Q fever in Egypt:
Epidemiological Survey of
Coxiella burnetii-Specific Antibodies
in Cattle, Buffaloes, Sheep, Goats and Camels

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Q fever in Egypt: Epidemiological Survey of *Coxiella burnetii*-Specific Antibodies in Cattle, Buffaloes, Sheep, Goats and Camels

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„If you always do what you’ve always done,
you’ll always get what you’ve always got.”

Henry Ford
(1863 – 1947)

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Abbreviations

16S rRNA	16S ribosomal ribonucleic acid
ACCM	acidified citrate cysteine medium
APSW	abortion, premature delivery, stillbirth, weak offspring
ATP	adenosine triphosphate
BTM	bulk tank milk
Cb	*Coxiella burnetii*
CCV	*Coxiella*-containing vacuole
CDC	Centers for Disease Control and Prevention, Maryland, USA
CFT	complement fixation test
DIVA	differentiating infected from vaccinated animals
DNA	deoxyribonucleic acid
ELISA	enzyme linked immunosorbent assay
FLI	Friedrich-Loeffler-Institut
Fig	Figure
FUO	fever of unknown origin
ID50	infection dose of 50%: dose of a microorganism that may cause illness in 50% of exposed individuals
IF(A)	immunofluorescence (assay)
Ig	immunoglobulin
IHC	immunohistochemistry
IS	insertion sequence
Kbp	kilo base pairs
LCV	large cell variant
LPS	lipopolysaccharide

Mbp	mega base pairs
MLVA	multiple variable numbers of tandem repeats analysis
MST	multispacer sequence typing
OD	optical density
O-PS	O-polysaccharide
PCR	polymerase chain reaction
PhI	Phase I
PhI^+/PhI^-	Phase I positive/negative
PhII	Phase II
$PhII^+/PhII^-$	Phase II positive/negative
pI	isoelectric point
PMF	proton motive force
RFLP	restriction fragment length polymorphism
qPCR	quantitative real time polymerase chain reaction
SCV	small cell variant
SNP	single nucleotide polymorphism
TLR	toll-like receptor
WHO	World Health Organization

I INTRODUCTION

Coxiella burnetii (*Cb*) is the causative agent of Q fever in humans and animals and is classified as category B biological agent [1]. Q fever is an endemic zoonotic disease worldwide, except for New Zealand [2]. The pathogen is a Gram-negative, obligate intracellular bacterium and is able to infect various host species [3, 4]. In humans, clinical findings vary from asymptomatic, mild or acute symptoms (self-limiting flu, atypical pneumonia, hepatitis) to chronic life-long and life-threatening outcomes (endocarditis, vascular infections) [3, 5-7]. In animals, no clinical signs are seen usually and *Cb* infections are often subclinical. However, main clinical signs in domestic ruminants are reproductive disorders such as (late) abortion or weak offspring [8-10].

Cattle, sheep and goats are considered as main reservoirs for the Q fever agent and small ruminants are the most potential source of infection for humans [8, 10-12]. The bacteria are excreted in high numbers with birth products during parturition as well as with several body fluids (vaginal mucus, milk, feces) and lead to contamination of the environment [11, 13]. Inhalation of contaminated aerosols is the most common infection route in humans [14-16]. Several factors e.g. environmental conditions (wind, dry areas) facilitate spreading and dissemination of *Cb* and are associated with a higher infection risk for humans [17, 18].

In African countries, only few studies are available of *Cb*-specific antibody prevalences in humans and animals and little is known about the disease, diagnosis, animal disease control and public health countermeasures. Very limited information is available about the epidemiology of *Cb* in humans and animals, although African countries like Egypt may provide good conditions for *Cb* transmission: environmental factors, increasing livestock production and constantly growing human population, frequent animal movements and preserved traditions. Awareness rising measures in animal keepers, veterinarians and physicians are necessary to implement the “One Health” approach in African countries ensuring animal welfare and public health and decreasing the socioeconomic burden in agricultural countries.

Therefore, this nationwide present survey was carried out to describe the seroepidemiological situation of *Cb*-specific antibodies in ruminants and camels in Egypt (except the Sinai). Serum samples were investigated using a commercially available enzyme linked immunosorbent assay (IDEXX CHEKIT Q fever Antibody

ELISA Test Kit). Milk investigations were done independently of the study in consideration of the still ongoing debate about the unresolved correlation of antibodies found in blood and milk. Prevalence data of *Cb*-specific antibodies in milk of any farm animal species have not been reported in Egypt until today. The elucidation of the correlation of the results to specific risk factors may provide a baseline data for support and improvement of public health countermeasures and animal welfare. The research results may also be used to tailor further epidemiologically studies especially in humans.

II REVIEW OF LITERATURE

1 History of *Coxiella burnetii*

Q (query) fever was first described in 1937 by E. H. Derrick studying an outbreak of an unknown febrile illness among abattoir workers in Queensland, Australia in 1935 [19]. Experimental infection of guinea pigs but no visualization of the pathogen raised the later reversed assumption that the disease causing agent was an unknown virus [19, 20]. In 1938 F. M. Burnet and M. Freeman were able to reproduce the characteristic febrile disease in guinea pigs, using infected tissues provided by Derrick. They discovered *Rickettsia*-like bacteria in smears of spleen tissue from infected mice [20]. At the same time, G. Davis discovered *Coxiella* as infectious agent by accident during a field study on Rocky Mountain spotted fever (1935). He collected ticks near the Nine Mile Creek in Montana, USA. These ticks were fed on guinea pigs and induced a febrile illness that did not resemble Rocky Mountain spotted fever [21]. Further studies together with H. R. Cox on the isolation and cultivation of the “Nine Mile Agent” led to the decision that the agent had bacterial and viral characteristics [21-23]. Cox succeeded in cultivating the “Nine Mile Agent” in embryonated hen eggs, in which he observed *Rickettsia*-like bacteria in large numbers within the yolk sac membrane tissue [21, 24]. R. E. Dyer doubted Cox’s culturing method due to his own failure in this matter and visited Cox to work together with him in his laboratory. Dyer contracted a laboratory infection and showed symptoms of Q fever after his return. This suggested that the “Nine Mile Agent” and the Q fever pathogen discovered by Derrick were the same agent. The assumption was validated in cross protection studies by Dyer in 1939 [21, 25].

In 1948 the agent was named as “*Coxiella burnetii*” to honor the researchers F. M. Burnet and H. R. Cox characterizing the Q fever bacterium nearly simultaneously [26]. In recent years, *Cb* was reclassified several times based on modern taxonomy studies.

2 Etiology and Pathogenesis

2.1 Taxonomy

From the discovery of *Coxiella burnetii* up to the 1990s the agent was often reclassified due to advanced phylogenic analysis methods. The original name of the Q fever pathogen was "*Rickettsia burnetii*" because of similar characteristics to *Rickettsia* organisms e.g. having a reservoir in ticks and being an obligate intracellular small coccoid rod. Until the 1990s *Cb* was classified as an α-Proteobacterium, in the order Rickettsiales, family Rickettsiaceae with the tribes Rickettsieae (with the genus *Coxiella*), Wolbachieae and Ehrlichiae. Further analysis of six selected species out of the family Rickettsiaceae based on the 16S rRNA gene sequence resulted in the reclassification of *Cb* as member of the γ-subdivision of the Proteobacteria. This survey also revealed that the phylogenetic origin of *Cb* is closer to the genus *Legionella* than to the genus *Rickettsia*. Nowadays, *Legionella pneumophila* is known as the closest relative of *Cb* [27]. This finding was also confirmed by Stein *et al.* comparing five human *Cb* isolates and the *Cb* Nine Mile tick strain showing a 16S rRNA gene sequence identity of more than 99% [28].

At present, *Coxiella* is phylogenetically classified within the phylum of the Proteobacteria, class γ-Proteobacteria, order Legionellales, family Coxiellaceae and genus *Coxiella* with a single species C. *burnetii* (**Fig. 1**). With the recent description of *Coxiella cheraxi* sp. nov. and the discovery of *Coxiella*-like tick endosymbionts the single species status of the genus *Coxiella* has to be re-evaluated [14, 29, 30]. It was suggested that the *Coxiella*-like endosymbionts represent the ancestors of *Cb* with less or nearly none virulence factors making *Cb* capable of successful infection of vertebrates [31].

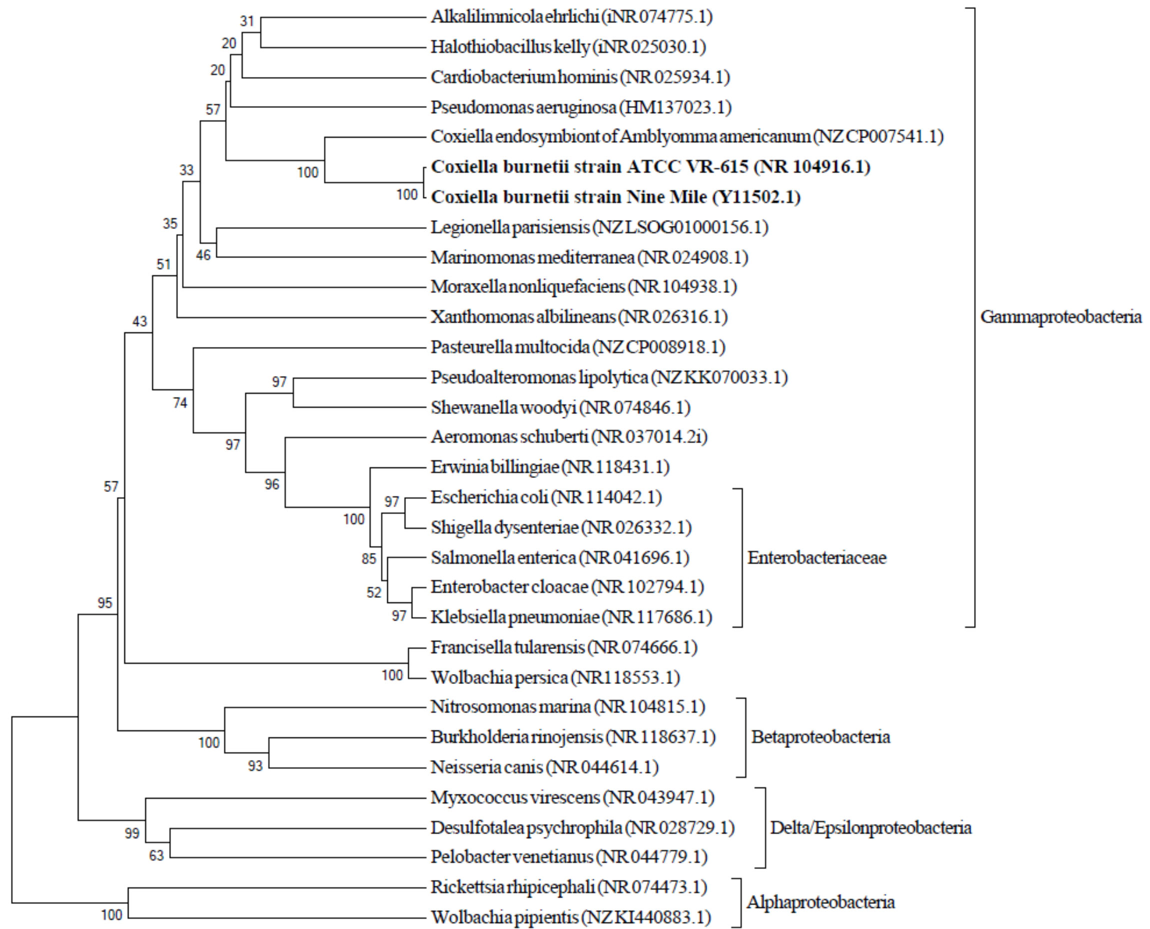

Figure 1. Current taxonomic classification *Coxiella burnetii*.

Created by P. Thomas (IBIZ, FLI).
Phylogenetic tree based on 16S rRNA gene sequences demonstrating the classification of *Coxiella burnetii* and *Coxiella*-like strains into the γ-Proteobacteria and showing the close relatedness of *Cb* and *Coxiella*-like organisms.

2.2 Bacteriology

2.2.1 Morphology

Coxiella burnetii is an obligate intracellular Gram-negative bacterium. It is a small, non-motile and pleomorphic coccobacillus with a width of 0.2-0.4 µm and a length of 0.4-1.0 µm [3, 4]. It has a typical Gram-negative cell wall with an inner and outer membrane, a periplasmic space and lipopolysaccharides (LPS) as major component of the outer leaflet of the outer membrane [4]. The bacteria stain Gram-variable and therefor Gimenez staining is the most used staining method [32].

Cb has a generation time of 8-12 h and undergoes developmental differentiation with two morphological distinct cell forms during its intracellular life cycle: a small cell variant (SCV) and a large cell variant (LCV; **Fig. 2**) [33, 34]. These morphotypes are distinguished by their ultrastructure and cell wall content, metabolic capabilities, protein compositions and physical resistances [33]. LCV have a size of 1 µm in diameter with a thin cell wall and an irregular shape. A dispersed nucleoid with a more scattered chromosome, a filamentous nucleoid region and granular or filamentous cytoplasm are characteristics of the LCV. Contrary, SCVs are 0.2-0.5 µm in length and are typically rod-shaped with a thick wall, characteristic condensed chromatin and an arrangement of intracytoplasmic membranes [4, 33]. Additionally, *Cb* displays two phase variations with different LPS length, designated as i.e. Phase I (smooth LPS) and Phase II (rough LPS) [35, 36].

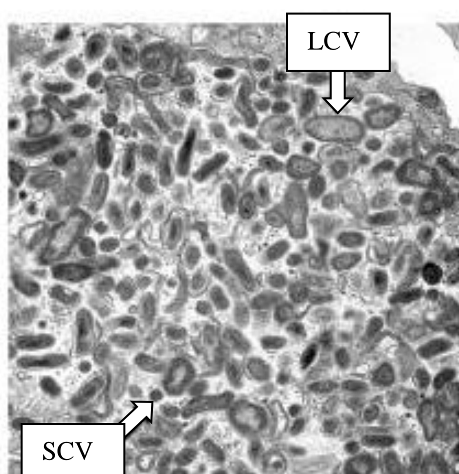

Figure 2. The two different morphotypes of *Coxiella burnetii*.

Cited from Coleman *et al.* (Coleman *et al.* 2004).
Small cell variant and large cell variant in cell culture (Vero cell) on day 6 post infection.

2.2.2 Genome and Genotyping

Cb Nine Mile Phase I (RSA 493) was the first isolate to be sequenced and the genome was published in 2003. Compared to other obligate intracellular bacteria *Cb* isolates have a relatively large circular genome of approximately 2 Mbp [37-39]. Additionally, each isolate harbor either one of five plasmids (QpH1, QpRS, QpDV, QpDG or a no further characterized plasmid from a Chinese isolate). They can also be free of plasmids with chromosomal integrated plasmid related sequences. Plasmid size ranges from 36 to 56 kbp except for chromosomal integrated homologous sequences with a size of 16 kbp [37, 39, 40]. Numerous pseudogenes indicate that genome reduction is an ongoing process reflecting the still ongoing adaptation to an obligate intracellular lifestyle [38, 41, 42]. Genetic elements such as three degenerate transposons and 29 insertion sequences (IS) are present [38, 43, 44]. These elements are transferred horizontally which is uncommon for obligate intracellular bacteria [43, 44]. The IS1111 transposase element is present in multiple copies and the copy numbers vary tremendously in different strains. Hence, this sequence is used in general for detection of *Cb* using polymerase chain reaction due to high sensitivity and specificity [45, 46]. Nevertheless, this method has to be re-evaluated since both *Cb* and *Coxiella*-like tick endosymbionts harbor this element.

Restriction fragment length polymorphism (RFLP) was used for early comprehensive genetic discrimination of *Cb* isolates from different species and geographical origin. Six genomic groups were described based on the correlation of plasmid content and manifestation of Q fever especially in humans [47, 48]. Some plasmid types have homologous regions [49, 50]. Consequently, the virulence of a *Cb* strain plays an important role. However, pathogenicity and disease outcome might be independent of plasmid type and depends also on predispositional factors of the host [51].

Genotyping of *Cb* for epidemiological investigations experienced a remarkable revolution due to several Q fever outbreaks in humans in the last decade. Today, newer genotyping methods are multispacer analysis, multiple variable numbers of tandem repeats analysis (MLVA), single nucleotide polymorphism analysis (SNP) and multispacer sequence typing (MST) analysis. Usually, a combination of MLVA, MST and SNP is currently used [52-55].

2.2.3 Developmental Life Cycle

Cb is not able to replicate outside host cells naturally [33, 34]. Despite the long generation time of up to 12 h replication follows a typical bacterial growth curve with a lag phase, exponential growth (log phase) and stationary phase [34]. Interestingly, *Cb* displays a biphasic developmental cycle with a replicative (LCV) and a spore-like dormant (SCV) cell form (**Fig. 3**) [4, 56]. During growth, these cell forms show characteristic appearance, gene expression, regulatory and structural components [34, 56-58]. Similarities with this particular life cycle and replication process were seen in other obligate intracellular bacteria e.g. *Chlamydia* spp. [33].

After internalization, the SCV reside in a parasitophorous vacuole [34]. Within the first two days during the lag phase the metabolic quiescent SCV differentiate into the replicative LCV form. Appearance of LCV indicates transition into the exponential phase with an increased transcriptional activity in the target cells. After approximately six days post infection with the onset of the stationary phase, LCVs start the morphological differentiation back into SCVs [34, 56]. The number of SCVs increases the following two days [34]. During this differentiation electron-dense, polar bodies can be observed in the LCVs [4, 33, 38, 57]. Differentiation into SCVs ensures survival within the environment [34]. LCVs are known as the replicative intracellular form of *Cb*. These cells are very unstable and fragile outside the host cell [33, 34]. Considering the high tenacity and resistance of the SCVs to environmental conditions and against chemical and physical factors, it is not surprising, that these are considered as “spore-like” extracellular survival forms. Resistance of the Q fever pathogen against heat, desiccation, pressure, high or low pH, UV rays, disinfectants and chemical agents has been demonstrated [4, 33, 59-61]. The main infectious stage of *Cb* is the SCV. Nevertheless, the infectious potential of LCVs should not be underestimated [33]. Therefore, the risk of infection for humans and animals is increased by remaining *Cb* in the environment.

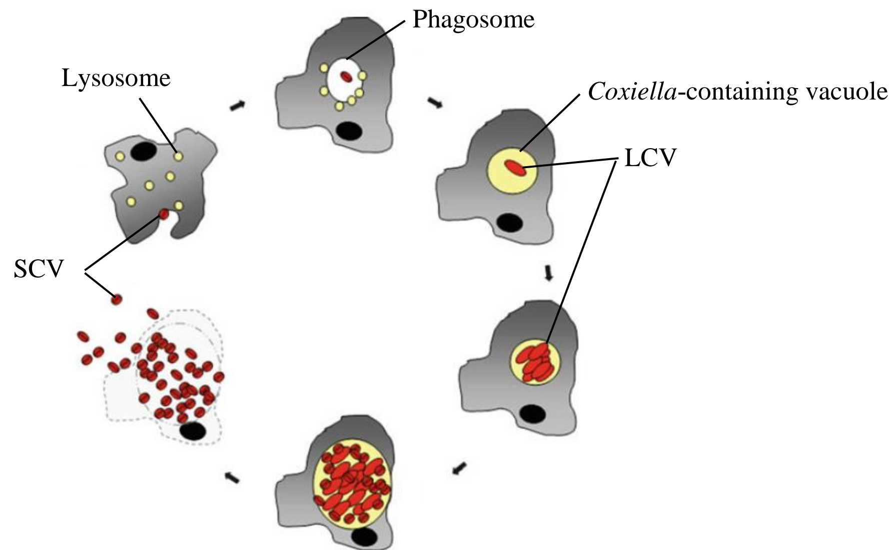

Figure 3. Proposed biphasic developmental cycle of *Coxiella burnetii*.

Cited and modified from Minnick *et al.* (Minnick *et al.* 2012).
SCV enters the host cell (e.g. alveolar macrophage) passively by phagocytosis. Acid activation of the SCV (due to the acidic environment in the *Coxiella*-containing vacuole) allows morphogenesis to LCV and subsequently replication. Morphological differentiation to SCV is initialized at least in the early stationary phase before they will be released from the host cell.

2.2.4 Intracellular Niche

A crucial step in the lifecycle of *Cb* is the establishment of an intracellular niche within the host cell. This niche is unique among intracellular bacteria [62, 63]. Other bacteria normally avoid the microbial degradative environment of this "niche", which shows characteristics of a terminal phagolysosome.

Natural transmission of *Cb* occurs mainly through inhalation of contaminated aerosols [3, 64]. Alveolar macrophages and monocytes are known as primary target cells [65]. After uptake via pathogen-induced phagocytosis, SCV resides in a parasitophorous vacuole, the so called *Coxiella*-containing vacuole (CCV) [34]. This CCV passes through the endocytic cascade fusing with vesicles of the endosomal and autophagic pathway [62, 66]. The CCV is highly fusogenic and finally fuses with lysosomal vesicles obtaining characteristics of a terminal phagolysosome such as an approximate pH value of 5 and degradative enzymes e.g. acid hydrolases [33, 62, 66, 67]. The acidic environment (pH ~ 5) triggers the differentiation of the SCV into the LCV, a mechanism termed as acid activation [33, 68, 69]. This acidification process also

enables active transport of nutrients and generation of ATP due to a proton motive force (PMF) driven by the pH gradient between the bacterial neutral cytoplasm and the acidic vacuolar environment [68, 70-73].

The terminal CCV can occupy almost the entire cytoplasm of the host cell. It is interesting, that this is not affecting the host cell's viability [74, 75]. This underlines the assumption of a minimal cytopathic effect of *Cb*. Further, it is assumed that infected eukaryotic cells are not actively lysed by *Cb* due to missing membranolytic exit systems as known for *Chlamydia trachomatis* [33]. It has been shown that *Cb* inhibits apoptosis of infected cells [76-78].

Evolutionary adaptation of *Cb* was necessary to ensure survival within the harsh environment within its unique niche. Therefore, *Cb* developed specific features. The genome of *Cb* is fitted with an unusual high number of genes encoding for basic proteins. These basic proteins with an isoelectric point (pI) value of ~8.25 buffer the excess of protons entering from the acidic environment [38]. Additionally, various types of secretion systems such as exchangers for protons and transporters for osmoprotection are expressed [38]. Especially the type IV secretion system (T4SS) is a noticeable virulence factor of *Cb* as it is involved in the stability and fusogenicity of the CCV and the inhibition of host cell apoptosis [79-81]. It has been shown that the Nine Mile isolate has over a quarter more drug-efflux systems than other γ-Proteobacteria [38]. This fact in combination with the acidic environment in the CCV makes a protracted therapeutic approach with antibiotics due to limited antibiotic effects.

2.2.5 Phase Variation

Cb displays antigenic variation, Phase I and Phase II. This phase variation refers to the length and complexity of lipopolysaccharides in the outer leaflet of the outer membrane. The virulent Phase I (PhI) bacteria may be isolated from infected hosts like humans, animals and ticks. Whereas the Phase II (PhII) avirulent form of *Cb* may be isolated from immune-incompetent hosts e.g. embryonated hen eggs after repeatedly passaging of PhI bacteria [35, 36]. The shift from virulent to an avirulent *Cb* population goes along with deletions in the chromosomal DNA finally resulting in, for example, the irreversible truncation of the LPS chain [36, 82]. Members of the Enterobacteriaceae family show a similar "smooth" and "rough" variation [35, 36].

LPS is considered as a virulence factor of *Cb*. Until today, chemical composition and structure of the LPS has not been completely resolved. Furthermore, it has to be mentioned that LPS analyses are mainly described for Nine Mile I and II strains only. PhI bacteria express a full length, smooth LPS. It consists of a lipophilic membrane anchor (lipid A) linked to a core oligosaccharide and an O-specific polysaccharide (O-PS) chain (**Fig. 4**). The O-PS chain is a heteropolymer and contains different sugars like virenose (Vir, 6-deoxy-3-C-methylglucose) and dihydrohydroxystreptose (Strep, 3-C-(hydroxymethyl)-L-lyxose) [82-85]. These unusual sugars are unique biomarkers as these are absent on the surface of any other enterobacterial species [84, 85]. The LPS of the avirulent form is severely truncated and resembles a deep, rough LPS chemotype. Major differences to the virulent form are the absence of the O-PS and of several sugar residues in the core oligosaccharide [82, 86].

LPS plays an important role during the host-pathogen-interactions by contributing to the immunogenicity and pathogenicity of *Cb*. Additionally, they are associated with evasion of specific immune defense mechanisms like complement-mediated killing and play a major role during phagocytosis [87-91]. The full length, smooth LPS of PhI bacteria has a low endotoxic potency and toll-like receptor 4 (TLR4) antagonistic properties, although it is considered a specific virulence factor of *Cb* [92, 93].

In immune competent hosts, PhII bacteria are eliminated rapidly. However, clinical signs such as splenomegaly may be seen in severe combined immunodeficiency (SCID) mice to a certain extent [94-96]. Interestingly, various PhII bacteria of *Cb* are known, differing in the surface (protein composition, charge, cell density) [97-100].

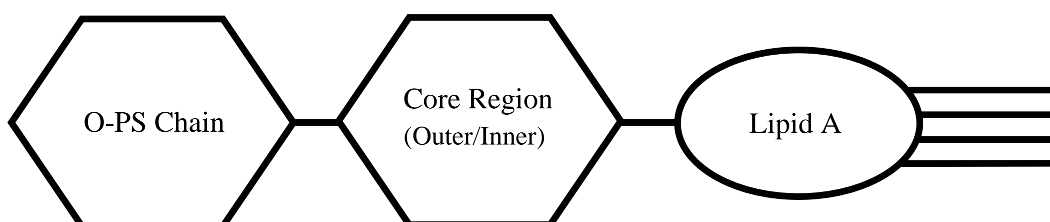

Figure 4. Schematic structural arrangement of the lipopolysaccharide of Phase I *Coxiella burnetii*.

The three units in the LPS consist of specific sugar residues in various number and sequence. These units may be seen in Gram-negative bacteria in general.

3 Epidemiology

3.1 Host Range, Reservoirs and Vectors

Q fever is a zoonosis, more precisely a zooanthroponosis. In consequence, *Coxiella burnetii* is transmitted from animals to humans. Human-to-human infections may be exceptional whereas animal-to-animal transmission is common.

Host range of *Cb* is impressively various and wide. Wild and domestic mammals are the main target hosts but also non-mammalian animals can carry the agent. Domestic ruminants like cattle, sheep and goats play an important role for the dissemination of *Cb*. They are considered the main reservoirs for human infections as shown during a nationwide Q fever outbreak in the Netherlands (2007-2010) and annually small scale outbreaks in Germany [12, 101-103]. The role of camels is unknown regarding bacterial shedding and risk for human infection, although DNA of *Cb* was demonstrated in several excretions [104]. *Cb*-specific antibodies or DNA was also found in cats, dogs, pigs and horses [105-110]. Wildlife animals like non-human primates, rodents and big game as well as reptiles, amphibians, fish and birds are described as further hosts for *Cb* [111-114]. *C. cheraxi* sp. nov. was isolated from a freshwater crayfish [30]. The exact role of ectoparasites like ticks or other hematophagous arthropods (fleas, mites, lice) as vectors in the transmission of Q fever is still under debate although *Cb* may be detected in arthropods worldwide [14, 25, 115-117]. Until now, *Cb* was detected in more than 40 tick species [118]. Bacteria were found in their gut, hemolymph and in high numbers in their feces.[23, 119-121]. In experimental settings it is possible to infect ticks and demonstrate transstadial and transovarian transmission of *Cb* [120, 122]. It seems like they do play a key role in the transmission of *Cb* between wildlife and humans e.g. in Spain, although it has not been proven yet [123]. Nevertheless, the reported prevalence in ticks of *Cb* (up to 10%) has to be reconsidered carefully, since several *Coxiella*-like endosymbionts have been described [124-126]. These bacteria are genetically highly similar to *Cb* and harbor the same multi-copy IS1111 element used for PCR detection [127, 128]. *Coxiella*-like bacteria are also widespread and common in ticks [125, 126, 129].

3.2 Global Distribution

Q fever is present worldwide, except New Zealand [2, 113, 130]. This zoonotic febrile disease is endemic due to the resistance of *Cb*, its spreading ways and the various susceptible hosts. Q fever outbreaks are reported for many countries worldwide such as Egypt, Germany, the Netherlands, Switzerland or Australia [12, 102, 131-133]. Q fever seroprevalence studies are available from nearly all northern, western, central, eastern and southern African countries [61, 134-136]. Nevertheless, only few surveys were conducted with random sampling or correlated prevalence in human and animal populations [136].

The seroprevalences in human surveys conducted in Africa vary strongly as seen in a study on data of seven African countries (1-37%) and is reported to be 10% in children in Niger [133, 134, 137-139]. Tissot-Dupont *et al.* assumed, that there might exist a correlation between high seroprevalences in humans in countries with developed livestock industries as demonstrated in data of West Africa, e.g. in Mali with 24%, where stock breeding is common [134]. Comparison of prevalence surveys from Mediterranean southern European countries to those of northern African countries showed similar results and it is speculated that epidemiological characteristics regarding Q fever transmission are more similar in both areas [134]. Similar findings in humans are not reported for Egypt [134, 136].

In 1952, Halawani *et al.* reported Q fever seroprevalence data in ruminants (cattle 9.3% [16/172], buffalo 33.3% [6/18], goat 26.7% [4/15], sheep 6.5% [14/217]) and humans (cattle farm workers 14.3% [11/77]) in Egypt for the first time [140]. Since then, seroprevalence of the Egyptian population was reported from different geographical areas between 10-32% in human adults and 22% in children [138, 141, 142]. In Egyptian livestock, *Cb*-specific antibodies were detected in up to 13% of cattle, 4% of buffaloes, 33% of sheep, 23% of goats and 70% of camels (**Tab. A1**). Whereas seroprevalences in livestock of other African countries was found to be as high as 55% in cattle, 4% in buffaloes, 33% in sheep, 24% in goats and 80% in camels [133, 139, 143, 144].

For epidemiological purposes, the use of genomic analyses of *Cb* isolates allowing regional distribution of genotypes is very helpful [40, 48, 51, 54, 118, 145, 146]. Nevertheless, the risk of infection depends on various parameters such as environmental factors or animal and human population density. Although Q fever seems to be present nearly worldwide it should be stressed that generalization of data is problematic. It has also been shown that the prevalence measured at herd/flock level in a country is often higher than the seroprevalence within a single herd/flock. This in turn means, that only few infected animals seroconvert in each herd, though a herd is considered positive when at least one animal was tested positive [103]. Findings using convenience sampling make comparison often difficult. **Table A1** gives a summary of different seroprevalence studies in Egypt and bordering countries highlighting the results and the diagnostic method and study design used.

3.3 *Coxiella burnetii*

3.3.1 Role as Biological Agent

The U.S. Centers for Disease Control and Prevention (CDC) classified *Cb* as Category B biological agent [147]. These agents are characterized by a moderately easy dissemination, low mortality and a modest morbidity. Additionally, a specific enhanced diagnostic capacity and disease surveillance is needed.

Dissemination of *Cb* is facilitated by different factors. First, a lot of easily accessible and susceptible hosts are available for *Cb* although the role of most hosts regarding dissemination is not clear. Excretion of the pathogen leads to environmental contamination. The special morphological characteristics of *Cb* mentioned allow surveillance in the environment for a long period of time. Consequently, *Cb* may be disseminated over a long period and thus increasing infection risk for humans and animals. The infection dose of 50% (ID50) is one to ten bacteria [113]. A “single organism theory” was created due to the low infection dose of less than 10 organisms in humans [1]. *Cb* has the ability to cause Q fever in large numbers of humans and animals [113]. Hence, Q fever has a high socioeconomic impact and is a serious threat for animals and civilians as well as for military personnel. This was shown during the last big Q fever outbreak in the Netherlands and in the military history of Q fever [64].

Consequently, *Cb* was produced and weaponized in high numbers during several programs of biological warfare [113].

Because of the rising number of Q fever outbreaks, the disease is considered as re-emerging infectious disease in humans as well as in animals. Nowadays, Q fever in humans is a notifiable disease in 17 and in animals in 14 EU member states including Germany [148]. Only specialized laboratories like reference laboratories are allowed to handle and further process *Cb* under biosafety level 3 conditions.

3.3.2 Excretion Routes

Domestic ruminants like cattle, sheep and goats are considered as primary animal reservoirs and the main source for Q fever outbreaks in humans [12, 101-103]. *Cb*-specific antibodies, however, can also be detected in many other hosts like horses, dogs, cats or pigs although their potential in bacterial shedding as well as their role in transmission is not clear yet [105-110]. For example, pigs can be infected with *Cb* but the assumption is that they pose a low infection risk for humans due to low prevalence (antibodies and DNA detection) and specific keeping system, although transmission could not be excluded [109]. Free roaming dogs from aboriginal communities had a 2.8 times higher risk of being seropositive than others (e.g. household dogs) in Australia but evidence regarding bacterial shedding is lacking [149]. In Egypt, 23% of dogs were seropositive [150]. Cats may play an important role in human infection, although their role is still not fully investigated [110, 151, 152]. DNA could be detected in infected (parturient) as well as in healthy cats [153, 154].

Various bacterial excretion routes were described for *Cb*. It has been shown that they are host dependent in particular in ruminants. Furthermore, it is suggested that shedding differs among herds and flocks of the same species [155].

It has been demonstrated that the Q fever pathogen shows organ tropism towards the placenta [11]. In intranasal infected pregnant goats, DNA of *Cb* could be detected first in the upper respiratory tract followed by the uterus and placenta. Afterwards DNA was detected in rising numbers in organs of the dams and the kids until parturition, but the amount of DNA decreased afterwards [11]. Roest *et al.* assumed that *Cb* mainly replicates in the placenta organ [11]. Up to 10^9 bacteria are excreted per one-gram placenta tissue [8]. Consequently, the most important shedding route in small ruminants

is via birth products such as placental membranes and birth fluids during parturition or abortion [3, 8, 11, 156, 157]. It should be emphasized that excretion of *Cb* is independent of the delivery of dead or living offspring. Hence, parturition of offspring born alive should be considered as further source of infection for humans as well as herds or flocks [11, 158]. *Cb* has also been detected in milk, feces and vaginal mucus of small ruminants [11, 155, 157, 159, 160]. Rodolakis *et al.*, however, found that the main excretion route in caprine herds was via milk whereas in ewes with an abortion background, DNA of *Cb* was mainly detected in feces and vaginal mucus and only to a lesser extent in milk [155, 159]. Joulié *et al.* demonstrated that bacterial burden was higher and shedding was longer in ewes with abortion or normal primiparous delivery than in non-aborting or multiparous females [159]. Hence, possible environmental contamination should be considered regarding results of feces and vaginal mucus samples to avoid misidentification of *Cb* shedders [11]. Experimental infected goats excreted *Cb* in milk until 38-52 days post inoculation depending on the milking pattern [8, 11]. It has been shown that *Cb* shedding in milk could be intermittent as well as continuously [155]. In naturally infected dairy sheep flocks *Cb* was shed at least two months in feces and three months in vaginal mucus [159].

In cattle the preferred shedding route of *Cb* is via milk although excretion routes via feces and vaginal mucus have been reported [13, 155]. Shedding could be independent of an abortion background and abortions are less prominent than in small ruminants [13]. Excretion might be irregular and may last for more than one year [155]. Little is known about excretion routes in buffaloes. One study showed that DNA could be detected in samples in all described routes in nearly the same numbers. In that study, milk was not the preferred route in buffaloes as seen for cattle [161].

In camels, little is known about the pathogenesis and excretion pathways of *Cb* despite the rising number of camel farms in many countries. *Cb* was detected in urine, blood, feces and in milk but no preferred excretion route was mentioned [104].

In summary, it can be said that despite all efforts dealing with pathogenesis of *Cb* and excretion routes in recent years both have not been discovered completely yet. Excretion routes are “used” to a variable extent and very often not simultaneously. Additionally, shedding is often discontinuously for all ruminant species in all described routes [13, 158, 159].

Comparison of the excretion routes in domestic ruminants shows that ewes shed *Cb* to a higher extent and over a longer period of time in vaginal mucus than goats. Both animal species can shed *Cb* during successive pregnancies [162, 163]. Milk shedding, however, is more frequent in goats and cattle [164]. The most important excretion route affecting the transmission of Q fever and the risk of infection for humans and animals is via birth products during abortion and parturition. High environmental contamination is a result of this massive bacterial excretion. After drying, *Cb* may become aerosolized and disseminated by the wind very effectively [3, 165]. It has been proven that small ruminants were more often related to human Q fever outbreaks than herds of cattle [10, 12, 101, 155]. These findings might also be explained by the different animal keeping systems e.g. ovine flocks are free ranged whereas dairy cows are kept and calve very often in stables. Therefore, the evaluation of infection risks is indispensable.

3.3.3 Ways of Transmission

As a zoonotic disease, Q fever can spill over to humans from infected animals or be transmitted from animals to animals. Certain characteristics of *Cb* allow survival for a long time outside the host e.g. in animal feces for two years or in dried blood for six month, respectively [166]. Hence, environmental contamination may last for years. Pathogen containing aerosols may be spread via the wind for probably several kilometers and may be inhaled [18]. Inhalation of contaminated aerosols such as water or dust is the main transmission route in humans as well as in animals [3, 11, 64, 167]. It has been shown that animals could also be infected through oral uptake or experimentally by subcutaneous inoculation [8]. In humans, transmission through oral uptake of unpasteurized milk and percutaneous or vertical infection has been documented [168]. Infection via tick bites has not been proven yet and the role of ticks in Q fever transmission is under discussion [120]. Recent studies focusing on *Cb* transmission via tick bite should be reflected critically, respectively. Infection risk for humans through drinking blood of infected animals and consumption of pathogen containing raw milk or unpasteurized dairy products is also still unclear [15]. Investigations of unpasteurized cheeses of small ruminants showed that 27/84 (32%) of cheeses were PCR positive and nearly two-thirds were non-handicraft (industrial) cheeses [16]. Whether the organisms in these products were infectious or not was not researched. Nevertheless, the result for industrial dairy products (cheeses, yogurts,

creams, butters) was also confirmed by Eldin *et al.* [169]. He detected *Cb*-specific DNA in 64% of examined dairy products (n = 201). Evidence of viable *Cb* was negative and dairy products made of unpasteurized milk contained more DNA than food made of pasteurized milk. Products made of small ruminant milk were not as frequently PCR positive than those made of cow milk [169].

Q fever transmission from "person to person" is seldom and only few cases were reported. Nevertheless, sources of infection varied including respiratory transmission after autopsy and infection after receipt of blood transfusion or a bone marrow transplant [170-172]. There was one report found of an obstetrician's infection and vertical transmission from a pregnant woman to her fetus [173].

3.3.4 Potential Risk Factors

In general, infection of humans and animals occurs through direct or indirect exposure. Factors like animal species, number of animals kept in an area or country and type of animal keeping system are closely connected to the risk of infection [12, 131, 156, 174, 175].

Q fever outbreaks in humans in the Netherlands (2007-2011) were mainly associated with goats due to the high number of goat farms [12]. In Germany, in contrast, more sheep than goats are kept. While sheep often range free through the landscape and graze in rural areas, goats and cattle are kept in dairy farms in villages or even small cities [12, 131, 175]. Since environmental contamination happens mainly through extensive animal keeping, farms in close proximity to urban areas increase the risk of infection for other animals as well as for humans. The inner-herd prevalence might be also affected by intensive keeping systems [175]. Therefore, it is likely that merging of herds or flocks from different animal keepers, as it can be seen in many African countries, facilitates spread of *Cb* within herds and in an area as well. *Cb* circulates in herds and flocks and it is assumed that synchronized production systems (with one main lambing season) in small ruminants result in seasonal peaks of human and animal infections [6, 18, 102, 165, 176]. Hence, the immune status of the whole flock or herd and of the individual animal itself plays an important role in *Cb* spreading [12, 156, 174]. Poor farm and hygiene management such as missing quarantine including health check-up of new animals brought to the farm and removal of afterbirths should be considered as

spreading factors. It has been shown that *Cb* DNA can be detected in dust and air samples from the animal houses for several months although individual shedding stopped before [159, 177].

Spillover to humans and other animals is also associated with risk factors such as specific environmental conditions or proximity to infected animals (especially small ruminants) as well as their production and processing sites. Environmental conditions include geography, landscape and climate conditions. Several Q fever outbreaks in humans were associated with close contact to goat farms or sheep flocks ranging and grazing near villages and contaminating the surrounding environment as mentioned above [12, 131, 165]. Investigations of Swedish dairy cattle herds revealed that herds in open landscapes with high temperature, low precipitation (high precipitation decrease aerosol formation) and high wind speeds have an increased risk of being seropositive [17]. However, a naïve cattle herd could only be infected when contaminated aerosols contain enough bacterial load and are transported from infected sources with high wind speed (>5.5 m/s) [178]. In Tanzania, onset of illness in humans was recognized during the dry season [179].

It seems that the risk of infection with *Cb* is often limited to rural areas. Transport or loss of contaminated animal bedding or manure during transport and animal movement, however, bear a high risk for further dissemination of *Cb* besides wind spreading [64, 113, 131]. Therefore, people in urban areas may become affected. Q fever bears a substantial risk for specific occupational groups with close contact to infected animals or their products such as veterinarians, abattoir workers and farmers [146, 180]. Sheep shearing and working in the wool industry is also correlated to a high risk of infection as pathogen containing tick feces is present regularly in the wool [181]. Consumption of raw milk and unpasteurized dairy products is considered as a risk factor, although evidence is missing [169]. Different African traditions such as consumption of raw milk or even blood of animals have been identified as further risk factors in Kenya [182].

4 Laboratory Diagnosis

Q fever diagnosis is a challenge in animals as well as in humans. Especially in ruminants, the appearance of clinical signs i.e. abortions initiate Q fever diagnostics usually. Diagnostic techniques for *Cb* infections are based on isolation and cultivation of the pathogen, detection of its DNA (direct diagnostic methods) and detection of *Cb*-specific antibodies using serological methods (indirect diagnostic methods) [11, 183]. Routine diagnostics usually involve serological methods and PCR only.

In humans the disease is often misdiagnosed due to various clinical presentations and confusion with influenza or malaria [179, 184]. Therefore, history of exposure and medical history should be investigated in detail. Serological methods are standard in human diagnostics and phase specific diagnostic techniques are available.

4.1 Q fever – Clinical Symptoms and Signs

Cb is the etiological agent of Q fever. Nowadays the term "Q fever" is accepted for the disease in humans and animals. In animals, Q fever was called coxiellosis before. Only one to ten bacteria are necessary to infect humans as well as animals. Incubation time is considered to be 2-3 weeks until the first symptoms in humans and clinical signs in animals become obvious. Usually the infection remains asymptomatic.

4.1.1 Human Disease

Humans at any age and gender may become infected with *Cb* and may develop Q fever. The infection can be asymptomatic or manifests with unspecific symptoms. Nearly every organ system may be affected [64]. Asymptomatic Q fever infections are common. Acute as well as chronic disease courses are known and may be differentiated through their serological profile [7].

Most (50.0-90.0%) of seroconverted people are asymptomatic [3, 131, 185]. Those asymptomatic patients may develop chronic disease. The risk to develop chronic Q fever increases, if treatment is not administered timely and is regularly seen in high risk groups such as patients with an (un)known pre-existing heart valve diseases. Additionally, Q fever is often misdiagnosed due to unawareness of physicians, the

nonspecific and various clinical manifestations and the lack of efficient rapid diagnostic methods resulting in delayed diagnosis several months after onset of illness [186, 187].

The acute stage is characterized by mild clinical symptoms (38%) and hospitalization is required in approximately 2% of infected patients [3]. The mortality rate for acute Q fever is approximately 2% [188, 189]. Clinical manifestations similar to influenza, pneumonia and hepatitis are seen [190]. It is assumed that manifestation of acute Q fever depends on the infection route [191]. Q fever is often described as a self-limiting flu-like disease with symptoms like cold, fatigue, malaise, myalgia, headache, shivers and sweats [19, 37]. Interestingly, fever is not always present but prolonged fever is often seen [23, 192]. Derrick *et al.* described a reappearing biphasic fever curve in one quarter of acute Q fever cases [192]. Additionally, it has been shown that Q fever is also a cause of fever of unknown origin (FUO) and thus a diagnostic challenge. In Africa, Q fever was the reason for hospitalization of 2-9% of all patients hospitalized with febrile illness [136]. In northern Tanzania Q fever is also one of the most common causes in febrile patients [179, 184]. Twenty-eight percent (n = 50) of Egyptian patients with FUO had *Cb*-specific antibodies in their first serum sample and further patients with FUO (12%) showed a seroconversion in their convalescence sera [138]. Atypical pneumonia is the most common manifestation, but clinically asymptomatic and rarely respiratory distress may be seen [3, 193]. Minimally auscultatory anomalies, nonproductive cough and fever are the main symptoms [193]. Hence, new diagnostic strategies focus on X-Ray [194]. Pneumonia is often noticed together with other symptoms. Hepatitis, i.e. hepatomegaly with rare jaundice, clinically asymptomatic or granulomatous hepatitis are noticed [195, 196]. Increased liver enzyme levels are the consequence. Many other manifestations of acute Q fever infection have been recognized in nearly every organ system but are less common. For instance, neurological (e.g. encephalitis, meningoencephalitis), cutaneous (e.g. rash, erythema nodosum) or cardiac (e.g. myocarditis, pericarditis) symptoms may be seen [3, 6, 189, 197]. Myocarditis (<1%) is a lifelong sequela and is described as the main cause for fatal outcome of Q fever [3, 6, 198, 199].

In chronic Q fever the disease is present longer than six months [200]. This chronic stage may be seen months and even years after an unrecognized or acute Q fever. Between 0.2% and 5% of infected patients develop chronic Q fever involving the cardiovascular system, the skeleton (osteoarticular) and the liver [5, 201-204]. Especially pregnant women and patients with predisposing factors as cardiovascular lesions, cirrhosis, immunosuppression or cancer are risk groups for developing chronic Q fever [6]. Several strategies have been discussed for early chronic Q fever detection but no standardized criteria could be identified for screening. Till today, there is no uniform case definition for chronic Q fever [205-207]. Assessment of possible pre-exposure of asymptomatic or unknown chronic infected humans and follow-up of acute Q fever cases could support diagnostics of chronic Q fever. For instance, at least one follow-up (six or nine months post infection) is recommended in acute Q fever patients with unknown risk [201, 208]. Patients with high risk of developing chronic Q fever such as patients with clinical cardiac abnormalities should be routinely examined using echocardiography to avoid fatality due to delayed diagnosis [208-210]. Nevertheless, it is recommended to examine patients with an indication only to avoid overestimation of cardiac disorders due to Q fever.

Endocarditis is the most common chronic manifestation (60-73%, 0.6-7% of acute Q fever cases), especially in people with unknown Q fever infection and/or predisposed (un)known cardiac valve or vascular defect or in immunocompromised patients [3, 6, 201-203, 211-213]. Several studies identified *Cb* as cause of endocarditis (3-5%) [214, 215]. In Africa, Q fever was responsible for 1-3% of all infective endocarditis cases [136]. Mortality rate for endocarditis cases decreased due to better diagnosis and treatment but is still approximately 5% [6]. Additionally, high relapse rates are described after withdrawal of antibiotics [187]. Further cardiovascular consequences e.g. arterial embolism, infection of aneurysms or vascular grafts are described [202, 216-218]. Chronic hepatitis or in its worst case cirrhosis, has been diagnosed in chronic liver manifestation [3, 202]. If the skeleton is affected, osteomyelitis, osteoarthritis and contiguous vertebral osteomyelitis (as consequence of aortic graft infection) are likely [219, 220]. Chronic fatigue syndrome is also discussed as a long term sequela [209, 221-224]. Cases of various rare manifestations e.g. chronic pulmonary infections were described [3].

Q fever in children manifests with nearly the same symptoms as described for adults. Acute cases are often self-limiting with fever or asymptomatic. Whereas chronic infections are characterized by osteomyelitis, endocarditis and fever relapse [225-227].

Pregnant women have a particular risk of contracting Q fever as it is known to cause reproductive disorders especially abortions. Most reported Q fever infections in pregnant women were asymptomatic with healthy infants [228, 229]. This might be one reason for the limited available case reports on reproductive disorders in women and thereof most described abortions [173, 199, 228-230]. In southern France, pregnant women (n = 23) suffered from premature birth (35%), abortion or neonatal death (43%), and intrauterine fetal death was also described [199, 229, 230]. Further complications such as placentitis, fever and thrombocytopenia are described and should be examined carefully in pregnant women for detection of *Cb* as possible cause [151, 173, 230-232]. Asymptomatic infection during pregnancy bears the risk of developing chronic Q fever and the risk of resurgence during further pregnancy [199].

More men than women are infected e.g. in France (2.5:1) and Australia (5.3:1) [6, 132]. These findings may be explained by occupational exposure as demonstrated for Egyptian cattle workers [138]. Nevertheless, it has also been demonstrated that causes for Q fever infection are not always linked to occupation or rural lifestyle.

Hospitalized younger patients developed hepatitis more often whereas pulmonary involvement was more frequent in older patients. Both manifestations were more frequently diagnosed in men [6].

Some clinical findings are predominant in different countries and within certain areas. It was suggested that these findings depend on the variable virulence of local *Cb* strains and the infection route. Pneumonia, for instance, is a major clinical manifestation in Nova Scotia, in the Basque region of Spain and in Switzerland, whereas hepatitis was prominent in France and southern Spain [189, 233-235]. In Australia, however, febrile manifestation was predominant [190, 192].

4.1.2 Disease in Animals

Cb infections in animals are often subclinical and asymptomatic although *Cb*-specific antibodies can be detected in various animal species. Interestingly, some specific *Cb* strains are correlated with different ruminant species [54, 236, 237]. Ruminants show clinical signs involving abortion as well as premature delivery, stillbirth and weak offspring. Agerholm designated these manifestations as APSW (abortion, premature delivery, stillbirth, weak offspring) complex [238]. In contrast, delivery of healthy offspring is also described and correlated with shedding of bacteria in high numbers [11, 13, 158]. These clinical signs are outcomes of the intrauterine infection due to the specific organ tropism of *Cb* (**Fig. 5**). Factors like gestation age, virulence of *Cb* strains, immune response (maternal and fetal), spreading (haematogenic or amniotic oral) and dissemination in the fetus may influence the disease outcomes [238].

The phenomenon of seropositivity but asymptomatic course of infection can be regularly seen in small ruminants (sheep and goats). If clinical signs are seen in pregnant small ruminants, the most conspicuous characteristics are stillbirth and abortion. This is especially evident during late pregnancy, whereas no clinical signs are observed in non-pregnant goats [3, 8, 9, 156]. Roest *et al.* assumed that premature death of fetuses is missing, because the trophoblast cells of the allantochorion (primary target cells of *Cb*) of pregnant goats covering the cotyledonary villi are not affected by *Cb*. This allows the maintenance of gas and nutrition exchange between maternal and fetal tissues. These findings are divergent to brucellosis or chlamydial infections [11]. Although placentitis, characterized by a purulent exudate on areas between cotyledons, may be seen (microscopically), the aborted fetuses look fresh and are rarely autolyzed [9, 239]. No clinical signs were recognized in most pregnant goats before abortion at late pregnancy [240]. Experimentally infected pregnant goats and ewes, however, showed fever and *Cb* could be detected in different organs (blood, lung, spleen, liver) during the acute phase [8, 9, 11, 241, 242]. It is still unclear whether *Cb* affects these organs because only mild lesions have been recognized [3, 9]. Further clinical signs such as metritis are observed in goats with abortion background. Additionally, the living offspring was born weak with reduced body weight or with e.g. respiratory disorders [156]. Reproductive problems during the season following an abortion storm are not striking in sheep and goats [156, 162, 174].

Cattle are frequently asymptomatic. Experimental infections in cows (n = 6) and heifers (n = 12) demonstrated that they may develop acute Q fever with e.g. fever and self-limiting pneumonia [243, 244]. Two abortions in this group of infected animals only support the present idea, that the abortifacient potential of *Cb* in cows is still low and an infrequent cause (also on herd level) in field situations [243, 245-247]. Compared to the epidemic (herd level) occurrence in sheep and goats, reproductive disorders are sporadic in cattle. Abortions are more often correlated with other infections such as *Chlamydia* spp. or *Neosporum canis* [248-250]. *Cb*, however, can be detected in the placenta of asymptomatic and symptomatic cows as well as in birth products or aborted fetuses. Mild lesions as well as placentitis may be found in the placenta as well [13, 155, 245-247, 251]. Nevertheless, mastitis, especially subclinical, is the most common obvious Q fever manifestation in cows [252]. Clinical signs like metritis or infertility are discussed as consequence of placental infection of *Cb*, but evidence is often lacking due to missing case definitions and specific examinations [238]. Knowledge about *Cb* infections and their effects on bulls are lacking. Although, *Cb* can be detected in semen [253].

Antibodies of *Cb* were detected in other animal species like horses, pigs, dogs and cats. But the role of *Cb* regarding reproductive disorders, especially as cause of abortion, in those species is unclear [105-109, 151, 152]. One Q fever outbreak in humans was described and associated with a parturient dog losing all puppies within one day after birth [254]. Parturient cats with clinical signs like stillborn or unhealthy kittens, however, are correlated to several human Q fever outbreaks [110, 152, 255, 256]. However, DNA was isolated from healthy cats as well [154].

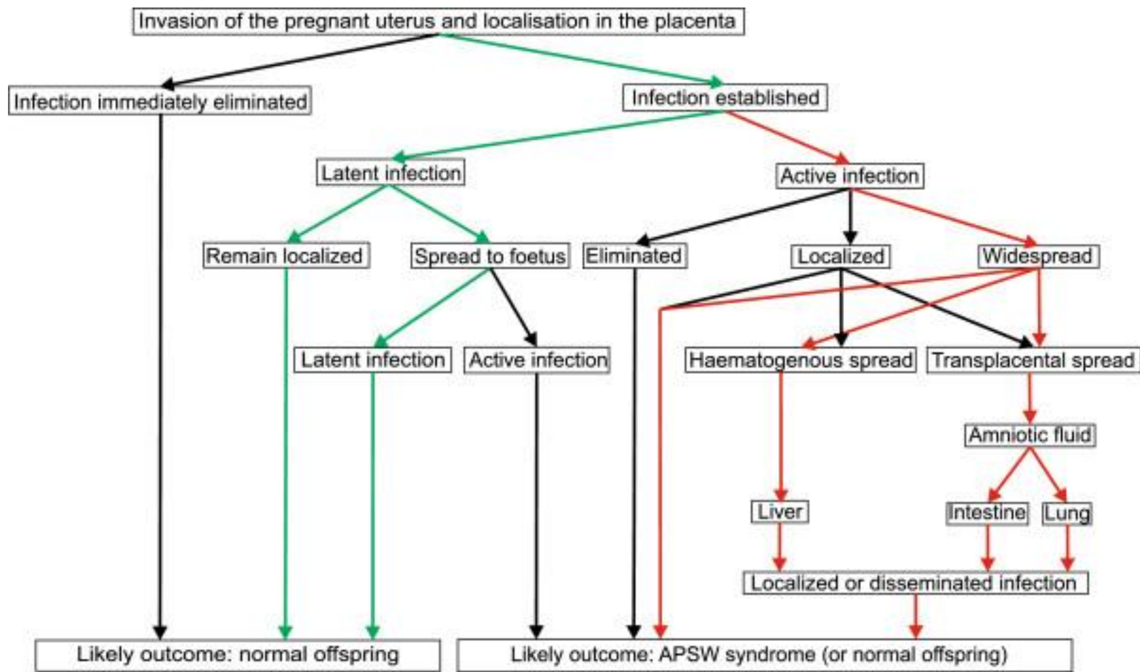

Figure 5. Estimated consequences of a *Coxiella burnetii* infection in the uterus of pregnant animals.

Cited from Agerholm, 2013.

This schematic figure is based on the present knowledge indicating that an intrauterine infection with *Cb* may have different outcomes in pregnant animals. The green arrows indicate the route of a latent infection of which the outcome is very common, at least in cattle. Whereas an active infection, marked by red arrows, most likely affects the fetus after hematogenous or amniotic-oral spreading.

APSW = abortion, premature delivery, stillbirth, weak offspring complex

4.2 Diagnostic Methods for Q fever Detection

4.2.1 Cultivation of *Coxiella burnetii*

Cb may be isolated and propagated in different cell lines or under axenic conditions. Nowadays, the use of laboratory animals in Germany is subject to strict legal regulations according to the animal protection law considering animal welfare. Thus, laboratory animals are not used for cultivation any longer despite their very successful use in the past [19-21]. The use of laboratory animals e.g. mice or guinea pigs had specific benefits: guinea pigs are very susceptible to *Cb* infections, the influence of microbial contamination of a sample is restricted and the pathogen can be isolated from spleen tissue for further processing [20, 257, 258].

Cb may also be propagated in high numbers in the yolk sac endoderm of embryonated hen eggs [24, 259]. A drawback herein is the shift of the bacterium to Phase II associated with loss of virulence. Additionally, purification of the Q fever pathogen is a long and difficult process [260].

In vitro the Q fever agent replicates in different cell lines such as epithelial, fibroblast and macrophage like cell cultures. Such cultures were used since the first *Cb* cultivation experiments in 1937. Cell lines like Vero cells (African green monkey kidney cells) or L929 mouse fibroblast cells are used often [6, 20, 33, 62, 261]. The sensitivity of *Cb* against some antimicrobial substances should be taken into account to ensure success of cultivation [262].

A milestone in *Cb* cultivation was the development of an axenic growth medium, the acidified citrate cysteine media (ACCM), in 2008 [257, 263]. *Cb* can be cultured over 14 days and undergoes developmental differentiation in this medium. ACCM cultivation allows direct cultivation and phenotypic and genotypic characterization of *Cb* isolates [264]. It is still unclear whether the virulence of *Cb* isolates is affected by repeated axenic propagation. ACCM is a useful tool for isolation from clinical samples [257, 265-267]. Nevertheless, success will always depend on bacterial load of the sample, virulence of *Cb* isolates and microbial contamination [257, 267].

It should be emphasized that cultivation method can only be performed under biosafety level 3 laboratories by specialized staff. It is time consuming and not useful for quick routine diagnosis.

4.2.2 Polymerase Chain Reaction

Cb may be detected using polymerase chain reaction (PCR) technique. Especially quantitative real-time PCR (qPCR) assays are used [268]. In general, PCR is based on the detection of specific DNA sequences and proven to be a sensitive and rapid detection method. Specific single copy genes like *com1* and *icd* or the multi copy IS1111 element of the pathogen can be targeted [43, 268]. The copy number of the IS1111 element varies in different *Cb* isolates and it can also be found in *Coxiella*-like bacteria. These findings hamper the use of the IS1111 assays for quantification and identification of *Cb* in environmental samples although it is known as the most sensitive assay [43, 128, 269]. For PCR analysis samples such as milk, feces and vaginal mucus or birth products of animals can be used. It has to be kept in mind that environmental contamination of samples such as feces or vaginal mucus, might give false results and serological diagnostic methods are used frequently instead or both in combination [11, 183]. Blood samples will only test positive in the initial bacteremic stage of infection before antibodies are detectable. In humans, it has also been shown that PCR is not useful for early Q fever diagnosis whereas a high sensitivity was noted for pathogen containing tissues like heart valves [270, 271].

4.2.3 Antigen Detection

The use of specific antibodies reflects a wide field of diagnostic techniques including immunohistochemistry (IHC) or immunofluorescence (IF). IHC is applied in Q fever diagnostics for examination of tissues and detects specific cell structures or antigens (epitopes) of *Cb* with specific antibodies conjugated to dyes. In the IF technique fluorescent dyes are applied. Histological slides may be prepared from tissue samples of aborted fetus or placenta [9, 11]. Gram or Gimenez staining of smears and other sample preparations are unspecific and variable. Other staining techniques like the modified Ziehl-Neelsen stain are also unspecific and might result in misdiagnosis, e.g. *Chlamydia abortus* or *Brucella abortus* also appear as red colored coccobacilli after staining and are indistinguishable from *Cb* [32, 64]. Histological sections and smears can also be analyzed by indirect IF. IF or IHC methods are helpful tools in research to reveal etiology and pathogenesis of *Cb*, but not in the veterinarian routine diagnostic laboratory.

4.2.4 Antibody Detection

For Q fever diagnosis complement fixation tests (CFT) and enzyme linked immunosorbent assays (ELISA) are routinely used [64]. Indirect immunofluorescence assays (IFA) are used less frequently and are not commercially available for veterinary medicine. In general, ELISAs have certain advantages over methods mentioned above. They are easy to handle, time- and cost-efficient. Milk and blood samples can be analyzed in a high throughput format. Especially, examination of bulk tank milk (BTM) by ELISA allows easy herd screening and monitoring of dairy cattle, goat and sheep farms with a fast turnaround [155, 239, 272]. Drawbacks of Q fever ELISAs are the use of mixed whole cell antigens from *Cb* (PhI and PhII) and the detection of only IgG isotypes instead of IgG and IgM [273]. CFTs are based on PhI, PhII or PhI and PhII mixed antigens and can detect IgG as well as IgM antibodies. Several studies comparing CFT and ELISA revealed that some animals may be seronegative in the ELISA technique whereas low positive CFT titers are found [273-276]. This finding was explained by the reactivity of the CFT to IgM and the lack of the PhII response (associated to IgM because of early production of PhII antigens in infection) [274, 275]. Based on these observations the sensitivity of current commercial available ELISAs is intensely debated although ELISAs are considered to have a better sensitivity and specificity than CFT in general [272, 275, 277, 278]. Furthermore, EFSA recommended ELISAs in combination with PCR for Q fever diagnosis in animals [164]. Anti-complementary activity is a known problem in CFTs leading to untestable results [148].

4.2.5 Human Medicine

All the above-mentioned diagnostic techniques are also applied in human medicine. In contrary to veterinary medicine, phase specific ELISAs are available and supported by various diagnostic cutoffs and case definitions [7, 164]. During an acute Q fever infection, PhII specific IgM antibodies and IgG are detected within the second week post infection. Whereas PhII titers steadily increase, low titers of PhI-specific IgM and IgG are detected during convalescence (successful treatment or spontaneously self-limiting) [7, 279]. Anderson *et al.* (CDC and Q fever working group) recommended for the diagnosis of acute Q fever besides clinically symptoms: a fourfold rise of PhII IgG (IFA) in a paired acute and convalescent blood sample (3-6 weeks), illness longer than

one week and a single positive convalescent serum sample with PhII specific IgG titers ≥1:128. For definitive diagnosis of acute Q fever, however, a combination of PCR and serological testing is necessary [7]. PCR testing is only useful in the first two weeks after infection and at least 24-48h after onset of antibiotic treatment. Chronic Q fever is mostly characterized and diagnosed by a constantly increasing PhI-specific IgG titer (titers ≥1:1024) and a comparable low PhII-specific IgG titer [7]. It has been shown that IgG titers ≤1:400 have a negative predictive value (100%) whereas IgG titers ≥1:1,600 (or IgG titers >1:800 and IgA titers >1:50) indicate a positive predictive value of 97% (or 94% for IgG and IgA titers) for chronic Q fever [6, 280]. PhI IgG titers ≥1:800 are a specific criterion for endocarditis [7, 279].

In human medicine, a skin test, an intradermal hypersensitivity test, is used besides serum antibody estimations before vaccination. This pre-vaccinating testing detects hypersensitivity reactions to the vaccine that may result from unrecognized exposure to *Cb*. Blood sampling and skin test should be done the same day. The skin test is read and the result is interpreted after seven days. Vaccination is indicated if both results are negative and the person has a negative history of previous infection with *Cb* [180, 281, 282].

4.3 Correlation of Diagnostic Results with Shedder Status in Animals

Correlation of diagnostic results with shedder status is a challenge in Q fever diagnosis. Rodolakis *et al.* observed bacterial shedding in ewes of which 80% were seronegative [155]. It has been shown that goats may also be seronegative and excrete *Cb* in e.g. milk or vaginal mucus during parturition [8, 11, 155, 158]. It was observed that antibody titers against PhII increased in infected goats at least ten days before delivery [283]. In dairy cattle a similar phenomenon was described and the pathogen was excreted intermittently or frequently in all possible routes [252, 272]. Approximately 35% of *Cb* shedders had a positive result in the ELISA only, but frequent shedding in milk was correlated to persistent high seropositive results [155]. In dairy cattle rising PhI-specific antibody titers were correlated to frequent shedding of *Cb* via milk [13, 274, 284]. Böttcher *et al.* found that chronic milk shedders may be correlated to a PhI titer ≥1:500, whereas puerperal shedding was indicated by a PhI antibody negative and PhII antibody

positive pattern in primiparous cows [284]. Muskens *et al.* mentioned that young cattle had negative fecal PCR results and only 1% was seropositive whereas higher prevalences were detected in the adult group. This finding was explained by the suggestion that young cattle become infected after re-grouping into the adult herd [272]. Re-grouping may be stressful for these young cattle and may cause immunosuppression.

Lack of commercially available and verified phase-specific diagnostic methods in veterinary medicine hampers differentiation between a previous and a present infection. Current serodiagnostic methods are unable to differentiate between Q fever infected and vaccinated animals [277]. Therefore, the development of DIVA vaccines is essential, since *Cb*-specific DNA from the current vaccine composed of the inactivated PhI *Cb* was detected in goat milk shortly after vaccination [285]. To solve this problem and to improve sensitivity and specificity, new development strategies in Q fever diagnostics concentrate on the identification of immunogenic proteins and peptides that will replace whole cell antigens [286, 287].

5 Therapy and Prevention

5.1 Therapy and Management of Q fever in Humans

Besides the fact that acute Q fever is often self-limiting within 2-3 weeks even without therapy, antibiotic treatment is recommended. The most effective antibiotic and first choice of treatment of adult acute Q fever patients and children ≥8 years is doxycycline administered for two weeks. If doxycycline is contraindicated, other antimicrobial chemotherapeutic regimes can be chosen (e.g. trimethoprim/sulfamethoxazole, clarithromycin, rifampin) [288-290]. To reduce the risk of further complications and to shorten the time of illness and accelerate recovery, treatment should be started at least on the third day after onset of symptoms [193, 289, 291, 292]. Consequently, treatment of suspected Q fever cases should start after clinical diagnosis without laboratory confirmation. It is not routinely recommended in asymptomatic cases or patients with mild symptoms [291, 292]. Additionally, patients with acute Q fever should be carefully examined by practitioners whether they belong to one of the groups having a higher risk to develop chronic Q fever e.g. patients with pre-existing (un)known cardiovascular diseases, immunosuppression or pregnant women. Those patients should carefully be monitored clinically and serologically in subsequent regular intervals to avoid non-recognition of chronic Q fever progression and early start of treatment. Especially untreated endocarditis is often fatal. Fenollar *et al.* recommend a doxycycline therapy for one year for acute Q fever patients with valvopathies [213]. Trimethoprim/ sulfamethoxazole should be considered for acute Q fever treatment in pregnant women as this antibiotic may have less negative effects on the developing fetus than doxycycline [7]. They should be monitored after delivery to detect postpartum progression to the chronic stage as well as during the next pregnancies to notice *Cb* recrudescent timely. In children aged <8 years with mild symptoms, doxycycline (5 days) or trimethoprim/sulfamethoxazole may be applied [7].

Diagnosed adult chronic Q fever patients should receive a combined antibiotic regime with doxycycline and hydroxychloroquine [3, 7, 187]. This combination ensures the bactericidal effect in vivo against *Cb* as hydroxychloroquine also raises the pH in the CCV. Hydroxychloroquine, however, should be used with caution and with additional ocular examination as it is known for its retinal toxicity (photosensitivity) during long-term use [187]. In patients with cardiovascular infections, treatment may last up to two years and decrease in antibody titers should be verified [187, 218, 293]. Surgical

interventions such as replacement of defect heart valves or removal of an infected graft may be necessary and live-saving.

5.2 Management of Q Fever in Animals

Therapy of Q fever in animals, in particular in ruminant herds and flocks, shall be based on farm management strategies and prevention measures rather than on chemotherapeutical treatment. During the Dutch Q fever outbreak, more than 50,000 pregnant goats and sheep were culled between December 2009 and June 2012 to inhibit further *Cb* spreading [12]. Additionally, goats were vaccinated with an inactivated PhI vaccine. Vaccination seems to be the most effective tool in Q fever control. Nevertheless, in Germany for instance, a licensed vaccine (Coxevac, inactivated Nine Mile Phase I) is only available for cattle and goats and its use in sheep requires specific authorization. Confirmation of a positive effect of a combined treatment (vaccination and antibiotic) of infected dairy cattle could not be demonstrated [294].

5.3 Prevention Measures

Primary objective in Q fever control is the reduction of the infection risk for humans and animals and the resulting positive impact on the socioeconomic burden. Therefore, prevention measures focus mainly on decreasing *Cb* shedding and dissemination to reduce environmental contamination within a farm as well as in the field. To achieve this, the implementation of a network relying on the One Health approach of epidemiologists, veterinarians and physicians is indispensable. In African countries, disease control efforts are missing, respectively, and implementation of diagnostic capabilities is needed [136].

5.3.1 Farm Management and Environmental Control Measures

On farms, management strategies should focus on reduction of *Cb* shedding in individuals as well as on prevention of *Cb* dissemination within herds, flocks and into the environment.

Epidemic control and hygiene measures should include the acquisition of new animals only from Q fever negative tested herds/flocks and should include quarantine of animals during serological testing. Within herds/flocks, separation of pregnant animals and those that aborted, is recommended [113, 159]. Joulié *et al.* also proposed to separate primiparous ewes from the flock [159]. During the Q fever outbreak in the Netherlands, many pregnant goats and sheep but also bucks were culled and a vaccination program was introduced [12]. Arricau-Bouvery *et al.* also recommend an animal disposal program involving vaccination and elimination of shedders [241]. It has been shown that the current vaccines reduce excretion (especially in vaginal mucus, feces and particularly in milk) and abortions in goats, whereas effects in sheep could not be confirmed [241, 295-299]. Several studies showed, that vaccination in pregnant animals (cattle, goats) or parous goats is largely ineffective. Newborn kids and naïve bovids cannot be protected from Q fever infection [295-298]. Ineffectiveness in pregnant animals was presumed, because of the induced immunomodulation during pregnancy [295]. Infections in previously infected animals (goats, cattle) were also not cleared by vaccination [241, 295-298]. Consequently, like for diagnostics, new approaches in vaccine development are needed to induce a comprehensive immune response. Nevertheless, in uninfected and young animals future bacterial shedding may be decreased and vaccination is recommended [164, 272, 296]. Vaccination of herds/flocks next to infected herds/flocks may be helpful as well.

Manure management is also an important and effective in-farm measure and includes e.g. composting of contaminated litter and manure, which reduces viability of *Cb* [300]. Animal birth and abortion products should be removed and eliminated conscientiously to prevent further dissemination of *Cb*. Legal requirements may be imposed including ban of animal movement and transport of infected animals, or even farm animal breeding as seen in the Netherlands [12]. Notification of increased farm animal abortion was made obligatory in the Netherlands and France [12, 164]. Hygiene management e.g. of personnel, disinfection of transport vehicles, utensils or even paths and indoor housing need to be considered.

Control and observation strategies such as regular testing of BTM are helpful to identify *Cb* shedders, but are not yet compulsory [155, 239, 272]. Additionally, data about the circulation of *Cb* within herds and flocks are useful for implementation and assessment of optimal farm management strategies [159]. It has been shown that the analysis of cloth samples from the environment e.g. of a barn is effective. Areas along fences or window ledges were wiped up with small cloths moistened with distilled water to collect dust [159].

5.3.2 Decrease of Risk of Infection and Impact of Q fever in Human Beings

The One Health approach is based on cross-sectional collaborations (epidemiologists, physicians and veterinarians) addressing risks originating from the relation between humans, animals and the ecosystem. To decrease risk of infection and further impact on human health, public information and awareness rising measures are indispensable and must be guaranteed.

To avoid further dissemination of *Cb* and spill-over, several restrictions may be implemented in high risk areas. Those may affect visiting of affected farms, livestock markets, human assemblies or blood donation [164, 301]. In France, restrictions also focus on trade of raw milk: sale of milk is forbidden for one year after diagnosis of Q fever in an animal out of a raw milk producing farm. Additionally, the milk of an aborted animal in a cheese producing farm has to be eliminated and pasteurization of the milk harvested from the remaining flock is needed [302]. Thus, pasteurization or sterilization of raw milk and prevention of raw milk and raw dairy products consumption decreases the risk of infection.

It is necessary to raise awareness in physicians regarding Q fever diagnosis and anamnesis. Q fever is often not considered as differential diagnosis in particular in febrile patients. Thus, diagnosis especially in late and chronic cases is often delayed because of unrecognized illness rather than of long incubation time.

Nowadays, the most used vaccine in humans is Q-Vax. It is only produced and licensed in Australia and is composed of whole-cell PhI bacteria (formalin-inactivated Henzerling strain) [180, 303]. In humans with previous exposure to *Cb* local and severe systemic reactions were noticed. Hence pre-examination is indicative using the cutaneous test [180, 281, 282]. Vaccination is normally not performed in Europe, but this vaccine was used during the Dutch Q fever outbreak in people at risk of chronic Q fever [103]. Persons with an occupational risk may receive vaccinations compulsorily as a protective effect was seen in slaughterhouse workers in Australia [180, 303].

III PUBLICATION

RESEARCH ARTICLE

Q fever in Egypt: Epidemiological survey of *Coxiella burnetii* specific antibodies in cattle, buffaloes, sheep, goats and camels

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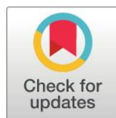

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Abstract

Q fever is a zoonotic disease caused by the bacterium *Coxiella burnetii*. Clinical presentation in humans varies from asymptomatic to flu-like illness and severe sequelae may be seen. Ruminants are often sub-clinically infected or show reproductive disorders such as abortions. In Egypt, only limited data on the epidemiology of Q fever in animals are available. Using a stratified two stage random sampling approach, we evaluated the prevalence of *Coxiella burnetii* specific antibodies among ruminants and camels in 299 herds. A total of 2,699 blood samples was investigated using enzyme-linked-immunosorbent assay (ELISA). *Coxiella burnetii* specific antibodies were detected in 40.7% of camels (215/528), 19.3% of cattle (162/840), 11.2% of buffaloes (34/304), 8.9% of sheep (64/716) and 6.8% of goats (21/311), respectively. Odds of seropositivity were significantly higher for cattle (aOR: 3.17; 95% CI: 1.96–5.13) and camels (aOR: 9.75; 95% CI: 6.02–15.78). Significant differences in seropositivity were also found between domains (*Western Desert*, *Eastern Desert* and *Nile Valley and Delta*) and 25 governorates ($p < 0.001$), respectively. Animal rearing in the *Eastern Desert* domain was found to be a significant risk factor (aOR: 2.16; 95% CI: 1.62-2.88). Most seropositive animals were older than four years. No correlation between positive titers and husbandry practices or animal origin were found ($p > 0.05$). Only 8.7% of the interviewed people living on the farms consumed raw camel milk and none reported prior knowledge on Q fever. Findings from this nationwide study show that exposure to *Coxiella burnetii* is common in ruminants and camels. Disease awareness among physicians, veterinarians and animal owners has to be raised. Future epidemiological investigations have to elucidate the impact of Q fever on human health and on the economy of Egypt.

collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Q fever is a zoonotic disease in humans and animals affecting a wide range of hosts. The causative agent, *Coxiella (C.) burnetii*, is a Gram-negative obligate intracellular bacterium and is known for its high tenacity and infectivity [1, 2]. *C. burnetii* has a worldwide distribution with the exception of New Zealand [3, 4]. Q fever in humans is most often a self-limiting, flu-like illness with symptoms such as headache, myalgia or atypical pneumonia. Hepatitis or endocarditis may be long lasting sequelae in chronic cases [5–10]. Animals are often sub-clinically infected but naïve small ruminants (infected in the last trimester of gestation) may present reproductive disorders such as (late) abortion, premature delivery, stillbirth and weak offspring. Cattle often suffer from sub-clinical mastitis resulting in reduction of milk production and final break down of the quarter [11]. Ruminants shed bacteria in high numbers in birth products and to a lower extent with milk, vaginal mucus and feces or urine [12, 13]. Abortions or lambing in small ruminants have been linked to subsequent human Q fever outbreaks because birth products are heavily contaminated and can easily contaminate the environment [14, 15]. Infection in humans usually occurs via inhalation of contaminated aerosols such as dust or tick feces. In general, risk of infection is increased for people living in rural regions or with occupational risk such as people employed in veterinarian clinics, abattoirs and wool industry due to close proximity to ruminants [16, 17]. Infection risk is also elevated in areas with a high population of ruminants or movement of reservoir animals. Egypt's hot and dry climate with little total precipitation as well as open landscapes with high wind speed may favor spreading of *C. burnetti* via contaminated aerosols [18]. The role of camels in transmission of *C. burnetii* to humans remains poorly understood [12, 19].

In Egypt like in many other developing countries, Q fever is not a notifiable disease although seroprevalences of up to 32% in adults, 22% in children and 16% in veterinarians and farmers have been reported [20–22]. Hence, a high socioeconomic impact of this disease is very likely [23].

Nevertheless, to date only limited data on the epidemiology of *C. burnetii* in animals are available for a few Egyptian districts although first serological evidence in Egyptian animals and humans was reported in the 1950's [4, 17, 24–26]. Therefore, this study was carried out to describe the seroepidemiological situation of *C. burnetii* specific antibodies in ruminants and camels and its potential impact in Egypt (except the Sinai). This study will provide a baseline for further research into the public health impact of Q fever and implementation of public health interventions.

Materials and methods

Study area

The territory of the Republic of Egypt encloses over 1,001,449 km^2 and is divided into 27 governorates. Based on its physical surface characteristics Egypt was divided into three large domains, the *Western Desert*, the *Eastern Desert*, and the *Nile Valley and Delta* region. The majority of the *Western Desert* and *Eastern Desert* domain are dry desert and steppe with scattered oasis. The *Nile Valley and Delta* region is green land with wet or muddy soil conditions. As a result of these differences in surface characteristics there is a distinct non-proportional spatial distribution of animal species and numbers within the different domains.

Study population and study design

Cattle, buffalo, sheep, goat and camel herds in Egypt except those of the Sinai (governorates in the *Eastern Desert* domain) due to ongoing political and security instability were investigated.

From October 2015 to March 2016 a cross-sectional study with a stratified (by governorates) two stage random cluster sampling strategy was conducted. In the first stage 80 villages were randomly selected from 25 governorates. The villages sampled are shown in Fig 1, whereas the governorates are listed in Fig 2 and S1 Table. During the second sampling stage one or two herds/farms were randomly selected without replacements from each sampling site. Thus, a total of 299 herds/farms had to be tested. Due to a full census of the village livestock population was not available sampling was distributed across all identified villages per domain. The number of animals to be tested was calculated using the two stage sampling formula. The calculated number of animals was divided by the total number of villages of each domain to obtain the final number of animals to be sampled per village. The animals sampled in the study were older than 1.5 years to avoid false positive results due to maternal antibody cross reactions in the ELISA test used. The estimated age of the animal was obtained from the farmer.

Sample collection

Blood (5 ml) was collected from the jugular veins of sheep, goats and camels and from the tail veins (*Vena caudalis mediana*) of cattle and buffaloes. Blood samples were collected using disposable needles (18 and 19 gauges) and 50/60 ml three part syringes (AMECO, Egypt). Blood samples were then stored at room temperature for one hour to allow clotting. After centrifugation (1,449 x g, 10 minutes) serum was aliquoted into cryo-vials and stored at -20˚C before being shipped to the Friedrich-Loeffler-Institut (FLI), Germany.

Questionnaire design and data collection

A questionnaire was used to obtain information covering a wide range of factors including information about the animal (age, species, origin) and on the husbandry system practiced. The animal husbandry systems were classified as follows: (a) stable/stationary: animals were kept in an open stable with fences and a partial roof for sun protection, (b) pasture: animals were kept on pasture/steppe in a fenced area and (c) nomadic: animals ranged free, might have been guarded by a person and were occasionally moved from one area to the next. The animal owners were interviewed about their general knowledge on Q fever including transmission, clinical signs in animals and application of countermeasures such as removal of birth products to reduce risk of infection with *C. burnetii*. Furthermore, they were asked if they consume raw milk. The teams interviewed the respondents in Arabic language. Moreover, GPS data were determined to identify the positions of the sampled villages.

Serological testing

The collected serum samples were screened for *C. burnetii* specific antibodies at the Q fever reference laboratory of the FLI. An indirect ELISA (IDEXX CHEKIT Q fever Antibody ELISA Test Kit, IDEXX Laboratories, Switzerland) was used and the results were evaluated according to the manufacturer's recommendations. Briefly, results with an optical density (OD) of $\geq$40% or $<$30% of (PK$\bar{x}$NK$\bar{x}$) (PK = positive control, NK = negative control, $\bar{x}$ = mean) were considered as positive or negative, respectively. Samples with a value between $\geq$30% and $<$40% were considered equivocal and were re-tested. The manufacturer reported sensitivity and specificity of the kit to be approximately 100% [27]. The test is certified for use in sheep, goats and cattle (ruminants). Cattle and buffaloes share a closely related immune system allowing the use of this ELISA for samples from buffaloes [28]. The IDEXX ELISA is commonly used in serum samples of camels although a final validation of this test in camelids is still missing [25, 29].

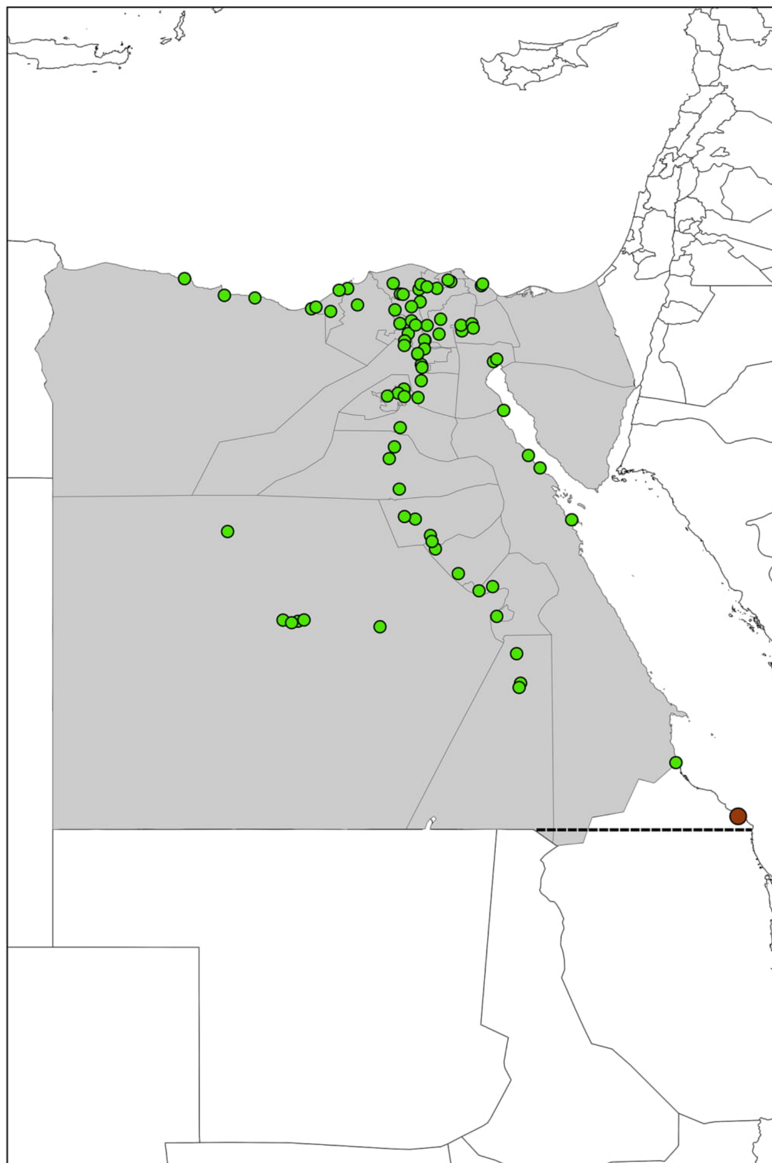

Fig 1. Positions of the sampled villages all over Egypt. The map of Egypt showing the position of each randomly selected sampling site (green dots) in each governorate (grey) where animals were sampled. The sampling site 'Halayeb', highlighted by a brown dot, is located in the territory disputed between Egypt and Sudan.

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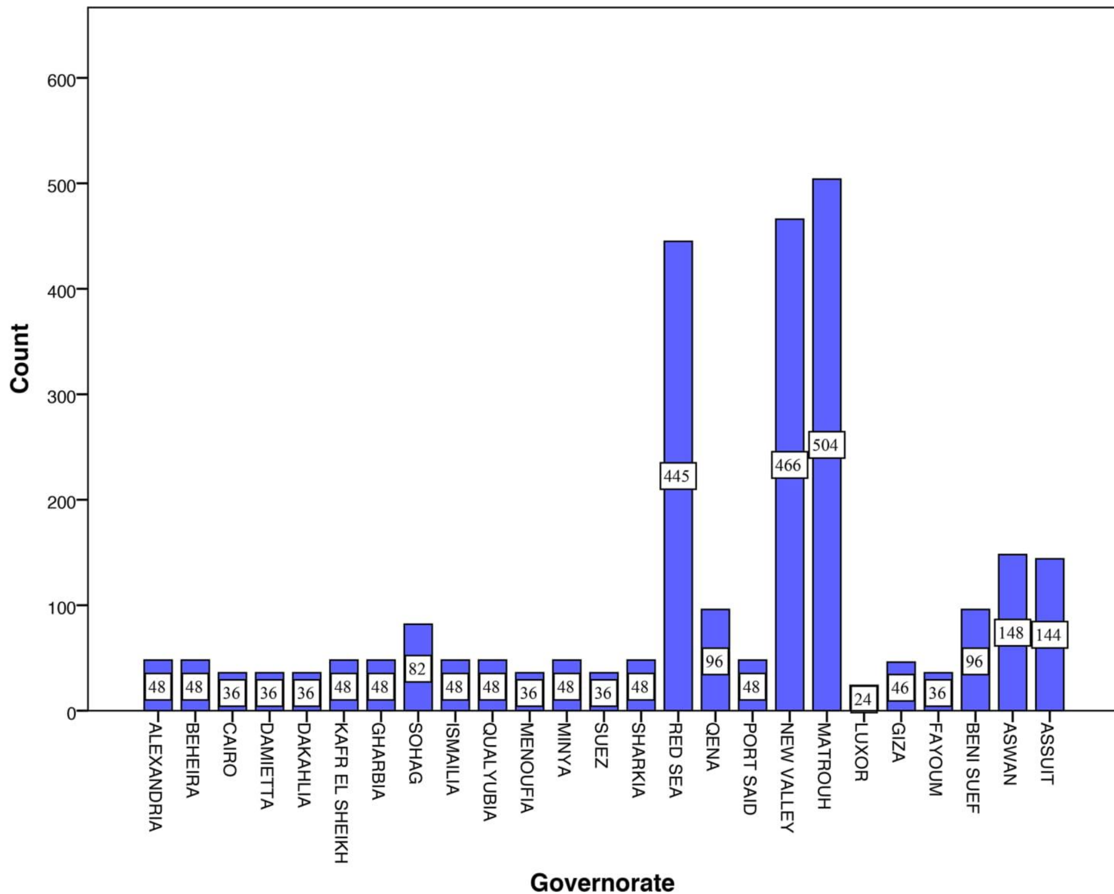

Fig 2. Numbers of animals sampled per governorate.

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Statistical analysis

Analyses were performed using SPSS Statistics software® (Armonk, IBM Corp, USA, version 19). Missing values were coded and included in the analysis as 'missing'. The chi-square or Fisher's exact test was used to determine differences in seropositivity among groups categorized by age, species of the animal, location of collection, animal husbandry system and origin of the animal. Stepwise logistic regression analyses were done to examine the association between variables with $p < 0.2$ in univariable analysis (animal age group, animal species, origin, housing and domain) and seropositivity with adjustment for the other variables. Logistic regression models were also run for each animal species separately. Age was categorized into two groups (up to four years and over four years) and husbandry conditions were categorized into two groups (nomadic vs. 'others' which combined pasture, stables and missing). Odds ratios (ORs) and corresponding confidence intervals (CIs) for each category compared with

the reference group were calculated. P values < 0.05 were considered significant. The map displaying the sampled villages was created using ArcGIS (ESRI, version 10).

Ethical considerations

This study was carried out in strict accordance with the recommendations of the Egyptian Network of Research Ethics Committees (ENREC), which complies with the international laws and regulations regarding ethical considerations in research. The ENREC approved this research work. For purposes of this study all animal owners consented to sampling.

Results

Study population

A total of 2,699 livestock (31.1% cattle, 26.5% sheep, 19.6% camels 11.5% goats and 11.3% buffaloes) were sampled on 299 farms of 80 villages. The majority of the animals sampled was from the *Nile Valley and Delta* (47.6%) and *Western Desert* (35.9%) regions. Animals from the *Eastern Desert* domain accounted for 16.5% due to missing samples of the Sinai. Goats were only sampled in 19 of 25 governorates. The number of goat samples collected differed from the sample size calculated prior to the study, especially in the *Western Desert* and *Eastern Desert* region. One thousand six hundred and thirty-nine (60.7%) animals were nomadic, 262 (9.7%) on pasture and 685 (25.4%) stationary/stables. In the *Western Desert* region, most animals were nomadic (936/2,699 [34.7%]) whereas stationary placement (18.1%) and pasture husbandry (8.0%) were mainly found in the *Nile Valley and Delta* domain. More than eighty-eight percent (88.5%) of all sampled livestock were bred in Egypt and only 311 animals (11.5%) were imported. Camels were the only imported animals and all of them originated from Sudan (58.9% [311/528]). Nine hundred and seventy (28.5%) animals were younger than 4 years and 1,729 (64.1%) were older than 4 years. Fig 2 and Table 1 summarize the characteristics of the study population. None of the livestock owners interviewed reported prior knowledge on Q fever or on any application of countermeasures. Twenty-six owners (8.7%) reported consumption of raw camel milk. Transmission of *C. burnetii* to humans via consumption of raw milk is still unknown.

Seroprevalence

The seroprevalence in goats was 6.8%, in sheep 8.9%, in buffaloes 11.2%, in cattle 19.3% and in camels 40.7% (Table 2). The differences in seroprevalence among the animal species were significant ($p < 0.001$) (Table 3). Multivariable analysis showed significantly higher odds for seropositivity for cattle (aOR: 3.17; 95% CI: 1.96–5.13) and camels (aOR: 9.75; 95% CI: 6.02-15.78) (Table 4). Cattle, sheep and camels of the *Eastern Desert* region had highest seroprevalences. Seroprevalences in buffaloes and goats were highest in the *Nile Valley and Delta* domain ($p < 0.001$) (Table 3). Seropositivity in the final logistic regression model was significantly associated with animals from the *Eastern Desert* domain (aOR: 2.16; 95% CI: 1.62–2.88) (Table 4). This was also evident in the analyses per animal species (S3 Table).

Seroprevalences at governorate level ranged from 4.2% to 36.4% in cattle, from 3.3% to 100% in buffaloes, from 5.3% to 25.0% in sheep, from 4.0% to 41.7% in goats and from 12.5% to 75% in camels ($p < 0.001$) (S1 Table). Seroprevalences determined for the villages were in the range of 4.8%-66.7% in cattle, 4.0%-100.0% in buffaloes, 3.3%-50.0% in sheep, 8.3%-50.0% in goats and 16.7%-78.6% in camels ($p < 0.001$). Fifty-three percent (157/299) of all sampled herds had at least one seropositive animal.

Table 1. Numbers of animals sampled per domain with age group, numbers of animals of a particular animal husbandry system and origin of animals.

Variable	Domain *n* (%)		
	Western Desert	Nile Valley a. Delta	Eastern Desert
Animal species			
Cattle	340 (40.5)	360 (42.9)	140 (16.7)
Buffalo	120 (39.5)	124 (40.8)	60 (19.7)
Sheep	262 (36.6)	314 (43.9)	140 (19.6)
Goat	48 (15.4)	238 (76.5)	25 (8.0)
Camel	200 (37.9)	248 (47.0)	80 (15.2)
Total	970 (35.9)	1284 (47.6)	445 (16.5)
Animal husbandry			
Nomadic	936 (57.1)	467 (28.5)	236 (14.4)
Pasture	34 (13.0)	215 (82.1)	13 (5.0)
Stationary/stable	0 (0)	489 (71.4)	196 (28.6)
Missing	0 (0)	113 (8.8)	0 (0)
Origin of animal			
Egypt	970 (40.6)	1053 (44.1)	365 (15.3)
Sudan	0 (0)	231 (74.3)	80 (25.7)
Animal age group			
$\leq$ 4 years	326 (33.6)	484 (49.9)	160 (16.5)
$>$ 4 years	644 (37.2)	800 (46.3)	285 (16.5)

n = number of animals

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Seroprevalence was found to be higher in animals from stationary/stable (135/685 [19.7%]) and nomadic (318/1639 [19.4%]) farming than from animals kept on pastures (26/262 [9.9%]) ($p = 0.002$) (Table 2). However, this variable was not significant in the multivariable analysis.

Higher seroprevalence 389/1729 (22.5%) was found in animals older than four years. Most sheep and goats with a positive result were younger than four years. The difference in seroprevalence between these age groups was statistically significant ($p < 0.001$) in the univariable analysis, but not in the multivariable analysis.

Of the imported camels 42.1% (131/311) were seropositive. Eighty-four (38.7%) camels from Egyptian origin were tested positive but the difference was not statistically significant ($p = 0.432$).

Discussion

This first nationwide cross-sectional study in ruminants and camels was conducted to provide a deeper understanding of the epidemiology of Q fever in Egypt. An overall seroprevalence of 40.1% in camels, 19.3% in cattle, 11.2% in buffaloes, 8.9% in sheep and 6.8% in goats was found. Seroprevalences were influenced by the geographical location, type of animal husbandry and age of animal, however not by the origin of an animal. Potential risks associated with seropositivity are animal species and the geographical location. Thus, Q fever is endemic throughout Egypt in ruminants and camels.

Over the last 65 years, to the best of our knowledge, ten prevalence studies have been conducted in Egypt. These studies had limitations in study design (missing or inadequate), study area (locally restricted) or size of test specimens. Thus, all major farm animal species which might serve as natural reservoirs were investigated using reliable study design, probabilistic

Table 2. Context of seropositivity and investigated factors of the study populations.

Variable	Total *n*	Seropositive *n* (%)	95% CI	*p* value
Animal species				< 0.001
Cattle	840	162 (19.3)	16.8–22.1	
Buffaloes	304	34 (11.2)	8.1–15.2	
Sheep	716	64 (8.9)	7.1–11.3	
Goats	311	21 (6.8)	4.5–10.1	
Camels	528	215 (40.7)	36.6–45.0	
Domain				< 0.001
Western Desert	970	165 (17.0)	14.8–19.5	
Nile Valley a. Delta	1284	211 (16.4)	14.5–18.6	
Eastern Desert	445	120 (27.0)	23.1–31.3	
Animal husbandry				0.002
Nomadic	1639	318 (19.4)	17.6–21.4	
Pasture	262	26 (9.9)	6.9–14.1	
Stationary/stable	685	135 (19.7)	16.9–22.9	
Missing	113	17 (15.0)	9.6–22.8	
Origin of camels				0.432
Egypt	217	84 (38.7)	32.5–45.3	
Sudan	311	131 (42.1)	36.8–47.7	
Animal age group				< 0.001
≤ 4 years	970	107 (11.0)	9.2–13.2	
> 4 years	1729	389 (22.5)	20.6–24.5	

n = number of animals

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sampling approaches and a representative sample size. A bias may be caused by the lower number of samples collected than calculated prior to the study. This is due to the missing samples from the Sinai and a lower sample size from goats. Nevertheless, the results reported for cattle, buffaloes, sheep and camels are representative for the serprevalences in Egypt.

Gwida et al. (2014) examined dairy cattle and detected *C. burnetii* specific antibodies in 13.2% (158/1,194) of cattle from nine farms from Dakahlia, Damietta and Port Said governorates [24]. Their results are in agreement with the data of this study corresponding to 11.1% in

Table 3. Prevalence of *Coxiella burnetii* specific antibodies in Egyptian livestock in relation to their geographical origin.

Domain	Animal species SP [%], (95% CI)				
	Cattle	Buffaloes	Sheep	Goats	Camels
Western Desert	17.6 (14.0–22.1)	4.2 (1.8–9.4)	7.3 (4.7–11.1)	6.3 (2.2–16.8)	39.0 (32.5–45.9)
Nile Valley a. Delta	14.2 (10.9–18.2)	17.7 (12.0–25.4)	8.0 (5.5–11.5)	7.1 (4.5–11.1)	38.7 (32.9–44.9)
Eastern Desert	36.4 (28.9–44.7)	11.7 (5.8–22.2)	14.3 (9.4–21.0)	4.0 (0.7–19.5)	51.3 (40.5–61.9)
Total	19.3 (16.8–22.1)	11.2 (8.1–15.2)	8.9 (7.1–11.3)	6.8 (4.5–10.1)	40.7 (36.6–45.0)

SP = seroprevalence, CI = confidence interval

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Table 4. Multivariable logistic regression analysis of factors associated with seropositivity.

Variable	Regression Coefficient	Standard Error	Significance	aOR	95% CI
Animal Species			<0.0001		
Goats[a]				1.00	
Sheep	0.21	0.27	0.429	1.23	0.73–2.07
Buffaloes	0.46	0.29	0.117	1.58	0.89–2.82
Cattle	1.15	0.24	<0.0001	3.17	1.96–5.13
Camels	2.28	0.24	<0.0001	9.75	6.02–15.78
Domain			<0.0001		
Western Desert[a]				1.00	
Nile Valley a. Delta	0.06	0.12	0.63	1.06	0.84–1.34
Eastern Desert	0.75	0.15	<0.0001	2.16	1.62–2.88
Constant	-2.74	0.25	<0.0001	0.06	

[a]reference (group with lowest risk), aOR = adjusted Odds Ratio, CI = confidence interval

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these three governorates. Another study did not detect *C. burnetii* specific antibodies in slaughter cattle using IDEXX ELISA in central Egypt [25]. We found antibodies in livestock all across Egypt including central Egypt. Since the origin of the cattle was not defined, it may be assumed that the cattle came either from few Q fever free holdings or the animals had been recently infected and no specific antibodies had yet been produced. The same authors also failed to detect antibodies in buffaloes older than six months. We found a seroprevalence of 11.2% in buffaloes and at least one seropositive buffalo in 47.2% of buffalo herds (25/53). Our findings show that the situation in the field may have changed. The seroprevalence of *C. burnetii* specific antibodies in sheep in our study is in accordance with the data of a study in livestock for slaughter (8% [14/174]) [25]. In contrast, a study on farm animals from the Giza, Cairo and Fayoum governorates showed remarkably high seroprevalences in sheep and goats (32.7% [18/55] and 23.3% [7/30]), respectively [17]. This difference could be explained by the high small ruminant density of this rural region and the fact that infected small ruminants may shed bacteria in high numbers [15, 20]. In goats, the bias discussed before may also be a reason for this difference.

Aridification of many regions of Africa and Asia has increased the relevance of camels as farm animals. In Egypt, camels are kept at high numbers in dry areas for milk and meat production or as pack animals. This can result in greater transmission rates of *C. burnetii* and has been demonstrated in this study with the high seroprevalence found in camels. Several studies from Saudi Arabia, UAE, Iran and Chad showed that camels have antibodies against *C. burnetii* and are able to shed the bacteria via secretions and excretions [12, 19, 25]. Indeed, Schelling et al. (2003) found that pastoralist camel breeders in Chad had increased odds of exposure when compared to cattle breeders [19]. Thus, camels may even play the same important role in human disease as reservoir and source of *C. burnetii* as ruminants do.

Beside the overall seroprevalences for each animal species, significant differences in exposure were found for the domains ($p < 0.001$). Although the association between transmission and prevalence of *C. burnetii* is strongly influenced by landscape, climate, animal movement and high animal population density, no obvious explanations were found for these results [30]. It is likely that transmission of *C. burnetii* in the *Eastern Desert* domain may be favored by coastal winds. Conversely, the high seroprevalence in buffaloes in the *Nile Valley and Delta* region (aOR: 5.01; 95% CI: 1.83–13.71; S3 Table) may be explained by the high animal population density due to abundant feed and water supply. In Egypt, buffaloes are kept in stables and

this may face a higher infection pressure and transmission rate. Nevertheless, further research analyzing the impact of the described factors for a significant assessment are needed.

A clear statement can be made about the influence of the type of husbandry system on seropositivity. Nomadic animal keeping did not pose a significant risk although the majority (318/496 [64.1%]) of seropositive animals were nomadic. This finding may be explained by the construction of the stables (open roofs and fences) which favor transmission of *C. burnetii* via aerosolisation.

C. burnetii could have also been spread by animal movements particularly during uncontrolled import of infected animals. In this survey, camels were the only livestock found to be imported to Egypt, but no correlation was found in the multivariable logistic regression. Hence, the seroprevalence found in camels from Aswan governorate bordering Sudan were strikingly high (67.5% [27/40]). A study from Iran has associated high Q fever seroprevalence in border areas with the import of infected camels [29]. A high (maybe illegal) import rate with no control may be responsible for the high seroprevalence in Aswan. Thus, the impact on transmission of *C. burnetii* through importation of infected animals in this region is substantial and requires immediate action to combat the potential widespread public health effects of *C. burnetii* on animal and human health. Measures to control the importation of camels from Sudan and Somalia to Egypt need to be implemented.

In conclusion, *C. burnetii* specific antibodies are present in Egyptians most important livestock species throughout the country. Especially buffaloes and camels should be the focus of any further research to establish their role in the transmission of *C. burnetii* to humans and to identify any potential risk factors for exposure. In African countries, a classification of husbandry systems is not expedient to identify a risk factor for *C. burnetii* transmission due to the open construction of stables. Whereas the specific geographical characteristics and climatic conditions may influence the seroprevalences in the Western, Eastern and Middle Egypt. Importation of animals with unknown health status has come to the fore and should be tackled immediately. Other consequences on the economy and animal and public health could not be evaluated. Nevertheless, awareness rising is needed in animal owners, veterinarians, physicians and authorities.

Supporting information

S1 Table. Prevalence of *Coxiella burnetii* specific antibodies positive tested animals in Egyptian governorates. $p < 0.001$, n = number, n.a. = not available.
(DOCX)

S2 Table. Positive farm animals kept in different animal keeping systems in Egypt. n = number.
(DOCX)

S3 Table. Multivariable logistic regression analyses of factors associated with seropositivity per animal species. [a]reference (group with lowest risk), aOR = adjusted Odds Ratio, CI = confidence interval.
(DOCX)

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S1 Table. Prevalence of *Coxiella burnetii* specific antibodies positive tested animals in Egyptian governorates. $p < 0.001$, n = number, n. a. = not available.

Animal species	Domain	Governorate (*n* total sites/ *n* total farms)	Serum positive *n*/total	[%]	(95% CI)
Cattle	**Western Desert**	New Valley (6/6)	26/170	[15.3]	(10.66-21.47)
		Matrouh (4/4)	34/170	[20.0]	(14.68-26.65)
	Nile Valley a. Delta	Alexandria (3/3)	0/12	[0.0]	(0.00-24.25)
		Beheira (2/4)	3/12	[25.0]	(8.89-53.23)
		Cairo (2/2)	0/12	[0.0]	(0.00-24.25)
		Damietta (2/2)	1/12	[8.3]	(1.49-35.39)
		Dakahlia (2/2)	1/12	[8.3]	(1.49-35.39)
		Kafr El Sheikh (3/3)	1/12	[8.3]	(1.49-35.39)
		Gharbia (3/3)	3/12	[25.0]	(8.89-53.23)
		Sohag (2/2)	8/24	[33.3]	(17.97-53.29)
		Ismailia (4/4)	2/12	[16.7]	(4.70-44.80)
		Qalyubia (2/2)	0/12	[0.0]	(0.00-24.25)
		Menoufia (3/3)	2/12	[16.7]	(4.70-44.80)
		Minya (2/2)	2/12	[16.7]	(4.70-44.80)
		Suez (2/2)	4/12	[33.3]	(13.81-60.94)
		Sharkia (3/3)	3/12	[25.0]	(8.89-53.23)
		Qena (3/3)	0/24	[0.0]	(0.00-13.80)
		Port Said (2/2)	2/12	[16.7]	(4.70-44.80)
		Luxor (0/0)	n. a.		
		Giza (3/3)	1/12	[8.3]	(1.49-35.39)
		Fayoum (3/3)	1/12	[8.3]	(1.49-35.39)
		Beni Suef (2/2)	1/24	[4.2]	(0.74-20.24)
		Aswan (3/3)	10/60	[16.7]	(9.31-28.03)
		Assuit (3/3)	6/36	[16.7]	(7.87-31.89)
	Eastern Desert	Red Sea (5/5)	51/140	[36.4]	(28.92-44.66)
	Total	24 (69/71)	162/840	[19.3]	(16.76-22.09)
Buffaloes	**Western Desert**	New Valley (6/6)	3/60	[5.0]	(1.71-13.70)
		Matrouh (3/3)	2/60	[3.3]	(0.92-11.36)
	Nile Valley a. Delta	Alexandria (3/3)	2/4	[50.0]	(15.00-85.00)
		Beheira (2/2)	1/4	[25.0]	(4.56-69.94)
		Cairo (1/1)	0/4	[0.0]	(0.00-48.99)
		Damietta (1/1)	0/4	[0.0]	(0.00-48.99)
		Dakahlia (1/1)	0/4	[0.0]	(0.00-48.99)
		Kafr El Sheikh (2/2)	2/4	[50.0]	(15.00-85.00)
		Gharbia (2/2)	2/4	[50.0]	(15.00-85.00)
		Sohag (1/1)	0/8	[0.0]	(0.00-32.44)
		Ismailia (2/2)	2/4	[50.0]	(15.00-85.00)
		Qalyubia (1/1)	1/4	[25.0]	(4.56-69.94)
		Menoufia (2/2)	0/4	[0.0]	(0.00-48.99)
		Minya (2/2)	0/4	[0.0]	(0.00-48.99)

		Suez (2/2)	0/4	[0.0]	(0.00-48.99)
		Sharkia (2/2)	4/4	[100.0]	(51.01-100.00)
		Qena (1/1)	1/8	[12.5]	(2.24-47.09)
		Port Said (1/1)	0/4	[0.0]	(0.00-48.99)
		Luxor (1/1)	0/4	[0.0]	(0.00-48.99)
		Giza (2/2)	0/4	[0.0]	(0.00-48.99)
		Fayoum (2/2)	3/4	[75.0]	(30.06-95.44)
		Beni Suef (2/2)	3/8	[37.5]	(13.68-69.43)
		Aswan (3/3)	1/20	[5.0]	(0.89-23.61)
		Assuit (2/2)	0/12	[0.0]	(0.00-24.25)
	Eastern Desert	Red Sea (6/6)	7/60	[11.7]	(5.77-22.18)
	Total	25 (53/53)	34/304	[11.2]	(8.11-15.22)
Sheep	**Western Desert**	New Valley (4/4)	11/112	[9.8]	(5.57-16.73)
		Matrouh (4/4)	8/150	[5.3]	(2.73-10.17)
	Nile Valley a. Delta	Alexandria (3/3)	0/12	[0.0]	(0.00-24.25)
		Beheira (2/2)	2/12	[16.7]	(4.70-44.80)
		Cairo (2/2)	1/12	[8.3]	(1.49-35.39)
		Damietta (2/2)	3/12	[25.0]	(8.89-53.23)
		Dakahlia (2/2)	1/12	[8.3]	(1.49-35.39)
		Kafr El Sheikh (3/3)	0/12	[0.0]	(0.00-24.25)
		Gharbia (2/2)	0/12	[0.0]	(0.00-24.25)
		Sohag (2/2)	2/24	[8.3]	(2.32-25.85)
		Ismailia (4/4)	2/12	[16.7]	(4.70-44.80)
		Qalyubia (2/2)	1/12	[8.3]	(1.49-35.39)
		Menoufia (2/2)	1/12	[8.3]	(1.49-35.39)
		Minya (2/2)	1/12	[8.3]	(1.49-35.39)
		Suez (2/2)	0/12	[0.0]	(0.00-24.25)
		Sharkia (2/2)	2/12	[16.7]	(4.70-44.80)
		Qena (3/3)	2/24	[8.3]	(2.32-25.85)
		Port Said (2/2)	0/12	[0.0]	(0.00-24.25)
		Luxor (0/0)	n. a.		
		Giza (3/3)	0/12	[0.0]	(0.00-24.25)
		Fayoum (2/2)	3/12	[25.0]	(8.89-53.23)
		Beni Suef (2/2)	0/24	[0.0]	(0.00-13.80)
		Aswan (2/2)	0/14	[0.0]	(0.00-21.53)
		Assuit (3/3)	4/36	[11.1]	(4.41-25.32)
	Eastern Desert	Red Sea (6/6)	20/140	[14.3]	(9.44-21.04)
	Total	24 (63/63)	64/716	[8.9]	(7.06-11.25)
Goats	**Western Desert**	New Valley (4/4)	1/24	[4.2]	(0.74-20.24)
		Matrouh (3/3)	2/24	[8.3]	(2.32-25.85)
	Nile Valley a. Delta	Alexandria (3/3)	2/12	[16.7]	(4.70-44.80)
		Beheira (2/2)	1/12	[8.3]	(1.49-35.39)
		Cairo (0/0)	n. a.		
		Damietta (0/0)	n. a.		
		Dakahlia (0/0)	n. a.		
		Kafr El Sheikh (3/3)	1/12	[8.3]	(1.49-35.39)

		Gharbia (2/2)	3/12	[25.0]	(8.89-53.23)
		Sohag (1/1)	0/10	[0.0]	(0.00-27.75)
		Ismailia (2/2)	5/12	[41.7]	(19.33-68.05)
		Qalyubia (2/2)	0/12	[0.0]	(0.00-24.25)
		Menoufia (0/0)	n. a.		
		Minya (2/2)	1/12	[8.3]	(1.49-35.39)
		Suez (0/0)	n. a.		
		Sharkia (2/2)	0/12	[0.0]	(0.00-24.25)
		Qena (2/2)	0/24	[0.0]	(0.00-13.80)
		Port Said (2/2)	0/12	[0.0]	(0.00-24.25)
		Luxor (2/2)	0/12	[0.0]	(0.00-24.25)
		Giza (2/2)	0/10	[0.0]	(0.00-27.75)
		Fayoum (0/0)	n. a.		
		Beni Suef (2/2)	3/24	[12.5]	(4.34-31.00)
		Aswan (2/2)	1/14	[7.1]	(1.27-31.47)
		Assuit (2/2)	0/36	[0.0]	(0.00-9.64)
	Eastern Desert	Red Sea (4/4)	1/25	[4.0]	(0.71-19.54)
	Total	19 (45/45)	21/311	[6.8]	(4.46-10.10)
Camels	**Western Desert**	New Valley (6/6)	41/100	[41.0]	(31.87-50.80)
		Matrouh (4/4)	37/100	[37.0]	(22.78-40.63)
	Nile Valley a. Delta	Alexandria (3/3)	1/8	[12.5]	(2.24-47.09)
		Beheira (2/2)	6/8	[75.0]	(40.93-92.85)
		Cairo (2/2)	4/8	[50.0]	(21.52-78.48)
		Damietta (2/2)	2/8	[25.0]	(7.15-59.07)
		Dakahlia (2/2)	3/8	[37.5]	(13.68-69.43)
		Kafr El Sheikh (3/3)	1/8	[12.5]	(2.24-47.09)
		Gharbia (3/3)	3/8	[37.5]	(13.68-69.43)
		Sohag (2/2)	7/16	[43.8]	(23.10-66.82)
		Ismailia (4/4)	1/8	[12.5]	(2.24-47.09)
		Qalyubia (2/2)	2/8	[25.0]	(7.15-59.07)
		Menoufia (2/2)	0/8	[0.0]	(0.00-32.44)
		Minya (2/2)	1/8	[12.5]	(2.24-47.09)
		Suez (2/2)	4/8	[50.0]	(21.52-78.48)
		Sharkia (2/2)	3/8	[37.5]	(13.68-69.43)
		Qena (3/3)	4/16	[25.0]	(10.18-49.50)
		Port Said (2/2)	2/8	[25.0]	(7.15-59.07)
		Luxor (2/2)	4/8	[50.0]	(21.52-78.48)
		Giza (2/2)	0/8	[0.0]	(0.00-32.44)
		Fayoum (2/2)	3/8	[37.5]	(13.68-69.43)
		Beni Suef (2/2)	9/16	[56.3]	(33.18-76.90)
		Aswan (3/3)	27/40	[67.5]	(52.02-79.92)
		Assuit (3/3)	9/24	[37.5]	(21.16-57.29)
	Eastern Desert	Red Sea (5/5)	41/80	[51.3]	(40.49-61.89)
	Total	25 (67/67)	215/528	[40.7]	(36.61-44.96)

S2 Table. Positive farm animals kept in different animal keeping systems in Egypt. *n* = number.

Animal Species	Animal Keeping System *n* Serum positive [%] pasture	stationary/ stable	nomadic	missing
Cattle (*n* = 840)	6 [0.7]	102 [12.1]	54 [6.4]	0 [0.0]
Buffaloes (*n* = 304)	0 [0.0]	25 [8.2]	5 [1.6]	4 [1.3]
Sheep (*n* = 716)	8 [1.1]	5 [0.7]	47 [6.6]	4 [0.6]
Goats (*n* = 311)	3 [1.0]	0 [0.0]	18 [5.8]	0 [0.0]
Camels (*n* = 528)	26 [1.0]	135 [5.0]	318 [11.8]	17 [0.6]

S3 Table. Multivariable logistic regression analyses of factors associated with seropositivity per animal species. [a]reference (group with lowest risk), aOR = adjusted Odds Ratio, CI = confidence interval.

Cattle

Variable	Regression Coefficient	Standard Error	Significance	aOR	95% CI
Domain			<0.0001		
Western Desert[a]				1.00	
Nile Valley a. Delta	-0.27	0.21	0.200	0.77	0.51-1.15
Eastern Desert	1.01	0.23	<0.0001	2.75	1.76-4.29
Constant	-1.52	0.14	<0.0001		

Buffaloes

Variable	Regression Coefficient	Standard Error	Significance	aOR	95% CI
Domain			0.007		
Western Desert[a]				1.00	
Nile Valley a. Delta	1.61	0.51	0.002	5.01	1.83-13.71
Eastern Desert	1.11	0.61	0.068	3.04	0.92-10.01
Constant	-3.14	0.46	<0.0001		

Sheep

Variable	Regression Coefficient	Standard Error	Significance	aOR	95% CI
Domain			0.052		
Western Desert[a]				1.00	
Nile Valley a. Delta	0.11	0.32	0.725	1.12	0.60-2.08
Eastern Desert	0.76	0.34	0.026	2.13	1.10-4.14
Constant	-2.55	0.29	<0.0001		

Goats

Variable	Regression Coefficient	Standard Error	Significance	aOR	95% CI
Constant	-2.62	0.23	0.073		

Camels

Variable	Regression Coefficient	Standard Error	Significance	aOR	95% CI
Housing			0.002		
other[a]				1.00	
nomadic	0.86	0.28	0.002	2.37	1.36-4.12
Age category			0.024		
≤ 4 years[a]				1.00	
> 4 years	-0.75	0.33	0.024	0.47	0.25-0.91
Constant	-0.38	0.36	0.290		

IV DISCUSSION

1 Assessment of Seroprevalences of *Coxiella burnetii*-Specific Antibodies in Egyptian Livestock

1.1 Evaluation of Serological Findings of *Coxiella burnetii*-Specific Antibodies

Available data on the epidemiology of Q fever in animals of African countries are limited although Q fever is a re-emerging zoonotic disease in humans and animals. In Egypt, only a few seroprevalence data of *Coxiella burnetii*-specific antibodies in livestock are available and restricted to a few districts [133, 135, 142, 304, 305]. Therefore, this nationwide cross-sectional survey was conducted to evaluate the prevalence of *Cb*-specific antibodies among the most important livestock species that might serve as natural reservoirs in Egypt: cattle, buffaloes, sheep, goats and camels. Further, this study aimed to provide a deeper understanding of the epidemiology of Q fever in Egypt presenting a baseline for further research into the public health impact of Q fever and implementation of public health intervention.

1.1.1 Seroprevalence Results in Animals

An overall seroprevalence of *Cb*-specific antibodies of 18.4% (496/2,699) was found in Egyptian livestock. In detail, *Cb*-specific antibodies were detected in 40.7% of camels (215/528), 19.3% of cattle (162/840), 11.2% of buffaloes (34/304), 8.9% of sheep (64/716) and 6.8% of goats (21/311). The high seroprevalence results of camels and cattle found were reflected in the high odds for seropositivity for cattle (aOR: 3.17; 95% CI: 1.96-5.13) and camels (aOR: 9.75; 95% CI: 6.02-15.78) of the multivariable analysis. Comparable studies about *Cb*-specific antibodies in Egypt or bordering countries do not exist (**Tab. A1**). This study was conducted with a reliable study design, a probabilistic sampling approach and a representative sample size, except for goats. Comparisons with other Egyptian studies could only be realized by calculating the seroprevalence for the governorates in question. Especially the results for small governorates in the *Nile Valley and Delta Area* should not be considered itself because the study is based on three classified domains. Hence these results, often calculated from two farms for each animal species, were not representative for a small governorate. This is supported by the wide ranges of the seroprevalence results for each

animal species determined for each governorate and village of this study.

Nevertheless, the overall results of Egypt and each domain indicate that Q fever is endemic throughout Egypt. Camels and buffaloes stand out as reservoir for *Cb* besides cattle. However, only the presence of antibodies was detected indicating a prior contact to the pathogen. Hence, further research should concentrate on detection of *Cb* to assess infection risk and public health impact.

1.1.2 Seropositivity of Animals Correlated to Specific Risk Factors

Besides the risk factor "animal species", "geographical location" was associated with seropositivity in livestock (**Fig. A7-A9**). Several studies identified risk factors like high wind speed, dry and hot climate and open landscapes facilitating *Cb* spreading and increasing infection risk for humans and animals [17, 178, 179]. In this study, landscape conditions were taken in consideration in so far as Egypt was divided into three domains. The *Western Desert Domain* mainly consist of desert whereas the *Nile Valley and Delta Area* is, corresponding to Egyptian conditions, more or less green land. The *Eastern Desert Area* is dry but has mountain-like ranges and is bordering the Red Sea.

In the *Eastern Desert Area* seroprevalence in cattle, sheep and camels was higher compared to those in the other domains. The final logistic regression associated seropositivity with animals from the *Eastern Desert Area* (aOR: 2.16; 95% CI: 1.62-2.88). These findings may be explained by the missing samples from the Sinai that could not be collected due to ongoing political instabilities and security risks. The lower number of samples may cause a bias. In contrast, the results for buffaloes and goats were highest in the *Nile Valley and Delta Area.* A scientific explanation for these findings could not be found. Therefore, future studies should focus on causes for the risk factor "geographical location" and specify them.

In this study, animal husbandry system, as well as the age and the origin of an animal were no potential risks in the multivariable risk factor analysis. The seroprevalence results for animal husbandry system and age, however, were statistically significant ($p = 0.002$, $p < 0.001$). A classification of husbandry systems to identify a risk factor for *Cb* is not useful in Egypt and possibly other countries. This finding may be explained by the dissemination of *Cb* via wind and the open construction of stables in Egypt. Stables are constructed of fences and open roofs also allowing *Cb* spreading through

wind. Most positive animals, however, are kept nomadic (318/496 [64.1%]). Maybe, environmental contamination is higher because excretions of the animals were not removed and the same paths were used for animal ranging. Widespread distribution of aerosols contaminated with *Cb* and thus an increase of risk of infection for animals and humans may be the results.

In general, it is not known if a specific age of animal is correlated to seropositivity of *Cb*. Therefore, seroprevalences depending on the age of animals was calculated in this study to be 11.0% (107/970) in ≤4-year-old and 22.5% (389/1729) in >4-year-old animals. It is, however, more reasonable to consider each animal species itself due to their specific characteristics, e.g. life expectation or type of use (**Tab. 1**). In consequence of the very limited data available about this specific issue, comparison with other study results is restricted. No research is available for Egypt and to the best knowledge of the author, only one study is available from an African country. Investigations with the same ELISA used in this study in small ruminants (goats and sheep) in the Gambia showed that 10% (n = 256) of <1 year-, 26% (n = 379) of 1-3-year- and 27% (n = 244) of ≥4-year-old animals had *Cb*-specific antibodies [306]. In Egypt, most small ruminants with a positive antibody result were between two and four years old (**Tab. 1**). This finding may be explained by the fact that most small ruminants sampled were younger than four years. It is not clarified if most small ruminants older than four years served for meat production and thus were not available for sampling. Sampling was done without consideration of animal age. Additionally, it is suggested that age of animals was often only estimated by the animal owner which makes a classification and thus realistic comparison difficult. On the other hand, the number of positive animals compared to the number of animals sampled is too low to point out a fundamental statement. A clearer figure is shown by the seroprevalence results in camels and cattle. The majority of positive tested animals of these species were older than four years. While no comparable data are available for camels, Böttcher *et al.* investigated 3,965 cattle from 105 farms in Bavaria with the same ELISA used in this survey. Seroprevalence results of 5% in 1-2-year-, 15% in 2-3-year- and 25-30% in ≥4-year-old cattle were obtained [274]. These results support the findings that seroprevalence in cattle >4 years of age shows a plateau and is higher than in younger animals. This comparison is hampered by the fact that the conditions in both countries are different, e.g. husbandry system, climate and landscape. Furthermore, the immune

system and hence the immune status of each group of age play an important role, not only in cattle. In that study, it was suggested that cattle younger than one year of age showed a positive ELISA due to maternally received antibodies [274]. In general, animals younger than 1.5 years were not sampled in this survey to avoid maternal antibody cross reactions. Maternal antibodies might explain the positive reactivity to PhII but transient infection could not be excluded [274]. Interestingly, Böttcher *et al.* found that $PhI^-/PhII^+$ 1-2-year-old heifers frequently seroconverted to negative, whereas primiparous cows with the same pattern changed to negative, PhI and PhII positive or stayed $PhI^-/PhII^+$ (persistent) equally [274]. These findings give new insights into the probable systemic immunotolerance to and subsequent persistence of Q fever infection but have to be determined further on for each animal species.

Table 1. Numbers of seropositive Egyptian livestock correlated to their estimated age.

Animal Species	**Estimated Animal Age** (years)						
n	**2-3**	**3-4**	**4-5**	**5-6**	**6-7**	**7-8**	**8-9**
Cattle (*n* = 162)	6	7	51	34	45	12	7
Buffaloes (*n* = 34)	3	0	3	7	9	8	4
Sheep (*n* = 64)	24	26	13	1	0	0	0
Goats (*n* = 21)	7	10	4	0	0	0	0
Camels (*n* = 215)	8	16	34	57	72	23	5
Total (*n* = 496)	48 9.7%	59 11.9%	105 21.2%	99 20.0%	126 25.4%	43 8.7%	16 3.2%

n = number of animals tested seropositive

1.1.3 Possible Effects and Evaluation of the ELISA Test Used

The IDEXX ELISA Checkit was applied in this study. Milk, blood and serum specimens from cattle, sheep and goats can be used as samples. The manufacturer states a 100% specificity and sensitivity compared to CFT by analyzing serum samples of field and experimentally infected goats [307]. Several studies, however, question the efficiency, especially the sensitivity of this ELISA [273, 274, 277, 284]. Additionally, this ELISA is used for routine testing samples of buffaloes and camels although it is not authorized for these animal species. Therefore, the discussion about the evaluation of this ELISA and its possible statistical effect is indispensable.

Sensitivity of commercially available ELISAs may be affected by the *Cb* whole cell antigen used. The ELISA used is based on *Cb* PhI and II purified whole cell antigens of the Nine Mile strain (isolated from ticks) and detects total IgG. It has been demonstrated that an ELISA using an ovine strain had the best overall performance compared to those using a bovine and a tick one [277]. Whereas Emery *et al.* did not notice a significant increase in sensitivity [308]. A lower sensitivity may also be caused by the lack of IgM detection and the missing differentiation between PhI and PhII specific antibodies [273, 274, 309, 310]. Additionally, it has been shown that a PhI and PhII mixed ELISA is insufficient in detecting the PhII-specific response that is associated to IgM class [101, 274].

This ELISA seems to be specific enough for its use in veterinary medicine [247, 310]. No cross reactions were recognized testing samples of *Chlamydia abortus* infected sheep when ovine derived *Cb* antigens were used [277]. It is noteworthy, that IFAs used in human medicine showed cross reactions with samples of patients infected with e.g. *Legionella* spp. (micro IFA) and *Bartonella* spp. (indirect IFA) although they had low titers and misdiagnosis should be improbable [311, 312].

Several studies were conducted on Q fever in buffaloes and camels by using IDEXX ELISA, suggesting a wide acceptance of application for buffalo and camel samples [104, 133, 139, 305, 313, 314]. As mentioned, this assay is not authorized for samples of these animals. Phylogenetic relationship of buffaloes and cattle enables the plausible use of this ELISA in buffaloes [315]. For camel samples, Kittelberger *et al.* recommend the use of complement fixation tests (CFTs) due to the missing evaluation of the ELISAs [275]. However, in this survey detection of *Cb*-specific antibodies in serum samples of camelids using the conjugated anti-ruminant antibodies was successful (**Fig. A1**). Surprisingly, the dilution ratio given by the manufacturer could be maintained. Nevertheless, it has never been proven that this test is valid for samples of the animals in question and no cutoffs were determined.

A cutoff value with an optical density (OD) of ≥40% is recommended by the manufacturer and the World Health Organization (WHO) for blood and milk samples, although Paul *et al.* calculated that no cutoff would be analytically perfect [310]. There will always be false positive and false negative results due to pipetting inaccuracy or possible cross reactions. Diagrams of the obtained OD results were created (**Fig. A2-A6**). In general, progression (hypothetical frequent distribution) should show a peak before the given negative (OD <30%) and after the given positive (OD ≥40%) cutoff for this indirect ELISA. The percentage of false results acceptable for a diagnostic application should be the basis for the chosen cutoffs (**Fig. 6**) [316]. Thus, sensitivity and specificity play an important role.

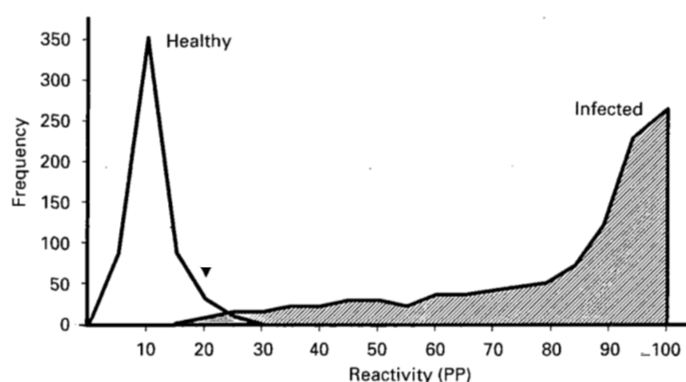

Figure 6. Determination of possible cutoffs for an indirect ELISA.

Cited from Wright *et al.*, 1993.
Samples from animals out of a healthy and an infected group should be investigated to obtain a hypothetical frequency distribution. The acceptable percentage of false positive and false negative determine the cutoffs, that should be chosen, the sensitivity and specificity.
PP = percent positivity

The obtained diagrams (**Fig. A2-A6**) show a similar distribution of the obtained OD values in cattle, sheep and goats. It is interesting, that the OD distribution in buffaloes (**Fig. A3**) is similar to that in cattle (**Fig. A2**), although less animals were investigated. The second peak determining the positive cutoff for buffaloes, however, is visible in the diagram at an OD value of 60% only and not at an OD value of 40% as usual in e.g. cattle. This finding and the high determined numbers of negative buffaloes might be explained by the hypothesis that buffaloes are not as susceptible to *Cb* infection as cattle and thus the immune response may not be strong. Furthermore, it might be possible that

Cb-specific antibodies of buffaloes are not as sensitive to the Nine Mile derived antigen and tests should be repeated with a bovine or ovine derived antigen to reveal false negative results. The diagram of camel samples also shows two peaks, but the equivocal area (OD 30-39.99%) includes more samples than seen in the other animal species (**Fig. A6**). Additionally, antibody response reactivity seems to be higher. This finding might be explained by the specific antibody structure of camelidae. Their immune system also produces antibodies with heavy chains only, besides conventional antibodies with heavy and light chains [317]. It has been shown that those antibodies have a specific extended structural feature suggesting that this allows and supports affinity to specific antigen structures when conventional antibodies fail [318]. Thus, false positive results cannot be excluded. In conclusion, the diagrams show that cutoffs for camel and buffalo samples should be reconsidered and the use of the IDEXX ELISA in these animal species has to be validated. Validation is hampered by the fact, that "true" negative (uninfected) and "true" positive (infected) animals are necessary to determine the exact cutoffs. As mentioned, animals with a seronegative status may also be *Cb* shedders. Lack of phase specific assays disallow antibody detection in samples taken from an animal during the early stage of infection [319]. Likewise, no further sampling was done to assess delayed antibody responses in this survey. Thus, it is likely that not all seropositive animals were detected.

1.2 Milk Samples

In general, milk samples have some advantages regarding animal welfare and personnel occupational safety as collection of milk is easy, non-invasive and not as stressful as blood sampling for (dairy) animals. Additionally, it is very cost efficient as DNA of *Cb* or *Cb*-specific antibodies can be investigated in bulk tank milk (BTM) samples detecting Q fever at herd level [239, 272]. Comparison of milk and blood ELISA analysis showed a negligible higher sensitivity of milk samples (0.86 to 0.84) in dairy cattle [310]. This finding was confirmed by Rodolakis *et al.* [155]. Specificity was 0.99 [310]. Therefore, investigation of BTM is a useful tool for surveillance programs in dairy farms. For non-lactating cows, young and male animals, nevertheless, blood sampling is still needed.

However, the use of serological methods to detect antibodies in milk and the obtained results are controversially discussed. It is not clear, if detected antibodies are produced due to systemic or local Q fever infection. If *Cb*-specific antibodies result from systemic infection, they have penetrated the blood-udder barrier. In general, penetration of the blood-udder barrier in bovines is a physiological process. Maternal antibodies accumulate in the mammary gland and the resulting colostrum ensures immune defense and thus promotes survival of the calf. Antibodies may also be excreted locally, if the mammary gland is infected only. It is also not clear if only parts of the mammary gland or the whole udder can be infected, because of the different anatomic structure in different animal species. Clear is, that *Cb* was detected in experimentally infected pregnant goats in the udder and mammary lymph node as well as it can be isolated from dairy cattle udders [8, 145]. It has been shown that *Cb* shedding in milk may be intermittent and may last for a longer period of time affecting antibody synthesis. Muskens *et al.* showed that out of 341 BTM samples that correspond to the number of tested dairy cattle herds in the Netherlands, most samples were ELISA positive but had negative (n = 81, 24%) or positive (n = 76, 22%) PCR result. Whereas the number of PCR positive animals rose in the second sampling round [272].

During this survey, milk samples from selected blood donors (n = 188) were collected and investigated with the IDEXX ELISA, additionally (**Tab. 2**).

Table 2. Correlation of the milk and serum ELISA results (paired samples) of selected animals (n = 188).

Animal Species	**ELISA results - serum/milk** *n*				**Total milk positive**	**Total serum positive**	**Total antibody positive**	**Kappa value**
	pos/pos	**pos/neg**	**neg/pos**	**neg/neg**	*n* [%]	*n* [%]	*n* [%]	
Cattle	7	7	17	37	24 [12.8]	14 [7.5]	31 [16.5]	0.15
Buffaloes	1	0	4	6	5 [2.7]	1 [0.5]	5 [2.7]	0.21
Sheep	1	3	9	18	10 [5.3]	4 [2.1]	13 [6.9]	-0.05
Goats	3	0	5	18	8 [4.3]	5 [2.7]	8 [4.3]	0.45
Camels	18	4	27	3	45 [23.9]	22 [11.7]	49 [26.1]	-0.07
Total *n* [%]	30 [16.0]	14 [7.4]	62 [33.0]	82 [43.6]	92 [48.9]	44 [23.4]	106 [56.4]	0.18

n = number of animals tested, pos = positive, neg = negative

No data are available about detection of *Cb*-specific antibodies in milk samples of animals from Egypt and only few studies dealt with detection of DNA in dairy cattle milk samples. Surprisingly, the findings (**Tab. 2**) show that one third of the animals had blood serum negative and milk positive ELISA results, especially cattle and camels. Hence, it is not possible to decide if all these animals had a previous exposure to *Cb* or if they had an actual Q fever infection nor if these antibodies were synthesized locally only. In a recent survey about Q fever in dairy cattle most sample pairs were positive (n = 249) or negative (n = 176) whereas only few had differing ELISA results [320]. Those findings were explained by assumed local antibody production in milk positive and serum negative sample animals (n = 8) with low titers, as well as by possible disturbance of the antibody transport from blood to milk or low antibody levels in pairs with serum positive and milk negative results (n = 15) [320]. In this study, kappa value calculations showed only little agreement in paired samples from Egyptian livestock except for goats (**Tab. 2**). In contrast, Guatteo *et al.* reported high agreement (Kappa = 0.89) between milk and blood ELISA of dairy cattle samples [320]. Differences might be explained by the fact, that their samples were investigated with an ELISA (LSI) based on ovine derived antigen. Further investigations e.g. PCR analysis and evaluations of the obtained OD values will be needed to assess possible correlations regarding *Cb* shedding in milk and antibody response.

1.3 Conclusion

In summary, the *Cb*-specific antibody seroprevalence results of each domain indicate that Q fever is endemic throughout Egypt, especially in cattle, buffaloes and camels. The only risk factors identified correlating to seropositivity are type of animal species and geographical location. Besides cattle, especially camels come to the fore as possible reservoir for *Cb* due to a high seroprevalence. It has to be determined, which effect the age and thus maybe the immune status of an animal has on seroprevalence in each animal species. Specific conditions such as animal population, climate and physical surface characteristics should be evaluated for each domain (geographical location) to identify possible risk factors facilitating *Cb* spreading. Whereas animal husbandry systems could be excluded as a risk factor in Egypt.

This study showed that many farm animals had previous contact to *Cb* or actually suffered from Q fever when sampled due to a positive antibody result. Future research, however, is needed to determine exact cutoff values especially for buffaloes and camels for the ELISA test used to avoid false results. Further, phase specific tests may be helpful to distinguish current and previous infections. Nevertheless, to identify Q fever shedders serology is not useful and combined investigation techniques are needed [158, 163, 321]. Additionally, other test materials such as milk or birth products should be considered besides blood samples. The evaluation of the presence of *Cb* in the field may finally help to give a new insight into the epidemiology of Q fever in Egypt and to assess infection risk and public health impact.

2 Socioeconomic Burden

“Socioeconomic burden” is defined as impact of a disease on social and economic factors and the combined influence of both. Little is known about the socioeconomic burden of Q fever in African countries and only one study in Egypt tried to analyze the impact on public health but failed [146]. It has been shown that Q fever infections in animals, particularly in livestock, have severe impacts on a country’s economy [322-324]. Livestock productivity is affected due to abortion epidemics in small ruminants leading to animal losses and thus affecting milk, meat and wool production. Further, an impact on the public sector is indispensable. Therefore, this study was conducted to give an initial assessment with the obtained seroprevalences of *Cb*-specific antibodies in Egyptian livestock concerning the present of *Cb* and thus the infection risk for humans and animals. In addition, animal owners were asked about their consumption of raw milk and their general knowledge on Q fever. The latter included transmission, clinical signs in animals and application of countermeasures to reduce risk of infection. The obtained data may provide a baseline for further research into the public health impact of Q fever and implementation of public health intervention.

The high seroprevalences in this study indicate that Q fever is endemic throughout Egypt. It is very likely that *Cb* is present in many regions. Especially in the *Nile Valley and Delta Area* and *Eastern Desert Area* it should be reckoned with a high infection risk for humans and animals due to a high animal population and the expanding economy. Further, tourism in these areas is a flourishing industry. In general, true impact on individual income is uncertain, but may be disastrous especially for farmers in resource-limited areas in African countries [322, 323]. Thirty thousand euros (0.04% of costs incurred in livestock sector) for all farms were calculated for production losses on infected farm animals during the big Dutch Q fever outbreak only [324]. Most costs (85 Million Euro/outbreak) were caused by the intervention program e.g. organization (58%), culling of infected animals (22%), breeding prohibition (14%) and vaccination (6%) [324]. It was hypothesized, however, that native Egyptian breeds might show infection tolerance to a certain extent as it was assumed that subclinical infections occur in Egyptian dairy animals mainly and abortions play a less prominent role. This hypothesis was justified by a single observation only: DNA of *Cb* was detected in the placental cotyledons and vaginal discharge of one goat out of 108 animals (26 cattle, 26 buffaloes, 27 sheep, 29 goats) after abortion [146]. The high seroprevalence numbers in

this survey contradict this assumption: *Cb*-specific antibodies were detected in 40.7% of camels, 19.3% of cattle, 11.2% of buffaloes, 8.9% of sheep and 6.8% of goats. These findings indicate that Egyptian livestock is susceptible to *Cb*. Only the low OD values in buffaloes might support the suggestion that these animals are not as susceptible to *Cb* infection or they simply do not show an immune response as strong as other animal species (**Fig. A3**). Further research should deal with comparing the sensitivity of ELISAs using an ovine and bovine strain for buffalo samples to answer this question. A further argument against the hypothesis of infection tolerance is that e.g. high-yield dairy cattle have been imported from Europe, especially Germany, and replace local breeds partially to increase milk production.

Import of animals with unknown health status has come to the fore in this study. Biosafety in Egypt is lacking. Thus, a (maybe illegal) high import rate with no control may have an impact on transmission of *Cb*. Interestingly, the only animal species found to be imported to Egypt were camels with origin in Sudan. *Cb*-specific antibodies were found in 42.1% of imported camels. Whether positive tested camels had contact with *Cb* prior or after import could not be shown. This study, however, supported the assumption that high seroprevalences in areas bordering Sudan are correlated to the import of infected camels (**Fig. A6**) [314].

Import or uncontrolled animal movement involves the risk of disease spread. Thus, infection risk is increased for animals as well as for humans. Although pets, equines or pigeons were not sampled in this survey, most Egyptian farmers keep those susceptible animal species on their farm additionally (data not shown). Their role in spreading of *Cb* is still not clear but infected animals may be a risk and thus may increase the risk of infection of people as well. A study in Egypt showed that the seroprevalence in farmers (30.8% [8/26]) was higher than that in veterinarians and their assistances (9.4% [3/32]). The difference was explained by the rural residence of the farmers in an area with high sheep and goat populations [146]. Interestingly, Abdel-Moein *et al.* assumed that only goats suffer from abortions, although prevalence is low [146]. Retrospective analyses of the Dutch Q fever outbreak showed a correlation between decrease in human Q fever cases and abortion storms in small ruminants. They showed that the number of human cases may be limited in time and the influence of contamination of environment may not induce an increase of the number of infections for a longer period of time [103]. Nevertheless, during the Q fever outbreak in the Netherlands between 2007-2011, the

total loss of the public sector was calculated to be 222 million Euro [324]. The number of chronic Q fever patients was high. Surprisingly, treatment costs were calculated to be low (<2% of the public health costs). The highest financial impact of public health costs was the loss in income due to chronic Q fever infection (34% of the public health costs). During long periods of sick leave, the losses accumulate over time especially in cases of chronic fatigue syndrome (63% of the public health costs) [324]. In African countries manpower is substantial especially for the agricultural sector. Unawareness of physicians, misdiagnoses and missing diagnostics in African countries influence the impact of Q fever on public health [134]. In a survey in Egypt (2011), Q fever was often not considered as differential diagnostic in hospitalized FUO cases although infections (42% [39/93]) were the most common cause headed by brucellosis (8/39) and infective endocarditis (6/39) [325]. Endocarditis is one of the most important sequelae of Q fever. These findings might influence public health as well as the agricultural sector enormously.

Socioeconomic assessment of individual Q fever cases and outbreaks is not easy even in developed countries. Obvious is that financial resources in third world countries are not available to establish sound diagnostic laboratories or to conduct solid research. Additionally, physicians, veterinarians and farmers are often unaware of the clinical manifestations of Q fever, spreading ways and risk factors facilitating dissemination of *Cb*. This study showed that none of the animal owners interviewed had any knowledge on Q fever or about any application of countermeasures like removing birth products. Further, 8.7% of animal owners reported to consume raw camel milk regularly. These findings, the lack in biosafety measures and the high seroprevalence found in the Egyptian livestock point out that Q fever most likely is a problem in Egypt. The problem and the far-reaching consequences, however, seem to be still unknown so far. The impact of this disease might be eminent for the economy, humans and animal populations. Therefore, further studies combining direct and indirect diagnostic techniques are needed to assess the presence of *Cb* and its true impact on Egyptian livestock. Changes in and development of animal husbandry and rising populations, solid public knowledge, efficient diagnostics and ongoing research are needed to implement intervention programs to contain Q fever spreading and disease burden. Therefore, first world countries have an important responsibility in contributing to set up laboratories, to provide awareness-rising measures and to develop cost-efficient

Q fever diagnostics. The conducted survey is also part of a German Partnership Program for Excellence in Biological and Health Security funded by the German Foreign Office, which intends to set up laboratories and awareness-rising measures e.g. training programs in Egypt to raise biosafety.

V SUMMARY

Coxiella burnetii (*Cb*) is an obligate intracellular bacterium and the causative agent of Q fever in humans and animals. The pathogen is classified as a category B biological agent and has a high socioeconomic burden as seen during the last big Q fever outbreak in the Netherlands. Little is known about the socioeconomic burden in Egypt and other African countries in general. Only limited data on the epidemiology of Q fever in Egyptian livestock are available and are restricted to few districts although Egypt has risk factors favoring spreading, persistence and transmission of *Cb*. Therefore, the objectives of this survey were to estimate the seroprevelance of *Cb* in cattle, buffaloes, sheep, goats and camels. Additionally, possible risk factors were identified by association with seropositivity in Egypt. The obtained data should build a solid basis for further interventions involving improvement of public health countermeasures and animal welfare in Egypt to contain risk of infection. Additionally, this survey may provide information for further African countries and future studies in risk factors.

Basis of this study was a solid epidemiological calculation of the size of test specimen using the two stage sampling method to evaluate the seroprevalence in the three domains of Egypt (*Western Desert Area*, *Nile Valley and Delta Area* and *Eastern Desert Area*). This method was used, because no actual data on animal populations nor animal data bases were available. Additionally, unequal distribution of animal populations was suggested due to the different agro-ecological zones (gegraphical characteristics, climate and water availability). Blood samples of the most important livestock animal species in Egypt were collected: cattle, buffaloes, sheep, goats and camels. A questionnaire was used to assess the origin of animals, animal keeping system and animal age. Additionally, personal details about knowledge on Q fever of the owner and consumption of raw milk were asked. Serum samples were investigated with a commercially available ELISA (IDEXX CHEKIT Q fever Antibody ELISA Test Kit, IDEXX Laboratories, Switzerland). The overall seroprevalence was 18.4% (496/2,699). *Cb*-specific antibodies were detected in 40.7% of camels (215/528), 19.3% of cattle (162/840), 11.2% of buffaloes (34/304), 8.9% of sheep (64/716) and 6.8% of goats (21/311) indicating that Q fever is endemic in Egypt. Animal species was identified as risk factor for seropositivity. Especially camels come to the fore as possible reservoir for *Cb*. The seroprevalence results for cattle, sheep and camels were highest in the *Eastern Desert Area*, those of goats and buffaloes in the *Nile Valley and Delta Area.* The geographical location was identified as second risk factor (*Eastern*

Desert Area, aOR: 2.16; 95% CI: 1.62-2.88). Future studies, however, should focus on cause for and specify the risk factor “geographical location”. The origin of and the age of an animal and animal keeping system were not correlated to seropositivity. The results for animal age and animal keeping systems were statistically significant. Stationary animal husbandry is common in Egypt showing changes in and development of animal husbandry systems. Nomadic animal keeping, however, is still the leading husbandry system for this agricultural country. Both systems lead to the suggestion that spreading of *Cb* is high, although excretion of the organism was not investigated. To assess excretion, spreading and impact on animal health further surveys have to be conducted with combined diagnostic techniques.

It has been shown that comparison with other serological surveys was difficult due to missing sampling schemes, differences in serological diagnostic methods (and cutoffs), low numbers of test specimen and partly regional sampling. Drawbacks such as lack in PhII reactivity (detection of early stage of infection) of the used ELISA are discussed, although this assay has a high sensitivity and specificity. The use of the IDEXX ELISA for samples of buffaloes and especially for camels, however, has to be further evaluated to avoid false results as the distribution of the OD values are different to those of the other animal species analyzed. Final validation is lacking. Thus, development strategies for new diagnostic techniques are indispensable.

At present, eradication of Q fever seems impossible. Therefore, elucidation and performances of training courses may help to raise awareness about the epidemiology of Q fever and correlated risk factors. This survey showed that no animal keeper interviewed has any knowledge on Q fever at all. Few animal keepers consumed raw camel milk showing their unawareness also. Nevertheless, further investigations would be needed to assess impact on the economy and public health as well as to show prevalence of Q fever as cause of FUO in humans in Egypt.

In summary, this nationwide epidemiological study in ruminants and camels highlights that the prevalence of *Coxiella burnetii*-specific antibodies is high in Egyptian livestock but the disease is neglected, although risk for transmission and human infection is high. Further studies are needed to provide meaningful data on the epidemiology of Q fever in Egypt and other African countries. Help and support from abroad is needed to contain Q fever dissemination with intervention programs and to raise awareness to improve public health countermeasures and animal welfare.

VI ZUSAMMENFASSUNG

Coxiella burnetii (*Cb*) ist ein obligat intrazelluläres Bakterium und Erreger des Q-Fiebers bei Menschen und Tieren. Das Pathogen ist in der Kategorie B der biologischen Wirkstoffe eingegliedert und birgt, wie man es während des letzten großen Q-Fieber Ausbruchs in den Niederlanden gesehen hat, eine hohe soziökonomische Belastung. Über die soziökonomische Belastung in Ägypten, wie auch generell in afrikanischen Ländern, ist wenig bekannt. Nur wenige epidemiologische Daten, zudem noch begrenzt auf wenige Distrikte, sind für Q-Fieber im ägyptischen Nutztierbestand vorhanden, obwohl in Ägypten einige Risikofaktoren erfüllt sind, die die Verbreitung, Persistenz und Übertragung von *Cb* begünstigen. Deshalb waren die Ziele dieser Studie, die Seroprävalenz von *Cb* spezifischen Antikörpern bei Rindern, Büffeln, Schafen, Ziegen und Kamelen einzuschätzen. Zusätzlich wurden Risikofaktoren in Ägypten identifiziert, die mit einem seropositiven Ergebnis assoziiert wurden. Die erhaltenen Daten sollten weiterhin eine solide Basis für weitere Interventionen bilden, die Gegenmaßnahmen für die öffentliche Gesundheit und Tiergesundheit involvieren, um das Infektionsrisiko einzudämmen. Zusätzlich stellt diese Untersuchung Informationen für weitere afrikanische Länder und zukünftige Studien über Risikofaktoren bereit.

Eine solide epidemiologische Berechnung der Probengröße mittels der Two-Stage-Methode war Basis dieser Studie, um die Seroprävalenz in den drei Gebieten Ägyptens (*Western Desert Area*, *Nile Valley and Delta Area* und *Eastern Desert Area*) zu evaluieren. Die genutzte Methode musste angewendet werden, da keine aktuellen Daten über die Tierpopulation und Tierdatenbanken zur Verfügung standen. Zudem wurde eine ungleiche Verteilung der Tierpopulation aufgrund der verschiedenen agroökologischen Zonen (Oberflächenbeschaffenheit, Klima und Wasserverfügbarkeit) angenommen. Blutproben wurden von den wichtigsten Nutztierarten in Ägypten gesammelt: Rinder, Büffel, Schafe, Ziegen und Kamele. Mittels eines Fragebogens wurden Daten zur Herkunft des Tieres, dem Tierhaltungssystem und dem Tieralter gesammelt. Zusätzlich wurden persönliche Daten über das Wissen zu Q-Fieber und den Konsum von Rohmilch abgefragt. Die Serumproben wurden mit einem kommerziell erwerblichen ELISA (IDEXX CHEKIT Q-Fieber Antikörper ELISA Test Kit, IDEXX Laboratories, Schweiz) untersucht. Die Seroprävalenzergebnisse beliefen sich auf 18,4% (496/2.699). *Cb* spezifische Antikörper wurden in 40,7% der Kamele (215/528), 19,3% der Rinder (162/840), 11,2% der Büffel (34/304), 8,9% der Schafe (64/716) und 6,8% der Ziegen (21/311) detektiert, und lassen die Annahme zu, dass Q-Fieber in

Ägypten endemisch ist. Die Tierart wurde als Risikofaktor für Seropositivität identifiziert. Vor allem Kamele rücken in den Fokus als mögliche Reservoire für *Cb*. Die Seroprävalenzergebnisse von Rindern, Schafen und Kamelen waren am höchsten in der *Eastern Desert Area*, die von Ziegen und Büffeln dagegen in der *Nile Valley and Delta Area.* Als zweiter Risikofaktor wurde die geografische Lage identifiziert (*Eastern Desert Area*, aOR: 2.16; 95% CI: 1.62-2.88). Zukünftige Studien sollten jedoch den Fokus auf die Ursache für die geografische Lage legen und diese spezifizieren. Die Herkunft und das Alter eines Tieres als auch das Haltungssystem wurden nicht mit der Seropositivität korreliert. Die Ergebnisse für das Alter und das Haltungssystem waren jedoch statistisch signifikant. Das stationäre Haltungssystem in Ägypten ist sehr gängig und zeigt die Veränderung und Weiterentwicklung in den Tierhaltungssystemen. Nichtsdestotrotz ist die nomadische Haltung führend für dieses agrikulturelle Land. Beide Haltungssysteme führen zu der Annahme, dass die Verbreitung von *Cb* sehr hoch ist, obwohl die Exkretion des Erregers nicht untersucht wurde. Weitere Studien, ausgeführt mit kombinierten Untersuchungsmethoden, wären nötig, um die Ausscheidung, die Verteilung und den Einfluss auf die Tiergesundheit zu beurteilen.

Der Vergleich mit anderen Seroprävalenzstudien gestaltete sich als schwierig, da dort Stichprobenpläne fehlten, andere serologische Diagnostikmethoden (und Cutoffs) angewandt wurden, die Probengröße zu klein war und teilweise örtlich begrenzt beprobt wurde. Die Nachteile des benutzten ELISAs, z. B. die fehlende Reaktion mit PhII Antigen (Erkennung des frühen Infektionsstadiums) werden in dieser Arbeit diskutiert, obwohl er eine hohe Sensitivität und Spezifität hat. Die Anwendung des IDEXX ELISAs bei Büffel- und vor allem Kamelproben sollte jedoch weiter evaluiert werden, um falsche Ergebnisse zu vermeiden, da die Verteilung der Werte der optischen Dichte nicht die gleiche ist wie bei den anderen untersuchten Tierspezies. Im Allgemeinen weist die Validierung Mängel auf. Deshalb sind Entwicklungsstrategien für neue Diagnosetechniken unabdingbar.

Zurzeit erscheint die Ausmerzung von Q-Fieber unmöglich, weshalb Aufklärung und Durchführungen von Schulungen helfen können, das Bewusstsein über die Epidemiologie von Q-Fieber und dessen Risikofaktoren zu erhöhen. Diese Studie zeigte, dass alle befragten Tierbesitzer kein Wissen über Q-Fieber besaßen. Die Unwissenheit zeigte sich ebenso dadurch, dass Tierbesitzer Rohmilch von Kamelen verzehren. Dennoch sind weitere Untersuchungen nötig, um den Einfluss auf die

Wirtschaft und die öffentliche Gesundheit, als auch die Prävalenz von Q-Fieber als Ursache für Fälle mit Fieber ungeklärten Ursprungs in Ägypten zu beurteilen.

Zusammenfassend hebt diese landesweite epidemiologische Studie in Wiederkäuern und Kamelen hervor, dass die Prävalenz von *Coxiella burnetii* spezifischen Antikörpern in ägyptischen Nutztieren hoch ist, die Krankheit jedoch vernachlässigt wird, obwohl das Übertragungs- und Infektionsrisiko für Menschen ebenfalls hoch ist. Weitere Studien werden benötigt, um aussagekräftige Daten über die Epidemiologie von Q-Fieber in Ägypten und anderen afrikanischen Ländern zur Verfügung zu stellen. Hilfe und Unterstützung von außen sind nötig, um die Verbreitung von Q-Fieber mit Interventionsprogrammen einzudämmen, das Bewusstsein zu erhöhen und somit Gegenmaßnahmen im Sinne der Gesundheit für Mensch und Tier zu verbessern.

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VIII APPENDIX

Table A1. Seroprevalences of *Coxiella burnetii*-specific antibodies in serum samples from humans and animals in Egypt and bordering countries.

Animal Species	Count.	Area	n	n Pos [%]	Diagnostic Method	Study Design	Ref.	Sample collection (year)
Buffaloes	Egypt	Giza, Cairo, Fayoum	45	0 [0]	IDEXX ELISA, Switzerland	n. a.	[133]	2012*
	Egypt	Muneeb abattoir, central Egypt	153	6 [4]	IDEXX ELISA, Westbrook, Maine	n. a., domestic and imported livestock for slaughter	[305]	2009
	Egypt	Sharkia, Cairo	40	3 [8]	CFT	n. a.	[326]	1975-1976
Camels	Egypt	Muneeb abattoir, central Egypt	10	7 [70]	IDEXX ELISA, Westbrook, Maine	n. a., domestic and imported livestock for slaughter	[305]	2009
	Egypt	different districts North Sinai	30	4 [13]	indirect IFA	n. a., animals from the patients' vicinity	[142]	2006
	Chad	Chari-Baguirmi, Kanem	142	114 [80]	IDEXX ELISA, Switzerland	livestock of the nomadic pastoralists	[139]	1999-2000
	Egypt	Sharkia, Cairo	54	3 [6]	CFT	n. a.	[326]	1975-1976
	Sudan	Central Sudan	110	16 [15]	capillary agglutination test	n. a., slaughter animals in Tamboul and camels in Butana plains to the northeast of Khartoum	[327]	1987*
Cattle	Egypt	Dakahlia, Damietta, Port Said	1,194	158 [13]	IDEXX ELISA, Switzerland	n. a., 9 farms, 4 farms with ≥500 and 5 farms with ≤200 Holstein Friesian dairy cows	[304]	2014*
	Egypt	Giza, Cairo, Fayoum	54	7 [13]	IDEXX ELISA, Switzerland	n. a.	[133]	2012*

	Egypt	Muneeb abattoir, central Egypt	161	0	[0]	IDEXX ELISA, Westbrook, Maine	n. a., domestic and imported livestock for slaughter	[305]	2009
	Egypt	Sharkia, Cairo	352	11	[3]	CFT	n. a.	[326]	1975-1976
	Chad	Southeastern shore of Lake Chad	561	44	[8]	ID Screen multi-species ELISA	n. a., 4 islands	[328]	2014*
	Chad	Chari-Baguirmi, Kanem	195	8	[4]	IDEXX ELISA, Switzerland	livestock of the nomadic pastoralists	[139]	1999-2000
	Sudan	South Sudan	52	21	[40]	MAT	n. a., 9 villages, region of Melut, Upper Nile province	[329]	1983
Goats	Egypt	Giza, Cairo, Fayoum	30	7	[23]	IDEXX ELISA, Switzerland	n. a.	[133]	2012*
	Egypt	different districts North Sinai	71	12	[17]	indirect IFA	n. a., animals from the patients' vicinity	[142]	2006
	Chad	Chari-Baguirmi, Kanem	134	18	[13]	IDEXX ELISA, Switzerland	livestock of the nomadic pastoralists	[139]	1999-2000
	Sudan	8 states	460	109	[24]	Lisvet ELISA, Nouzilly, France	selection of locations being main potential areas for livestock rearing, sampling of at least 4 groups	[144]	2011
	Sudan	South Sudan	42	22	[53]	MAT	n. a., 9 villages, region of Melut, Upper Nile province	[329]	1983
Sheep	Egypt	Giza, Cairo, Fayoum	55	18	[33]	IDEXX ELISA, Switzerland	n. a.	[133]	2012*
	Egypt	Muneeb abattoir, central Egypt	174	14	[8]	IDEXX ELISA, Westbrook, Maine	n. a., domestic and imported livestock for slaughter	[305]	2009
	Egypt	different districts North Sinai	89	20	[23]	indirect IFA	n. a., animals from the patients' vicinity	[142]	2006

	Egypt	Sharkia, Cairo	49	1 [2]	CFT	n. a.	[326]	1975-1976
	Chad	Chari-Baguirmi, Kanem	142	16 [11]	IDEXX ELISA, Switzerland	livestock of the nomadic pastoralists	[139]	1999-2000
	Sudan	South Sudan	32	20 [63]	MAT	n. a., 9 villages, region of Melut, Upper Nile province	[329]	1983
Humans	Egypt	Giza, Cairo, Fayoum	92	15 [16]	Vircell ELISA, Granada, Spain	n. a., veterinarians, veterinary workers and farmers from agricultural and urban districts	[133]	2012*
	Egypt	different districts North Sinai	180	9 [5]	indirect IFA	n. a., patients with FUO and healthy humans	[142]	2006
	Egypt	Kafr Ayoub, Sharkia	883	285 [32]	EIA	Proportional clustering strategy to select sample households randomly	[330]	1991
	Egypt	Nile river delta	418	93 [22]	EIA	n. a., schoolchildren (8-14 years) from 4 schools in 3 villages	[141]	1989
	Egypt	Nile Delta, Nile Valley, Suez Canal	1286	192 [15]	EIA/IFA	n. a., serum from past projects in northeast Africa	[138]**	1983-1989
	Egypt	Cairo	100	8 [8]	MAT, CFT	n. a., serum pairs (maternal and cord serum), most patients of the University Cairo Hospital were from rural, low income areas next to Cairo	[331]	1966
	Egypt	Western Desert	572	45 [8]	CFT	n. a., Arab Bedouin tribes, cooperation varied between the areas sampled	[332]	1962
	Egypt	18 locations	1.271	232 [18]	CFT	Spot sampling of various communities	[333]**	1959*
	Chad	Chari-Baguirmi, Kanem	368	4 [1]	IDEXX ELISA, Switzerland	3 nomadic communities: Fulani cattle breeders, Arab camel and cattle breeders	[139]	1999-2000

	Sudan	North Sudan	371	199 [54]	EIA/IFA	n. a., 2 locations (Merowie, Karima), samples from past projects in northeast Africa	[138]**	1983-1989
	Sudan	1 village	73	19 [26]	CFT	One Nilotic tribe (the Shilluk) in the village Akwajo-Pakwanythtor	[334]	1961-1962
	Sudan	11 locations in the South	401	28 [7]	CFT	Spot sampling of various communities	[333]**	1959*

Count. = Country, n = number of individuals tested, n. a. = not available, Pos = positive, Ref. = reference;
CFT = complement fixation test, EIA = enzyme immunoassay, ELISA = enzyme linked immunosorbent assay, IFA = immunofluorescence assay, MAT = microagglutination test;

*year published, no data available on period of sample collection

**Not all results listed.

No data are available for Libya.

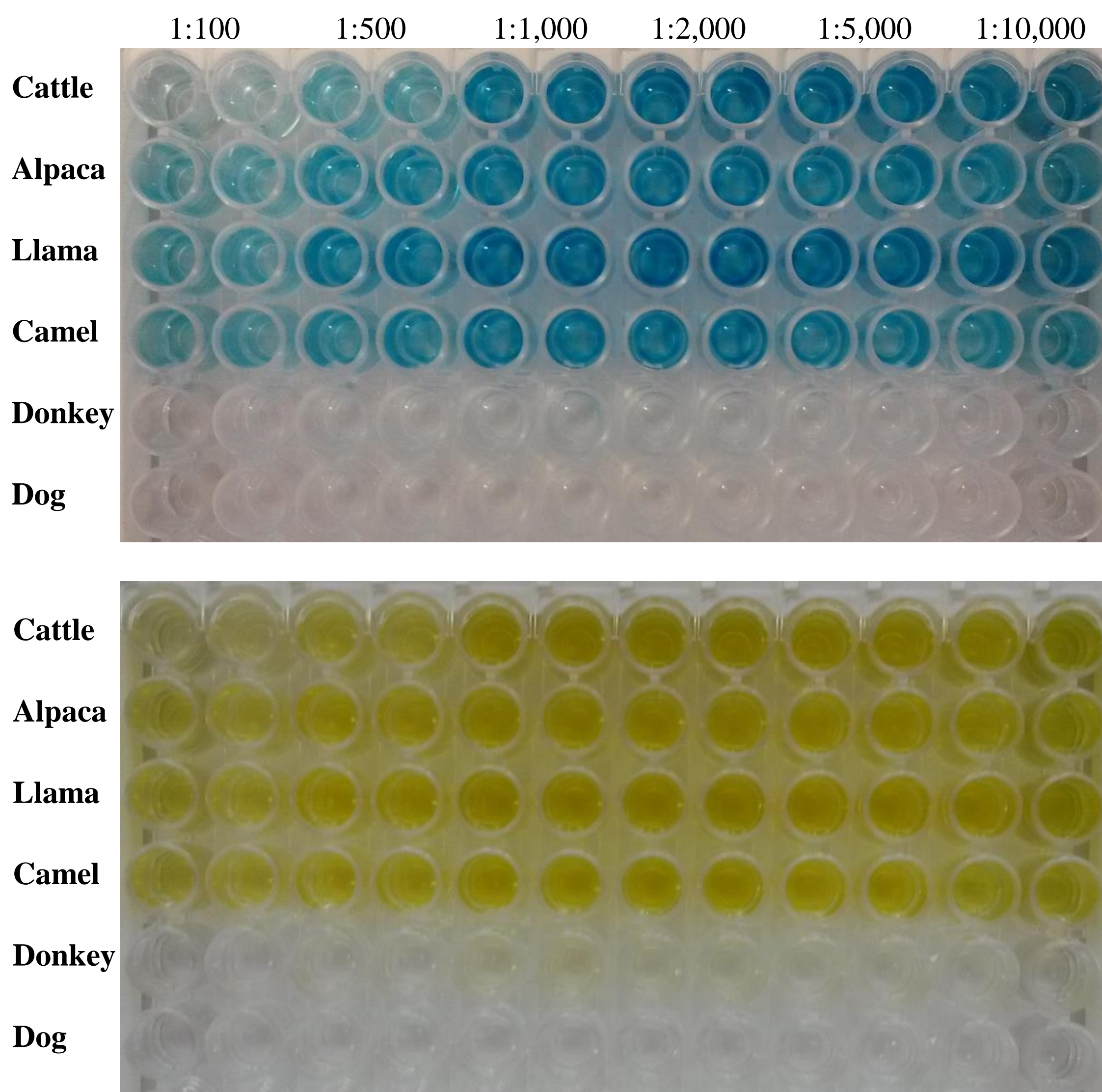

Figure A1. Verification of the efficiency of the IDEXX conjugated anti-ruminant antibody to detect antibodies in serum samples of camelidae.

An empty 96-well plate was coated with coating-buffer in-house to detect animal species specific antibodies. Further steps were performed as described in the manufacturer`s instruction (IDEXX). Serum samples of a seropositive cow and different camelidae and control groups (donkey, dog) were used. Various dilution ratios were performed to find the best ratio for camel serum samples (1:1,000). Pictures were taken briefly before the reaction was stopped (blue) and after stopping (yellow).

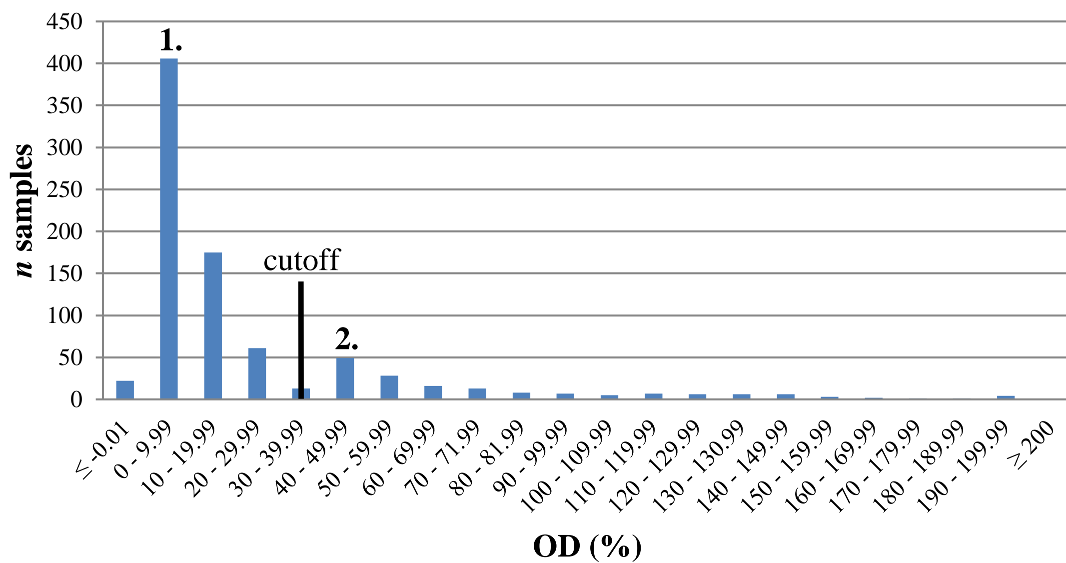

Figure A2. Distribution of the OD values obtained from cattle.

The figures A2-A6 show the distribution of the OD values obtained from the ELISAs of each animal species to assess cutoff values. The OD results include two peaks: 1. before the negative and 2. after the positive cutoff. Given cutoff values for the ELISA used (IDEXX, Switzerland) determine <30% to be negative and ≥40% to be positive.
Figure A2 shows a diagram of the obtained ELISA results of cattle.
n = number, OD = optical density

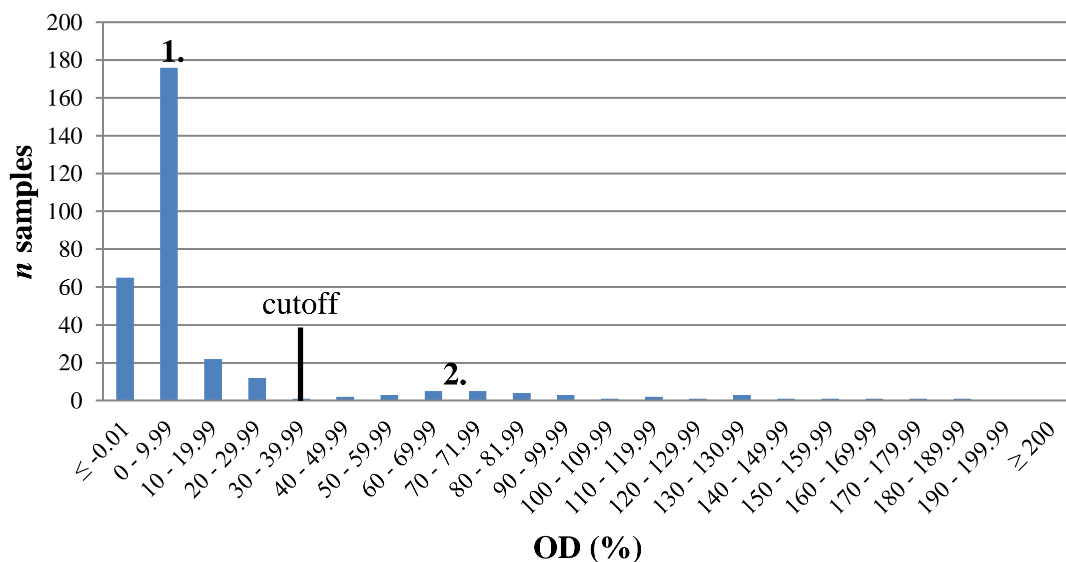

Figure A3. Distribution of the OD values obtained from buffaloes.

n = number, OD = optical density

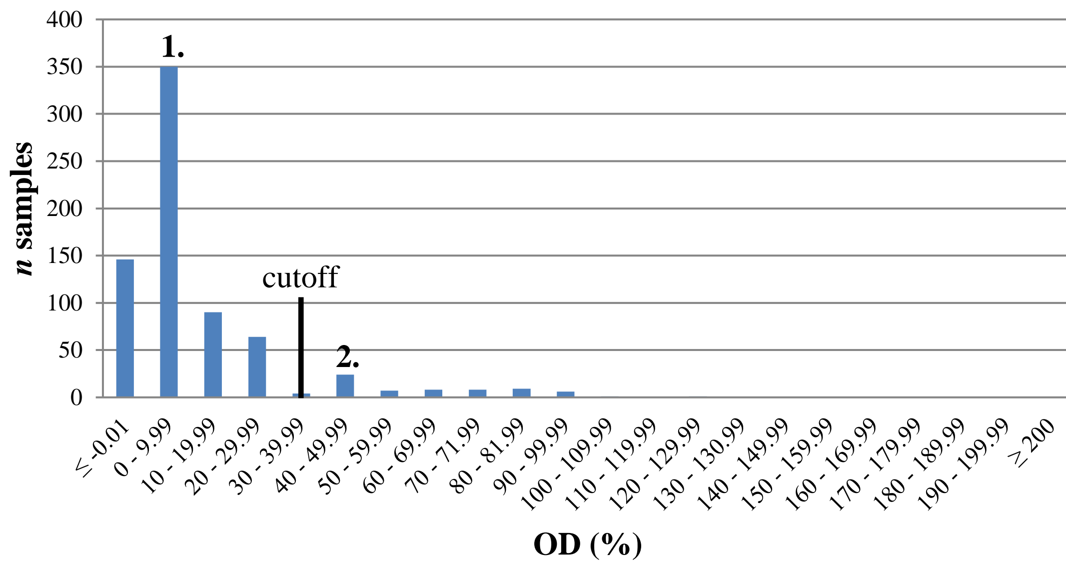

Figure A4. Distribution of the OD values obtained from sheep.

n = number, OD = optical density

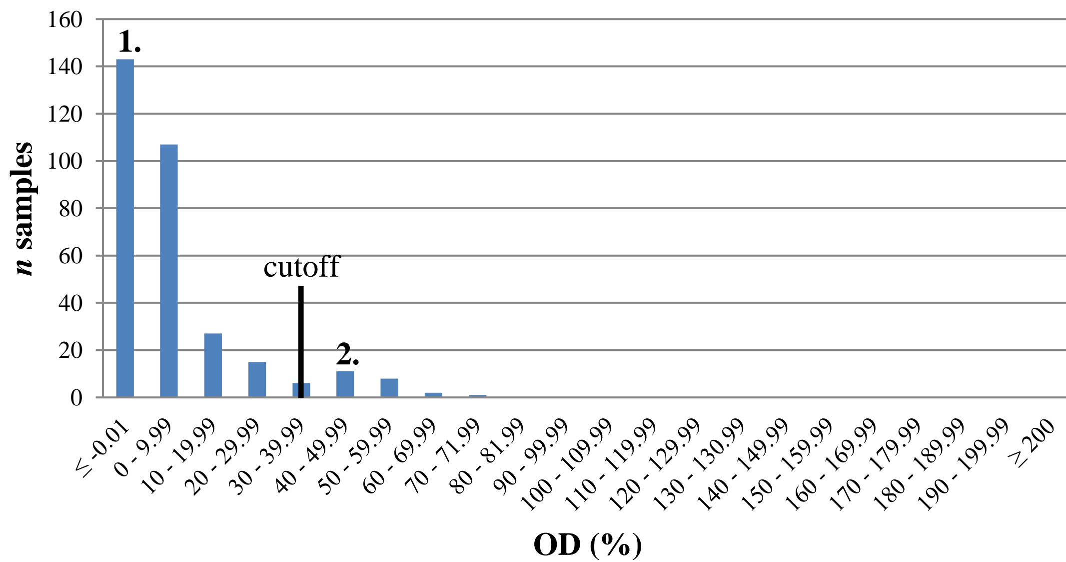

Figure A5. Distribution of the OD values obtained from goats.

n = number, OD = optical density

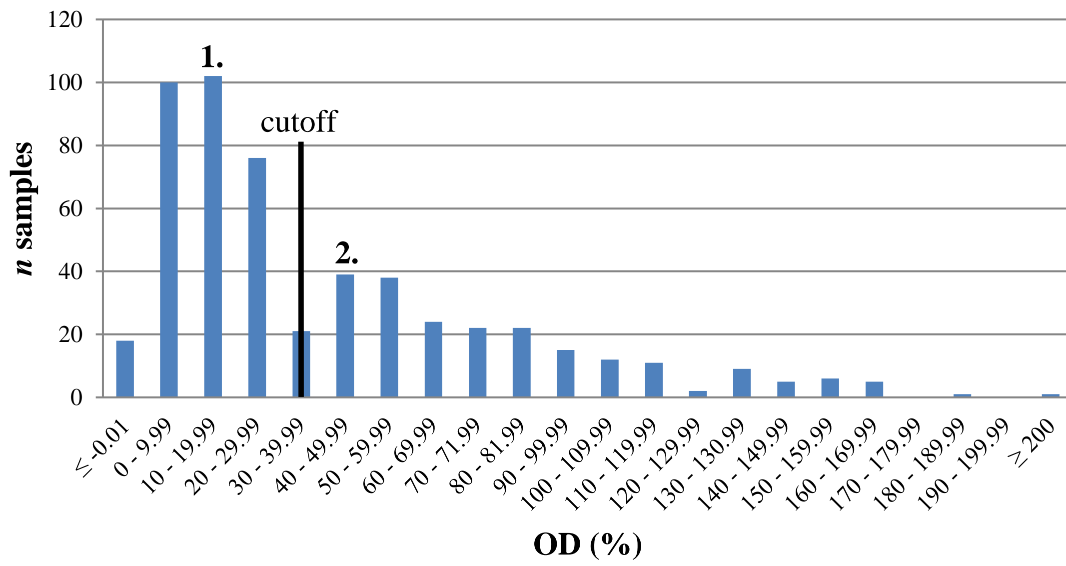

Figure A6. Distribution of the OD values obtained from camels.

n = number, OD = optical density

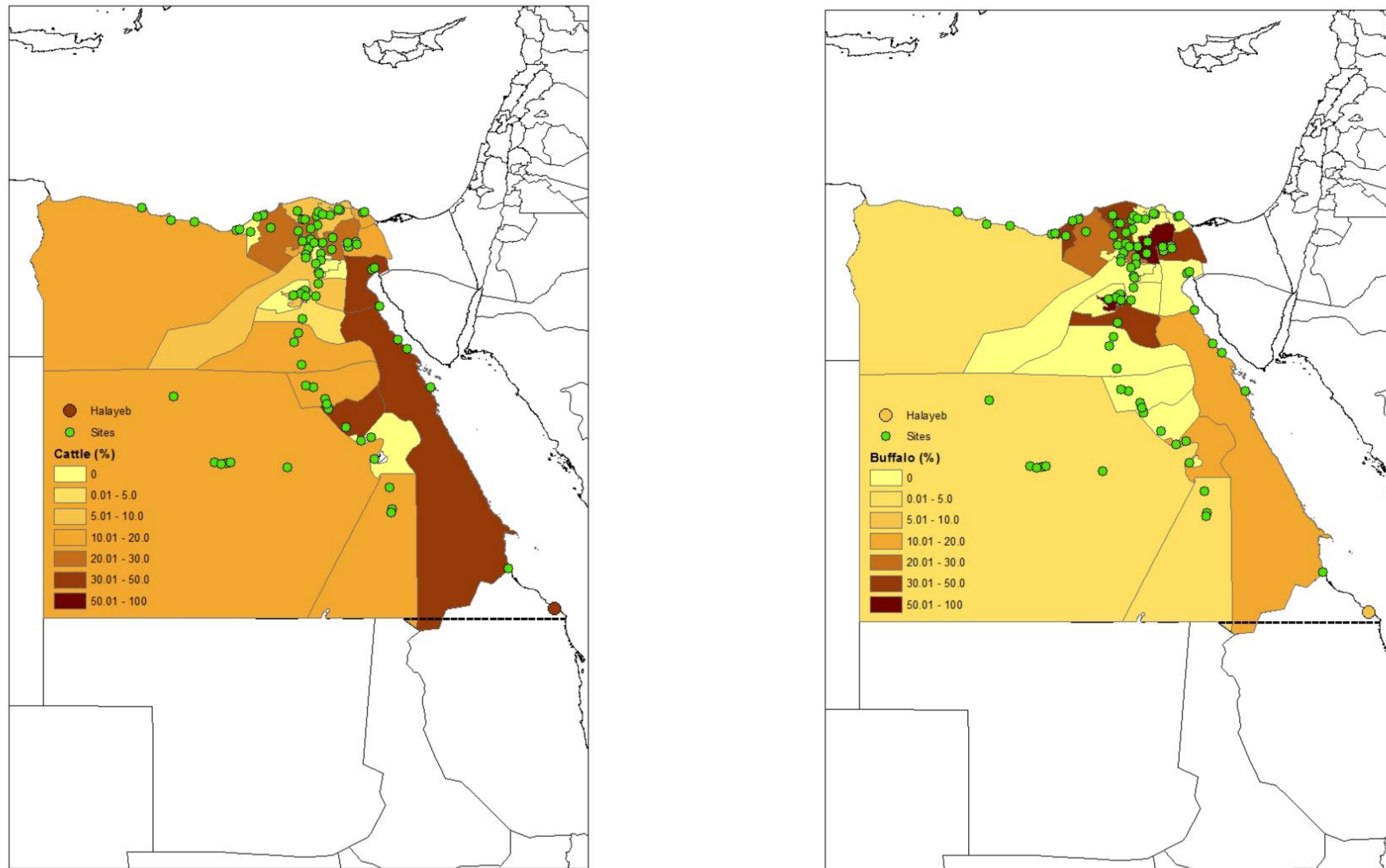

Figure A7. Seroprevalence in Egypt for cattle and buffaloes.

The maps of Egypt show the seroprevalence of *Coxiella burnetii*-specific antibodies for each animal species and in total. The green dots show the position of each randomly selected sampling site in each governorate. The sampling site 'Halayeb', highlighted by a brown dot, is located in the territory disputed between Egypt and Sudan.

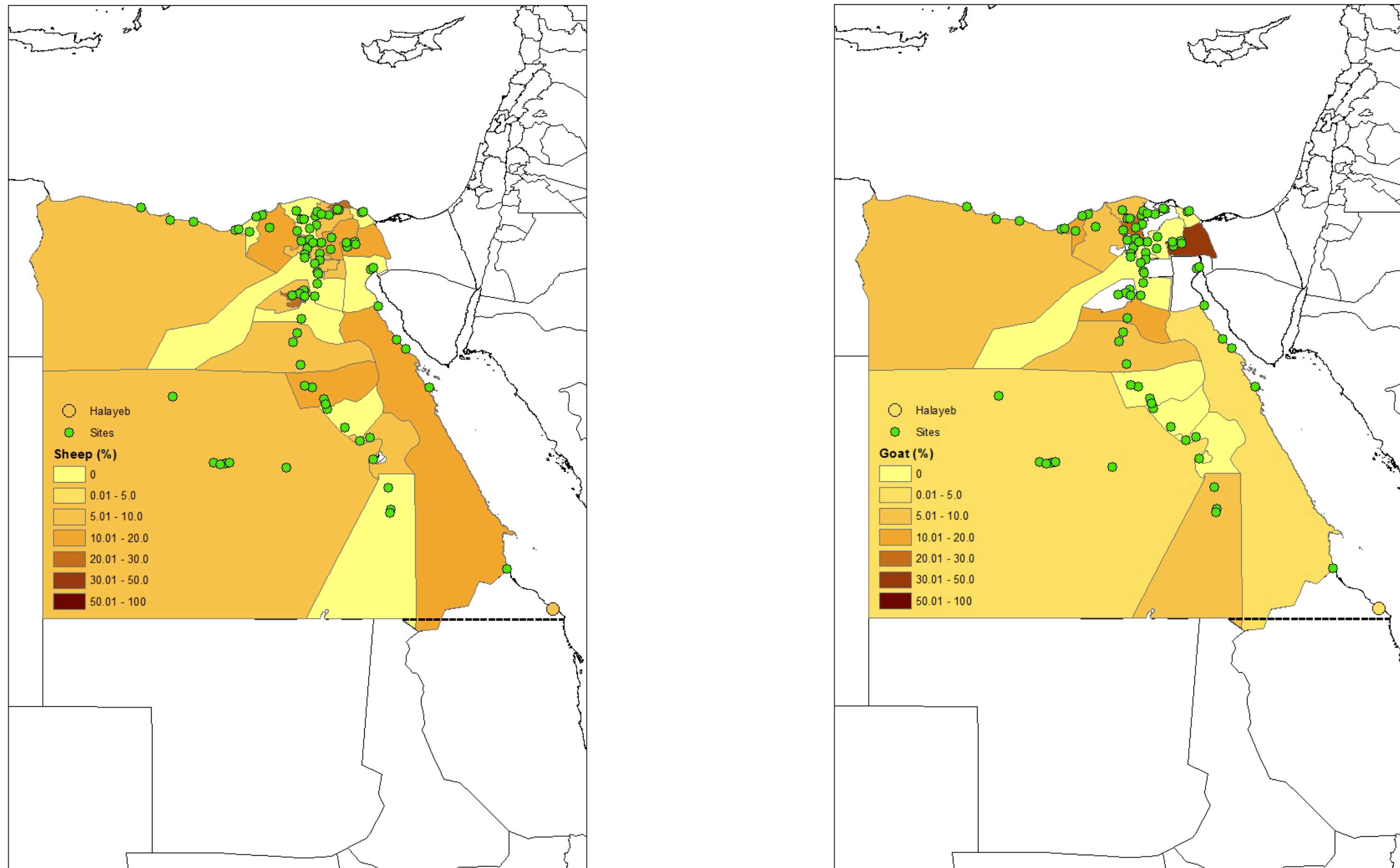

Figure A8. Seroprevalence in Egypt for sheep and goats.

The green dots show the position of each randomly selected sampling site in each governorate. The sampling site 'Halayeb', highlighted by a brown dot, is located in the territory disputed between Egypt and Sudan.

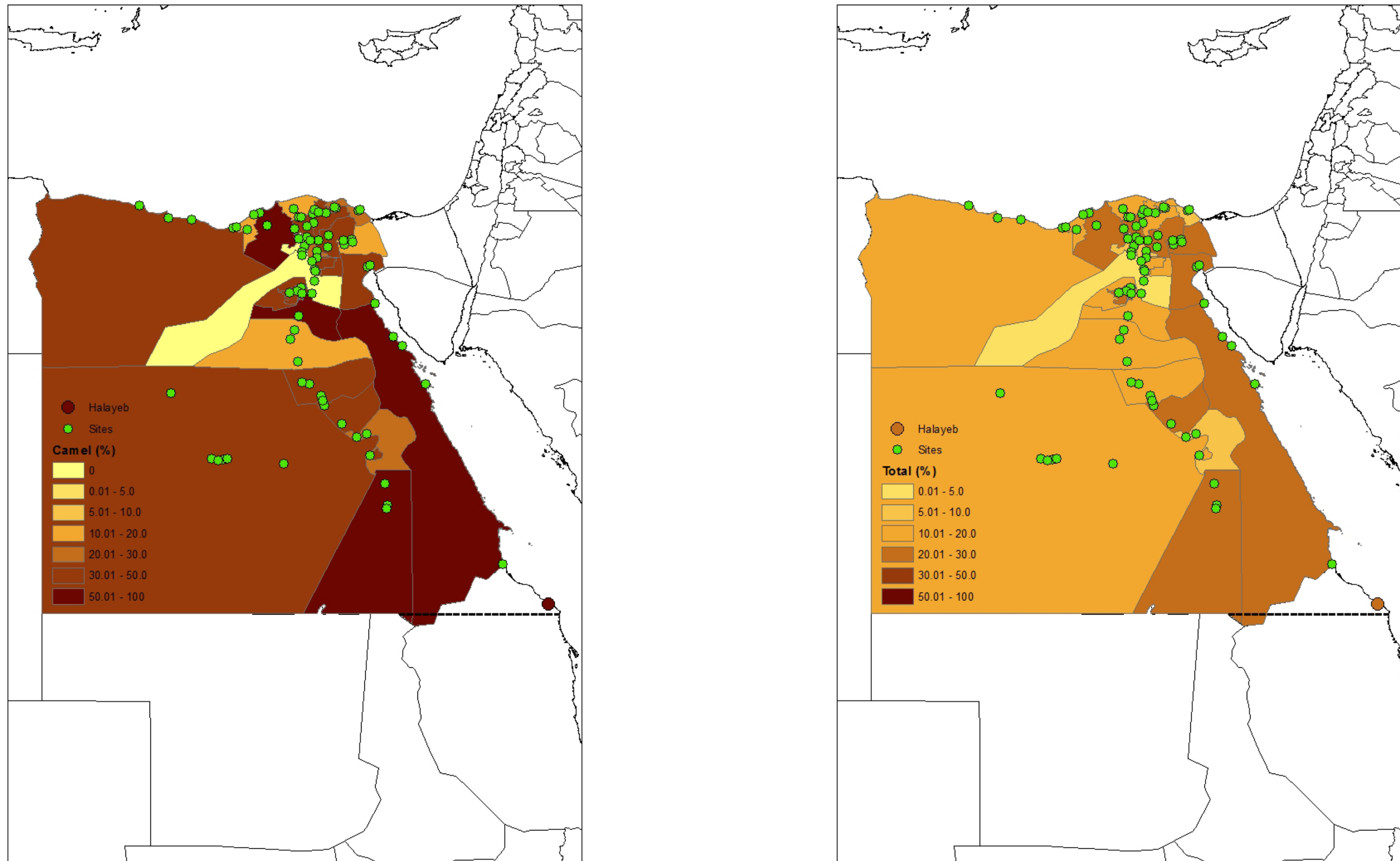

Figure A9. Seroprevalence in Egypt for camels and in total.

The green dots show the position of each randomly selected sampling site in each governorate. The sampling site 'Halayeb', highlighted by a brown dot, is located in the territory disputed between Egypt and Sudan.

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