110 × 1.8 = 198. For RVF: $D = 1 + \rho(m - 1) = 1 + 0.1(5 - 1) = 1.4$, therefore sample size = 139 × 1.4 = 195. Therefore, overall sample size for the study was calculated to be a minimum of 200 goats.

From the list of goat owners in each village compiled by animal health technicians, farmers were randomly selected. In each herd, animals were selected by convenience sampling stratified by age, i.e. selecting animals from 3 different age (0 - 6 months, 7 – 19 months, >19 months).

5.4. Blood sample collection

Clinically healthy animals were enrolled into the study. Blood collection was by jugular venepuncture with a 20-gauge needle vacutainer into an evacuated tube without anticoagulant 10 ml of blood were collected from each goat into a 10 ml serum tube which was then labelled with a sample number. Once clotted, the blood samples were transported on ice in a cooler-box to the University of Pretoria, Faculty of Veterinary Science in Onderstepoort where they were centrifuged at 1500 G for 10 minutes at room temperature and separated on the same day as collection.

5.5. Questionnaire

A questionnaire (Appendix 6) was used to collect data from goat owners. The questionnaire collected information such as age, sex, breed, history of kidding, history of abortion and the origin of each animal was determined. Management practices such as dipping of goats, routine buying and selling of goats, use of injectable tetracyclines in the goat herds as well as the vaccination against RVFV among others. Prior to administration of the questionnaire and blood collection from the goats, goat owners had to give informed written consent by way of a consent form (Appendix 7).

5.6. Laboratory analysis

Testing for *C. burnetii* antibodies was done using the LSIVet^{TM} Ruminant Q fever - serum/Milk (Life Technologies, Carlsbad, California USA) which is now being marketed by Thermofischer as Priocheck (Leylstad, Netherlands). This is an indirect ELISA kit for the detection of phase 1 and phase 2 anti-*C. burnetii* antibodies in ruminant serum or milk. The test was conducted in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, according to manufacturer's instructions. Following the antibody-antigen reactions, the ELISA plates were read by spectrophotometry at 450nm (monochromatic reading) on a microplate reader within 30 minutes of completing the test as per manufacturer's instructions. The average optical denstity (OD) of the positive control [PC (ODm PC)], and that of the negative controls [NC (ODm NC)] were calculated. For each sample, the Sample/Positive ratio (S/P ratio) was then calculated as: S/P = (OD Sample – ODm NC) / (ODm PC – ODm NC) where OD_{Sample} was the optical density of each sample tested. The Q fever antibody titer was then calculated as follows: Titer = S/P x 100 the interpretation of these results were antibody titers less than or equal to 40 were negative, titers between 40 – 100 were mild positives, 100 – 200 were moderate positives and greater than 200 were strong positives.

Rift Valley fever virus antibody testing was done by ID Screen® Rift Valley Fever Competition ELISA test kit (IDVet, Grabels, France). The test was run in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria. The ELISA was run according to manufacturer's recommendations and following the antibody-antigen reactions in the test, the results were interpreted according to the test data sheet. The optical density of each sample was read and recorded at 450 nm on a microplate reader. The OD was then

converted to S/N% based on the equation: S/P ratio (OD_{sample}/OD_{NC}) x 100% . The S/N% was then interpreted as less than or equal to 40 being positive, greater than 40 and less than or equal to 50 was doubtful and greater than 50 was negative for RVF antibodies as per manufacturer's guidelines.

Samples that tested positive and suspect-positive for RVFV antibodies on the IDVet ELISA were further tested using the virus neutralization test (VNT) which is the gold standard, in Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria. This was an in-house test adapted from the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 8th edition (2018), using the Smithburn vaccine virus.

5.7. Data analysis

Questionnaire data and serological test results were entered into a Microsoft Excel (Microsoft Corporation, Redmond, CA, U.S.A.) spreadsheet. After data cleaning it was transferred to Stata 14 (StataCorp, College Station, TX, U.S.A.). Prevalences and exact 95% confidence intervals were calculated, with standard error adjustment for the clustered sampling using the *svy* commands in Stata 14. The intracluster correlation coefficient (ICC or ρ) was calculated as follows (Fleiss et al. 2013):

$$\rho = \sum_{i=1}^{K} \{Y_{i+}(Y_{i+} - 1) - 2p(n_i - 1)Y_{i+} + n_i(n_i - 1)p^2\}$$

where K is the number of flocks, Y_{i+} is the number of seropositive animals in flock i, n_i is the number of animals tested in flock i and p is the overall seroprevalence. Univariate analysis of potential risk factors for seropositivity was done by cross-tabulation and the Fisher's exact test. Factors associated with the outcome at $p<0.25$ were selected for inclusion in multivariable logistic regression models, which were then developed by backward elimination until variables remaining in the model were significant at $p<0.05$. Herd was included as a random effect and adjustment for the multistage survey design was done using the *svy* command in Stata. Univariate associations between seropositivity and its potential consequences were assessed using logistic regression and odds ratios were calculated.

6. Results

A total of 216 goats belonging to 39 goat owners (5.5 goats per herd) were sampled across 8 villages, namely Dertig (3 goat owners), Thulwe (5 goat owners), Kontant (4 goat owners), One and ten (4 goat owners), Makapanstad (6 goat owners), Tladistad (4 goat owners), Dikebu (8 goat owners), and Moretele (5 goat owners). The locations of the herds are shown in Fig. 7.

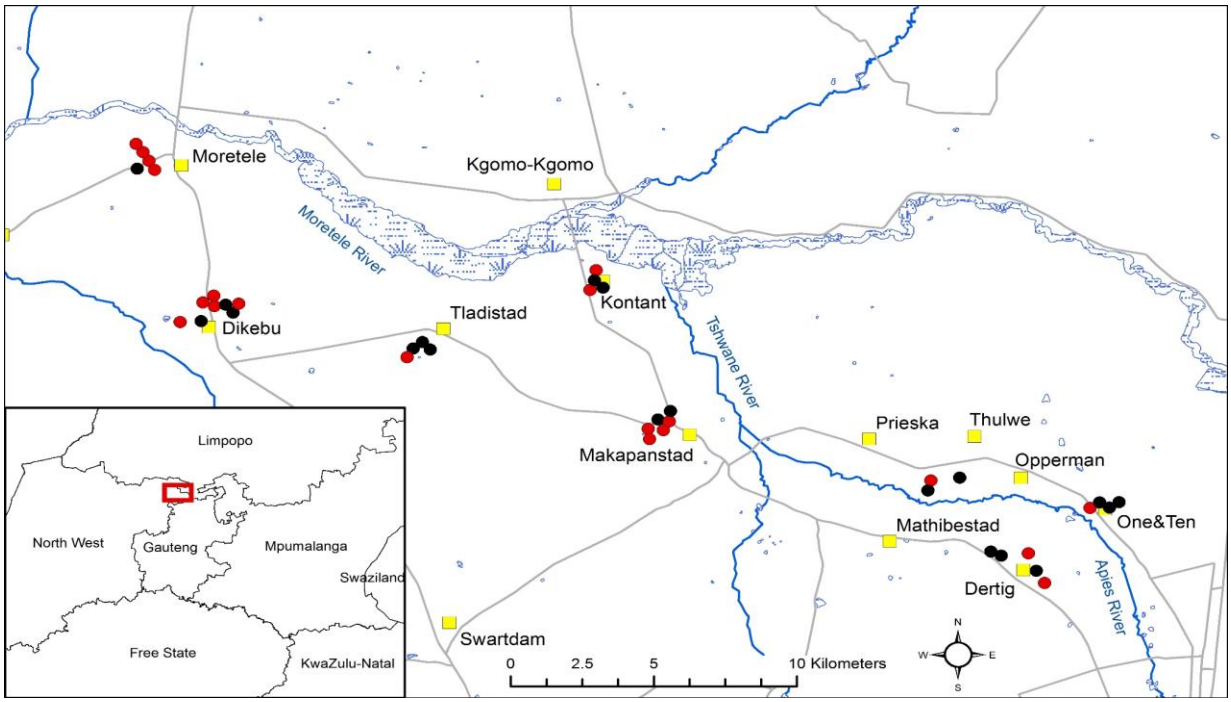

Fig 7: Locations of goat flocks that were tested for antibodies to *Coxiella burnetii* and Rift Valley fever virus. Red dots indicate properties on which *C. burnetii* positive goats were found and black dots are *C. burnetii* negative properties

The total number of *C. burnetii* antibody positive goats was 32/216 (estimated animal prevalence 16%, 95% CI: 10.6 – 23.6%) and this prevalence was adjusted for clustering. These goats belonged to 20/39 goat owners (51% herd prevalence; 95% CI: 35-68%). The intraclass correlation co-efficient (ICC) was 0.06, indicating low to moderate clustering of *C. burnetii* within the tested goat herds.

The total number of RVFV antibody positive goats on the IDVet® Rift Valley fever ELISA was 1/216 and 3 other samples tested as doubtful. The positive sample and the negatives were further analysed using the RVF gold standard diagnostic test, which is VNT, which revealed

0/4 samples to be positive. Therefore, the estimated RVF seroprevalence in the study area was 0% (95% CI: 0 - 1.4%).

Univariate associations ($p<0.2$) of potential predictor variables with *C. burnetii* seropositivity were found for age category ($p = 0.001$) and breed ($p = 0.159$) (Table 1). These variables were therefore selected for inclusion in the multivariable model. Due to small category sizes, breed was re-categorised as Boergoat, Mixed breed and Other (included Kalahari red, Angora and Saanen) (Table 1).

Table 1. Factors associated with seropositivity to *Coxiella burnetii*: univariate analysis

Variable and level		n	% positive	p value	OR
Herd size				0.764	
	<15	70	17		1 (base)
	15-19	79	13		0.7
	>19	67	15		0.9
Age class				0.001	
	1 (0 – 6 mths)	67	6		1 (base)
	2 (7 – 19 mths)	64	9		1.6
	3 (> 19 mths)	85	26		5.5
Sex				0.286	
	female	156	10		1 (base)
	male	60	17		0.6
Breed				0.159	
	Angora	1	100		1 (base)
	Boergoat	46	11		0.7
	Kalahari Red	18	22		1.6
	Mixed	143	15		1
	Saanen	8	0		1
Origin				0.233	
	Born on premises	202	16		1 (base)
	External origin	14	0		1
Tetracyclines given in past 12 months				0.684	
	No	145	16		1 (base)
	Yes	71	13		0.8
Buy animals					
	No	114	15		1 (base)
	Yes	102	15		1
Dipping					
	No	64	14		1 (base)
	Yes	152	15		1.1
Total		**216**	**16**		

The final multivariable, mixed-effects logistic regression model of factors associated with *C. burnetii* seropositivity is shown in Table 2. Odds of seropositivity to *C. burnetii* increased significantly with age, with goats >19 months old being 6.6 times more likely to be seropositive than goats 0 – 6 months old (p = 0.010). The odds of seropositivity to *C. burnetii* varied by breed and the breeds classified as “other” (Kalahari red, Angora and Saanen) were 6.3 times more likely to be seropositive than Boergoats (p = 0.033) (Table 2).

Table 2. Mixed-effects logistic regression model of factors associated with *Coxiella burnetii* seropositivity

Variable and level	Odds ratio	95% CI	p-value
Age class			
0 – 6 months	1 (base)	–	–
7 – 19 months	1.7	0.3 - 8.5	0.497
>19 months	6.6	1.6 - 26.8	0.010
Breed			
Boergoat	1 (base)	–	–
Mixed	2.6	0.6 - 11.6	0.203
Other	6.3	1.2 - 33.6	0.033

Univariate analysis showed an association between history of individual goat abortion and *C. burnetii* seropositivity (p = 0.051) (Table 3). The odds of goats in herds that experienced more than 2 abortions in the last 12 months to be seropositive were higher than those of those that had experienced no abortions (p = 0.007) (Table 3). Univariate associations of each potential consequence of infection with *C. burnetii* seropositivity are shown in Table 3. Goats from herds with mortalities in the last 12 months tended to be less likely to test *C. burnetii* positive than goats from herds without mortalities .

Table 3. Consequences associated with seropositivity to *Coxiella burnetii*

Variable and level		n	% positive	p-value	OR
Goats with history of kidding					
	No	26	15		1
	Yes	83	22	0.487	1.5
Goats with history of abortion					
	No	101	18		1
	Yes	8	50	0.042	4.6
Abortion Herd 12 months					
	No	139	19		1
	Yes	77	12	0.154	1.7
Herd number abortions 12 months					
	0	139	12		1
	1 - 2	50	16	0.501	1.4
	>2	27	26	0.071	2.5
Retained Foetal Membranes Herd					
	No	210	14		1
	Yes	6	33	0.216	3
Herd tetracyclines 12 months					
	No	145	16		1
	Yes	71	13	0.537	0.8
Herd sell animals					
	No	33	18		1
	Yes	183	14	0.555	0.8
Mortality herd 12 months					
	No	94	20		1
	Yes	122	11	0.119	0.5
Herd slaughter and consumption					
	No	54	17		1
	Yes	162	9	0.191	2
Total		**216**	**16**		

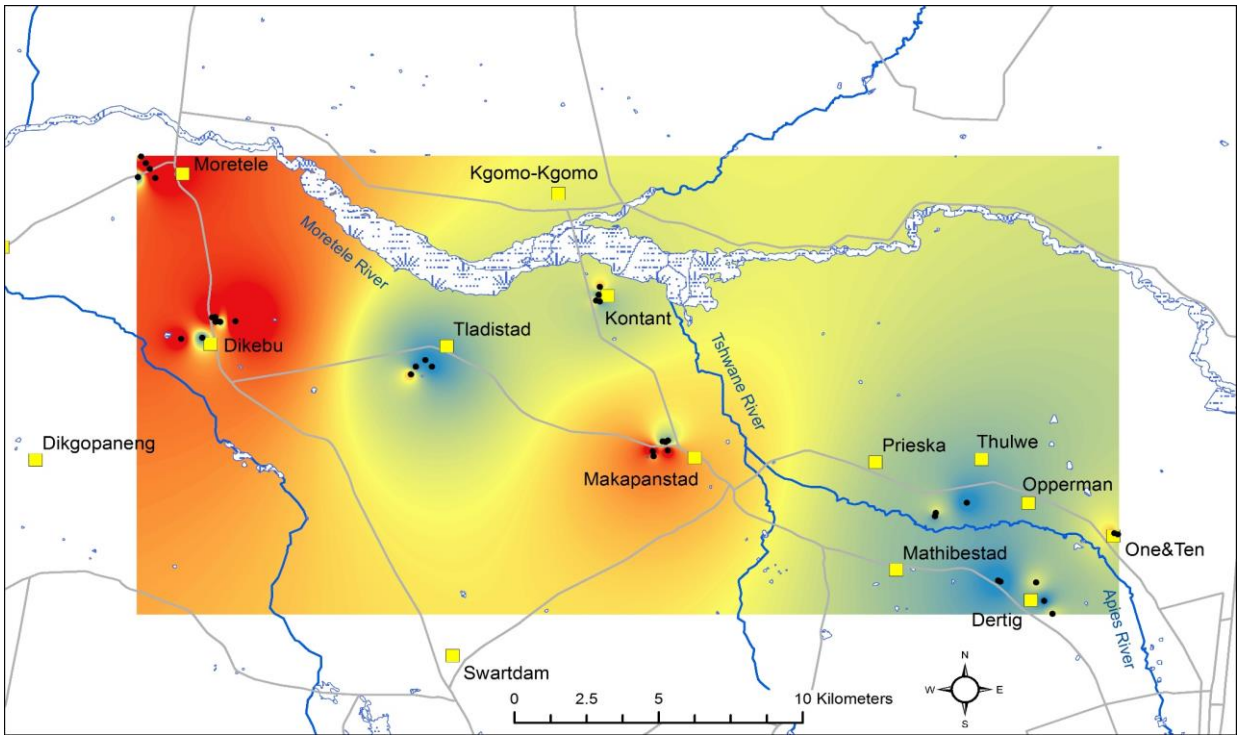

Fig 8. Risk surface for *C. burnetii* in the study area created by interpolation using inverse distance weighting. Blue represents low seroprevalence and red indicates high seroprevalence.

Fig. 8 shows a risk surface for *C. burnetii* in the study area, using the 31 measured within-herd seroprevalences and interpolation using inverse distance weighting. The *C. burnetii* seroprevalence appeared to vary geographically (Fig. 8), with animals more likely to test positive in the north-west of the study area. The highest seroprevalence was in the vicinity of Makapanstad and Dikebu villages. Animals in the Thulwe and Opeprman villages were less likely to test *C. burnetii* positive.

7. Discussion

This study showed that exposure to *C. burnetii* was widespread in the study area, with estimated animal level and herd-level seroprevalences of 16% and 51%, respectively. In this study, the individual animal Q fever seroprevalence was found to be 16% (adjusted for clustering). Menadi et al (2011) conducted a two-year serological survey in cattle in north-east Algeria, reporting an animal seroprevalence of 11%, which is similar to the 16% in our study This could be due to similarity in husbandry practices, stocking rates, tick populations and communal grazing with Algeria. A study of 110 sheep and 80 goats in Northern Egypt showed the overall animal seroprevalence of *C. burnetii* in both sheep and goats was between 15 and 20% which was similar to 16% found in this study (Selim et al. 2018). In an Australian study, Muleme *et al* (2017) reported an individual animal seroprevalence of 25% for *C. burnetii* and this increased to 43% during the kidding period dairy goats in Victoria. The seroprevalence reported by Muleme et al (2017) was most likely due to more intensive farming systems in dairy goats characterized by higher population density and periods of concentrated kidding which is the highest infection risk period for new animals.

The herd seroprevalence was 51% in this study, similar to the 47% which was reported by Guatteo et al (2011) in a study in northern Italy while another Italian study reported herd prevalence of 25% for small ruminants and 37% for cattle (Guatteo et al. 2011; Masala et al. 2004). A study in Northern Egypt reported that the seroprevalence of Q fever in the same locality is generally higher in goats than in sheep but the authors did not suggest reasons for this disparity (Selim et al. 2018). Our finding of 51% herd prevalence might be increase gradually over the years since in Moretele municipality, as with other rural communities, most animals are on communal grazing; this is speculative at this stage. As such, there is mixing of several animals from several goat herds and this increases the risk of herds becoming infected. Also, the abundance of ticks and the infrequent use of acaricides amplifies the risk of tick transmission of *C. burnetti* which is very important in the transmission of Q fever in animals . Menadi et al (2019) reported a cattle herd seroprevalence of 45.6% (95% CI: 35.27 – 55.84%). This value was quite similar to the 51% found for herd seroprevalence found in this study and this is possibly due to similarities listed above (Menadi et al. 2019).

Rizzo *et al* (2016) conducted a similar serological survey in north-western Italy and found that older and cross-bred goats were more likely to be seropositive (Rizzo et al. 2016) and these findings were somewhat similar to those of this study. Animals living in an environment with constant infection risk, they are more likely to test positive as they get older due to constant

exposure, infection and subsequent seroconverting which is demonstrated in our study as well as that of Rizzo *et al* (2016). In the study conducted by Menadi, the authors found association between introduction of animals into their herds (p = 0.016) compared to this study were there was no association (p = 0.966). The odds ratio for purchased animals testing positive for *C. burnetii* for the study by Menadi et al (2019) was (OR 2.05, 95 CI 1.14–3.68) compared to (OR 0.98, 95 CI 0.46- 2.09) in our study. The difference is likely to be due to the lack of concentrated birthing season in our study as well as low animal densities on the farms, which reduces the infection risk carried by actively shedding goats that are bought into the premises. Also, the fact that all of these animals use communal pastures in which they mix with animals from other herds makes all herds technically open herds even though they are housed separately.

This study found no herd-level factors to be significantly associated with *C. burnetii* seropositivity and this contrasts with the findings of Rizzo *et al* (2016). They demonstrated four herd-level risk potential risk factors (herd size, contact with other herds, mixed herds and geographical location of the herd) as being significantly associated with *C. burnetii* seropositivity. In this study, herd size did not have a significant association with seroprevalence of *C. burnetii* in both the univariate and the mixed-effects logistic regression. Goat herd seroprevalence to *C. burnetii* varied significantly between villages and this is most likely due to risk factors that were not assessed in this study (Fig 8).

The LSIVET Ruminant Q Fever - Serum/Milk ELISA detects *C. burnetii* Ig G in sera and milk samples for both phase 1 and phase 2 *C. burnetii.* In humans, serological typing of phase-specific antigens is done routinely to determine the course of infection but this is not done routinely in animals. Recent infections in humans are characterized by an increase in *C. burnetii* phase 2 antigen IgM and this occurs acutely within two weeks of infection while IgG to phase 2 antigens while IgG to phase 1 antigens appear about 114 days post infection in humans (Muleme et al. 2017). Since the test possesses both phase 1 and phase 2 *C. burnetii* antigen, it is was not possible in our study to detect animals that were actively infected versus those that were convalescing.

A study done by Tejedor-Juncor *et al* (2016) showed that intensity of farm management was significantly associated with seropositivity to *C. burnetii* antibodies with intensively and semi-intensively run goat farms having higher seroprevalence than farms that are extensively run. The authors reported an animal seroprevalence of 42% which was higher than the 16% animal-prevalence in this study. Intensive and semi-intensive management systems have higher

stocking densities and hence higher degree of contact and higher rates of infection. The considerably lower seroprevalence of *C. burnetii* antibodies (16%) in goats sampled in our study compared to what was found in intensively managed goat farms in Moretele municipality may be explained, in part, by the fact that all goats were from extensively managed farms (Tejedor-Junco et al. 2016). Intensive management on goat farms is associated with designated breeding and kidding seasons. Periods of concentrated kidding and/or abortions are associated with increased rate of infection in infected herds as *C. burnetii* has been shown to concentrate in foetal fluids and membranes. As such, environmental contamination increases exponentially in infected goat herds during periods of concentrated parturition and/or abortion making intensive breeding seasons a high risk factor at herd level (Muleme et al. 2017). All goats in this study were in extensive setups and no breeding seasons; this reduced the aforementioned risk of infection associated with concentrated parturition and/or abortion. A lower concentration of animals kidding down and/or aborting presents a lower infection risk or animals in infected herds (Muleme et al. 2017).

It is known that seropositive animals are not necessarily actively infected with *C. burnetii* but an indication that they have had an exposure experience of the organism at some point in the past which resulted in seroconversion to detectable antibodies to the pathogen (Selim et al. 2018). Consequently, our seroprevalence data demonstrate only evidence of exposure to *C. burnetii* and not necessarily active infection. Determination of shedders would require that PCR be used to detect *C. burnetii* RNA. It has also been reported that some infected animals never seroconvert and this was demonstrated in the study conducted by Selim et al (2018); where the overall seroprevalence in herds was between 15 – 20% while the prevalence of shedders confirmed by Rt-PCR of vaginal swabs was 20 – 30%. This means that, again in our study, the possibility exists that some infected goats were not detected, as they did not seroconvert to *C. burnetii.* It has also been estimated that up to 24% of *C. burnetii* infected goats do not seroconvert i.e. they shed bacteria but do not seroconvert and these can be missed in a “test and removal” scheme (Selim et al. 2018).

The intraclass correlation co-efficient (ICC) of 0.06, shows low to moderate clustering of *C. burnetii* within the tested goat herds. ICC is a descriptive statistic that can be used when quantitative measurements are made on units that are organized into groups (Otte and Gumm 1997). Q fever is a contagious disease and as such, the presence of one infected goat likely means several other goats in the same herd are also infected. High ICCs are usually associated with highly contagious diseases such as bovine viral diarrhoea (BVD), infectious bovine rhinotracheitis (IBR) and foot and mouth disease as well as Newcastle disease, among

others (Otte and Gumm 1997). ICC in our study would have been affected by factors that are owner-specific and environment-specific as the goats of all owners grazed communal pastures and were exposed to goats of other owners. Communal grazing, over and above the risk of aerosol transmission of *C. burnetii*, also carries the risk of infected ticks moving between goat herds.

No serological evidence of exposure to RVFV was demonstrated in this study despite the fact that the mosquito species *Aedes mcintoshi*, a competent vector of RVFV and capable of transovarial transmission to its offspring (Linthicum et al. 1985b), was present in large numbers in the floodplains of the Moretele river which runs in the study area (P. Thompson, personal communication). In the 2008-09 outbreak, areas like Pretoria and Bela-Bela were affected by RVF and these are geographically close to the study area. It is therefore possible that there may be a low level circulation of RVFV in the ruminant population in Moretele district; however, evidence of this could not be demonstrated in this study. The upper 95% confidence limit for RVFV seroprevalence in this study was 1.4% and in order to detect a lower seroprevalence of RVFV in goats in Moretele municipality, a larger sample size would be required. The study would also need to focus on more areas that are adjacent to the flood plains of the Moretele and Tshwane rivers, and other suitable habitat for *Aedes* spp. mosquitoes. Further studies are required to identify the exact locations of virus circulation in the inter-epidemic period. Despite no serological evidence of exposure to RVFV, it should still be considered during abortion outbreaks, particularly following periods of high rainfall, which usually precede an outbreak of RVF.

Limitations of this study

No information was collected on the presence of other ruminant species (cattle and sheep) on the properties tested, therefore complete epidemiology of Q fever in ruminants in Moretele municipality could not be established. There was also no information collected on the interaction of the goat flocks with other ruminant species such as sheep and cattle in the communal grazing areas. The serological test in this study does not distinguish between phase 1 and phase 2 antibodies to *C. burnetii*; therefore it was not possible to determine the animals that had active infections versus those that were convalescent. Due to the potential presence of exposed animals which did not seroconvert, approximately 24% (Selim et al 2018), i.e. false negative goats, some Q fever positive animals might have been missed, meaning that the true prevalence of exposure to *C. burnetii* may have been higher than the 16% detected in this study.

Recommendations for further research

A large-scale study (or studies) in all provinces in South Africa in ruminant animals and people that are occupationally at risk should be done to determine the true prevalence of exposure to *C. burnetii* in animals and humans and the occupational risk of infection for humans. Cattle, sheep and goats should be included in the studies and they could employ both serology and PCR in order to distinguish between current and past infections. If Q fever infection rates are found to be high and the human exposure rates reported to be very high, then there could be a long term plan to develop a cost-effective and efficacious vaccine for use in animals to reduce infection rate in animals thus mitigating against animal to human infection. Awareness campaigns can also be embarked on to educate farmers on the risks of Q fever and ultimately the benefits of eradicating infection by slaughter policy within their ruminant herds.

Larger studies need to be designed for RVF, including a range of different ecological regions across southern Africa These should not be restricted to goats but should ideally include all domestic ruminant species. Areas targeted for further studies should ideally be close to low-lying floodplains of rivers and other temporary wetlands. The inclusion of wildlife in these studies should also be considered to identify areas serving as potential reservoir locations for RVFV during the inter-epidemic period.

8. Conclusions

- Exposure to *Coxiella burnetii* was widespread in the study area and is present in more than 50% of goat herds.
- Increasing age was significantly associated with increased seropositivity and the highest seroprevalence was in the age category above 19 months of age.
- This study showed an association between seropositivity for *C. burnetii* and the number of abortions experienced in a herd over the previous year; *C. burnetii* should therefore be considered when investigating single or outbreaks of abortions in Moretele.
- *C. burnetii* should also be considered a potential cause of human disease in the study area.
- The presence of RVFV antibodies could not be demonstrated in this study despite competent vectors being present in the area.

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10. Appendices

Appendix 1: Animal Ethics Committee approval

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**A serological survey for Rift Valley fever and Q fever in goats in the Hammanskraal and Moretele communal areas and identification of factors associated with seropositivity**
PROJECT NUMBER	**V001-18 (Revised)**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. R Magadu**

STUDENT NUMBER (where applicable)	**U_04894406**
DISSERTATION/THESIS SUBMITTED FOR	**MMedVet (Bovine health and Production)**

ANIMAL SPECIES	**Goats (*Capra hircus*)**	
NUMBER OF ANIMALS	**200animals (x 10ml blood)**	
Approval period to use animals for research/testing purposes		**February 2018 – February 2019**
SUPERVISOR	**Prof. P Thompson**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 30 January 2018
CHAIRMAN: UP Animal Ethics Committee	Signature

. Appendix 2

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Humanities
Research Ethics Committee

13 June 2018

Dear Dr Magadu

Project:	**A serological survey for Rift Valley fever and Q fever in goats in the Hammanskraal and Moretele communal areas and identification of factors associated with seropositivity**
Researcher:	**R Magadu**
Supervisor:	**Prof P Thompson**
Department:	**Production Animal Studies (Veterinary Science**
Reference number:	**04894406 (GW20170928HS)**

Thank you for your response to the Committee's correspondence.

I have pleasure in informing you that the Research Ethics Committee formally **approved** the above study at an *ad hoc* meeting held on 13 June 2018. Data collection may therefore commence.

Please note that this approval is based on the assumption that the research will be carried out along the lines laid out in the proposal. Should your actual research depart significantly from the proposed research, it will be necessary to apply for a new research approval and ethical clearance.

We wish you success with the project.

Sincerely

Prof Maxi Schoeman
Deputy Dean: Postgraduate and Research Ethics
Faculty of Humanities
UNIVERSITY OF PRETORIA
e-mail: PGHumanities@up.ac.za

cc: Prof P Thompson (Supervisor)

. Appendix 3

read
Department:
Rural, Environment and Agricultural Development
North West Provincial Government
REPUBLIC OF SOUTH AFRICA

2030 NDP

AgriCentre Building
Cnr. Dr. James Moroka
and Stadium Rd
Private Bag X2039,
Mmabatho 2735

CHIEF DIRECTORATE: AGRICULTURAL SUPPORT
DIRECTORATE: VETERINARY SERVICES

Tel: +27 (18) 389 5102/5057
Fax: +27(18) 389 5090
E-mail: Lmadyibi@nwpg.gov.za

Ref. : 12/5/1

Dr Rungano Magadu

Clinical Assistant (Bovine Health and Production)

Department of Production Animal Studies

University of Pretoria

Pretoria

13th March 2018

Re: AUTHORIZATION TO CONDUCT RESEARCH ON SEROLOGICAL SURVEY FOR RIFT VALLEY FEVER AND Q FEVER IN GOATS IN THE NORTH WEST

Thank you for your request based on Section 20 of the Animal Diseases Act to conduct research work in the North West province in Hammanskraal and Moretele areas. My office has no objection to the request and therefore gives authorization. The National Director Animal Health has equally been informed of your intention.

Please make contact with the state Veterinarian (Dr T. Mlilo) of the area when your researchers arrive to inform them of your presence in the area. You are advised to observe biosecurity of utmost levels when visiting farms to collect samples.

WE BELONG WE CARE WE SERVE

REPUBLIC OF SOUTH AFRICA
Department of Rural, Environmental & Agricultural Development

2018 -03- 13

Dr. L.S. Madyibi
SAVC REG. NO. D 93/3478
NORTH WEST PROVINCE

Dr L.S. Madyibi

Director Veterinary Services

Date 13th March 2018

WE BELONG **WE CARE** **WE SERVE**

Appendix 4

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X138, Pretoria 0001

Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za
Reference: 12/11/1/1/6

Dr Rungano Magadu
Faculty of Veterinary Science
University of Pretoria

Dear Dr Magadu,

RE: Permission to do research in terms of Section 20 of the ANIMAL DISEASES ACT, 1984 (ACT NO. 35 of 1984)

Your fax / memo / letter/ Email dated 01 May 2018, requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers.

I am pleased to inform you that permission is hereby granted to perform the following research/study, with the following conditions:

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. All potentially infectious material utilised or collected during the study is to be destroyed at the completion of the study. Records must be kept for five years for audit purposes. A dispensation application may be made to the Director Animal Health in the event that any of the above is to be stored or distributed;
3. Samples may only be collected from the Hammanskraal and Moretele areas in the North West Province as per the approval from Dr Madyibi on 13 March 2018;
4. Prior to collection of samples, written approval must be obtained from the National Director Animal Health that collection may take place in view of the current Rift Valley fever outbreak;
5. The responsible state veterinarian, Dr T Mlilo must be notified upon arrival to collect samples and biosecurity of utmost levels must be adhered to when visiting farms to collect samples as per the approval from Dr Madyibi on 13 March 2018;
6. Samples must be packaged in triple packaging in compliance with the Regulations of the National Road Traffic Act, 1996 (Act No 93 of 1996) for transportation;
7. The ID Screen Rift Valley fever Competition Multi-species ELISA by IDvet, ID Screen Q Fever Indirect Multi-species ELISA by IDVet, PrioCHECK Ruminant Q Fever Ab Plate Kit by ThermoFisher, or the IDEXX Q-Fever (Coxiella burnetii) Antibody Test Kit must either be imported directly by the researcher after having obtained a veterinary import permit, or it must be ensured that the distributor

being purchased from has legally imported these kits with a veterinary import permit;

8. The results from these test kits may not be used for diagnostic purposes and hence results may not be given out to the owners of the goats;
9. Samples are to be loaded on the ELISA plates in the BSL2 biosafety cabinet in the DVTD Virology Laboratory after which the ELISA method may be performed in the DVTD Serology Laboratory;
10. Wastech must be used as accredited waste management company;
11. This Section 20 approval expires on 31 December 2018.

Title of research/study: A serological survey for Rift Valley fever and Q fever in goats in the Moretele communal area.
Researcher (s): Dr Rungano Magadu.
Institution: Faculty of Veterinary Science.
Your Ref./ Project Number:
Our ref Number: 12/11/1/1/6

Kind regards,

Maja

DR. MPHO MAJA

DIRECTOR OF ANIMAL HEALTH

Date: 2018 -05- 17

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X138, Pretoria 0001

Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za
Reference: 12/11/1/1/6

Dr Rungano Magadu
Faculty of Veterinary Science
University of Pretoria

Dear Dr Magadu,

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2. All potentially infectious material utilised or collected during the study is to be destroyed at the completion of the study. Records must be kept for five years for audit purposes. A dispensation application may be made to the Director Animal Health in the event that any of the above is to be stored or distributed;
3. Samples may only be collected from the Hammanskraal and Moretele areas in the North West Province as per the approval from Dr Madyibi on 13 March 2018;
4. Prior to collection of samples, written approval must be obtained from the National Director Animal Health that collection may take place in view of the current Rift Valley fever outbreak;
5. The responsible state veterinarian, Dr T Mlilo must be notified upon arrival to collect samples and biosecurity of utmost levels must be adhered to when visiting farms to collect samples as per the approval from Dr Madyibi on 13 March 2018;
6. Samples must be packaged in triple packaging in compliance with the Regulations of the National Road Traffic Act, 1996 (Act No 93 of 1996) for transportation;
7. The ID Screen Rift Valley fever Competition Multi-species ELISA by IDvet, ID Screen Q Fever Indirect Multi-species ELISA by IDVet, PrioCHECK Ruminant Q Fever Ab Plate Kit by ThermoFisher, or the IDEXX Q-Fever (Coxiella burnetii) Antibody Test Kit must either be imported directly by the researcher after having obtained a veterinary import permit, or it must be ensured that the distributor

Appendix 5 **Questionnaire**

Owner details

First name ……. Surname ……..

District …… Cell phone: …….

Property details

Coordinates: S……..…….. E……..…….

Tag number or name	Vaccination status RVF (Yes/No)	Distinguishing marks	Breed* (KR, BG, S, I, M)	Age (months)	Sex (M/F)	Born on premises (Yes/No)	If origin external, state origin	Duration on premises	Given birth before? (Yes/No)	Aborted previously? (Yes/No)	Number of previous abortions noted	Size of aborted foetus*1

* **KR- Kalahari red, BG – Boergoat, S – Saneen, I – indigenous, M – mixed breed**

*1 **small (S): <10cm, medium (M): 10-20cm, large(L): > 20cm**

Herd-specific information

Are the animals routinely vaccinated for any diseases? Y…….. N……

If so, which ones?.....

Do you sometimes sell animals? Y….. N…..

If yes, where to mostly?.....

Do you sometimes buy animals? Y….. N…..

If yes, where from mostly?.....

Do you ever slaughter your own animals for consumption? Yes……No……..

Have any animals ever aborted on the premises? Y…….. N…… Goats…….. Cattle…… None……

If yes, how many animals have aborted in the past 12 months?.....

how big were the foetuses?.....

what did you do with the aborted foetuses?.....

did the mothers have retained foetal membranes? Y…….. N……

did they show signs of uterine infection? Y…….. N……

Have any animals died in the last twelve months? Y…….. N……

If yes, how many?.....

how old were they?

what clinical signs did they show?

Are the animals ever or routinely treated with tetracyclines for any reason? Y…….. N……

If yes, how often?.....

Is any tick control instituted for the goats? Y…….. N……

If yes, which products are used?.....

If yes, how often is the product used?.....

Appendix 6 Research consent form

Rift Valley fever is an important viral disease of livestock that can also affect humans. It is transmitted by mosquitoes and causes losses through deaths and abortions in pregnant animals. People working directly with animals and their products are at increased risk of infection. Q fever is caused by the bacterium *Coxiella burnetii.* Ruminants are the reservoirs and do normally not show clinical signs, but occasional abortions occur. In humans, Q fever is usually a transient flu-like illness with less than 5% of cases becoming chronic. Human mortalities due to both Rift Valley fever and Q fever are low. The aim of the project is to establish if goats in the Hammanskraal and Moretele communal areas have been exposed to the causative agents of these two diseases and if so to identify risk factors for exposure.

The animals required for the study are goats older than 6 months of age. Each goat that is selected will undergo a physical examination and if clinically healthy, 6 ml of blood will be collected from the jugular vein. The goat will then be released and the blood sample will be tested in the laboratory for antibodies to Q fever and Rift Valley fever.

The results of this study will be aggregated and no individual owner or animal will be reported upon. There is no anticipated risk to participants. In the unlikely event of injury to a goat during sampling, it will be treated by the researcher at no cost to the owner.

Informed consent

I agree to participate in the research study being conducted by Dr. Rungano Magadu titled **A serological survey for Rift Valley fever and Q fever in goats in the Hammanskraal and Moretele communal areas.**

The purpose of the research has been well explained to me and I have agreed to enrol my animals voluntarily.

I have been offered the option of being notified of the results of the laboratory testing, and I have chosen to **ACCEPT/DECLINE.** As an added benefit, I have been given educational material on the two diseases under study.

I understand that the blood samples from my animals will be stored at the University of Pretoria and I give permission for the samples to be tested for other purposes in the future if required: **YES/NO**

I understand that I reserve the right not to answer any question should I choose not to.

I have been given explicit guarantees by the researcher that I will not be identified in any way in reports, publications or presentations associated with this or any other research project and that my confidentiality will remain secure.

_____	_____	_____
Participant's name	Participant's signature	Date

_____	_____
Researcher's signature	Date