University of Veterinary Medicine Hannover

Prevalence and risk factors of *Coxiella burnetii* in German sheep flocks and evaluation of a novel approach to detect an infection via preputial swabs at herd-level

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Glenz A, Bauer BU, Prüfer TL, Schoneberg C, Campe A, Runge M, Ganter M
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Bioland Praktikertage für Landwirte: Haltung kleiner Wiederkäuer im ökologischen Landbau: Die Gesundheit im Fokus, Medebach-Oberschledorn, Germany
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Wolf A, Bauer BU, Prüfer TL, Schoneberg C, Campe A, Runge M, Ganter M
Forschungsverbund Q-GAPS:
Risikofaktoren für eine Q-Fieber Infektion in Herden kleiner Wiederkäuer
Q-GAPS Verbundtreffen, Leipzig, Germany
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Bauer BU, Wolf A, Prüfer TL, Schoneberg C, Campe A, Runge M, Ganter M
Q-Fieber bei kleinen Wiederkäuern
VPH (Veterinary Public Health) Seminar: Gemeinsam sind wir stärker! Q-Fieber-Prävention und Bekämpfung einer Zoonose als gemeinsame Aufgabe von Human- und Veterinärmedizin, Hannover, Germany
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List of abbreviations

ap	apparent prevalence
BAV	Bavaria
BMBF	Bundesministerium für Bildung und Forschung
BMEL	Bundesministerium für Ernährung und Landwirtschaft
BTM	bulk tank milk
BW	Baden-Wuerttemberg
C. burnetii	*Coxiella burnetii*
CFT	complement fixation test
CI	confidence interval
Destatis	Internetseite des Statistischen Bundesamtes
DVG	Deutsche Veterinärmedizinische Gesellschaft e.V.
e.g.	exempli gratia
ECSRHM	European College of Small Ruminant Health Management
EFSA	European Food Safety Authority
ELISA	enzyme-linked immunosorbent assay
et al.	et alii
IBEI	Institut für Biometrie, Epidemiologie und Informationsverarbeitung
IFA	immunofluorescence assay
IfSG	Infektionsschutzgesetz
LAVES	Niedersächsische Landesamt für Verbraucherschutz und Lebensmittelsicherheit
LS	Lower Saxony
MOSS	monitoring und surveillance system
NDS	Niedersachsen
NRW	North Rhine-Westphalia/Nordrhein-Westfalen
OR	Odds Ratio
PCR	polymerase chain reaction
Q fever	Queensland/Query fever
Q-GAPS	Q fever GermAn Interdisciplinary Program for reSearch
qPCR	quantitative polymerase chain reaction
RKI	Robert Koch-Institut
SH	Schleswig-Holstein
spp.	subspecies
TKrMeldepflV	Verordnung über meldepflichtige Tierkrankheiten
VPH	Veterinary Public Health
vs.	versus
WHO	World Health Organization

1. Introduction

Q fever is a widespread zoonosis caused by the obligate intracellular living bacterium *Coxiella (C.) burnetii* (Eldin *et al.* 2017; Arricau-Bouvery and Rodolakis 2005). In Germany, the pathogen was first identified in 1947 during an outbreak in humans in Baden-Wuerttemberg (Heni and Germer 1948; Hellenbrand *et al.* 2001). Most human infections occur in southern Germany, but cases have also been reported in other parts of Germany including occasional larger outbreaks (Hellenbrand *et al.* 2001; Bauer *et al.* 2020; Runge *et al.* 2012; Porten *et al.* 2006; Gilsdorf *et al.* 2008). A human infection usually occurs through the inhalation of contaminated dust and aerosols (Angelakis and Raoult 2010; Arricau-Bouvery and Rodolakis 2005; Todkill *et al.* 2018; Eldin *et al.* 2017) and proceeds in 60% without clinical symptoms, but it can also lead to acute Q fever with self-limited febrile illness, atypical pneumonia or hepatitis. It may become chronic, usually manifesting as endocarditis (Maurin and Raoult 1999). In Germany, human infections are often linked to infected sheep (Hellenbrand *et al.* 2001; Bauer *et al.* 2020; Runge *et al.* 2012). Infected mammals and especially ruminants excrete large amounts of the pathogen mainly with vaginal excretions and birth products, such as the placenta and foetal membranes, during abortions or normal parturition, but also via milk, faeces and urine (Maurin and Raoult 1999; Arricau-Bouvery and Rodolakis 2005; Berri *et al.* 2001; Angelakis and Raoult 2010). The route and duration of shedding varies among ruminant species (Arricau-Bouvery and Rodolakis 2005; Bauer *et al.* 2020). In comparison to cattle and goats, sheep shed the pathogen via faeces and milk over a short period of eight days, while *C. burnetii* is detected in vaginal secretion up to 71 days (Berri *et al.* 2001; Arricau-Bouvery and Rodolakis 2005). Goats shed the pathogen for a period of 20 days in faeces and 52 days in milk, while it is detected in vaginal secretion for a short period of 14 days (Arricau-Bouvery and Rodolakis 2005). Data about the entry and transmission of *C. burnetii* in ruminant flocks are scarce. Ticks are known as a reservoir for *C. burnetii* and play an important role in transmission among wild animals (Maurin and Raoult 1999). Although they are suspected to be a vector in sheep in Germany and are considered to be involved in the infection cycle of *C. burnetii* (Sting *et al.* 2004; Liebisch 1977), the pathogen was rarely detected in ticks from endemic areas (Hildebrandt *et al.* 2011; Sting *et al.* 2004; Pluta *et al.* 2010). Data

about airborne transmission are scarce. Nevertheless, the pathogen could be isolated in the air of a sheep farm during shearing (Schulz *et al.* 2005) and spreading of *C. burnetii* between ruminant herds by wind and/or the introduction of infected animals into the flock are suspected (Nusinovici *et al.* 2015). Furthermore, data on the transmission between different ruminant species are limited. However, the same *C. burnetii* genotypes (C1, C7) were identified in different ruminant species kept on the same farm (Bauer *et al.* 2019; Bauer *et al.* 2020). In addition, wild animals are known as a reservoir for *C. burnetii* (Madariaga 2005; Eldin *et al.* 2017) and constitute a source of infection for livestock (González-Barrio and Ruiz-Fons 2019). Transmission by sexual intercourse could not be detected in small ruminants, despite the pathogen was detected in the semen of breeding sires (Ruiz-Fons *et al.* 2014).

C. burnetii infections are usually asymptomatic in non-pregnant ruminants (Roest *et al.* 2013; Eldin *et al.* 2017). However, clinical signs which might occur in infected sheep have been described with the main manifestation being reproductive disorders in the late pregnancy like abortions, premature delivery, stillbirths, weak offspring and placentitis (Agerholm 2013; Palmer *et al.* 1983; Zeman *et al.* 1989; Eibach *et al.* 2012; van den Brom *et al.* 2015). The proportion of abortions within the flock can vary considerably (Arricau-Bouvery and Rodolakis 2005; Agerholm 2013). Nevertheless, higher abortion levels were described particularly in infected goats (Álvarez-Alonso *et al.* 2018; Palmer *et al.* 1983). It should be noted that the bacterium can occur in combination with other infectious agents causing abortion such as *Chlamydia* spp. or *Toxoplasma* spp., which might lead to misinterpretation or failure to diagnose the cause of abortion (Runge *et al.* 2012; Hazlett *et al.* 2013; Agerholm 2013).

The Polymerase Chain Reaction (PCR), staining of prepared sample material according to Stamp, Gimenez or Machiavello and the cell culture are applied for direct detection of the pathogen (Arricau-Bouvery and Rodolakis 2005; Sidi-Boumedine *et al.* 2010). The use of the PCR is increasing due to its high sensitivity and the examination of different sample matrices within a short time (Sidi-Boumedine *et al.* 2010; Arricau-Bouvery and Rodolakis 2005; EFSA 2010). Suitable sample matrices sent in for direct detection are vaginal swabs and specimen of aborted material (placenta, dead foetus) due to the high bacterial burden, but also milk and faeces (Berri *et al.* 2001; Sidi-

Boumedine *et al.* 2010; Berri *et al.* 2000; EFSA 2010). In comparison to direct detection, serological tests are usually carried out for epidemiological studies and are suitable for screening herds (Arricau-Bouvery and Rodolakis 2005). Serological examinations can be conducted with serum and milk (Sidi-Boumedine *et al.* 2010) by the complement fixation test (CFT), the immunofluorescence assay (IFA) or the enzyme-linked immunosorbent assay (ELISA) (Arricau-Bouvery and Rodolakis 2005; Sidi-Boumedine *et al.* 2010). IFA and ELISA are more sensitive than the CFT (Sidi-Boumedine *et al.* 2010; EFSA 2010). However, the IFA is not authorised (Bauer *et al.* 2020) and rarely used for diagnosis in veterinary medicine (Arricau-Bouvery and Rodolakis 2005; Sidi-Boumedine *et al.* 2010). In comparison to the IFA, the ELISA is suitable for investigations of large numbers of animals/flocks due to its simple applicability (Sidi-Boumedine *et al.* 2010; Arricau-Bouvery and Rodolakis 2005). The phase-specific ELISA is a suitable test for the differentiation of phase-specific antibodies and could therefore allow the determination of infection dynamics within the flock and the stage of infection (Muleme *et al.* 2017; Bauer *et al.* 2020), but is not authorised for veterinary laboratories (Bauer *et al.* 2020). In general, flocks were investigated after the appearance of clinical symptoms (e.g. abortions), while preventive examinations and official monitoring and control programmes are rarely performed (Sidi-Boumedine *et al.* 2010). As an infection in animals is often subclinical or with unspecific clinical symptoms, the diagnosis depends on the detection of the pathogen and/or pathogen-specific antibodies (Lang 1988). Therefore, the occurrence of Q fever may be less frequently reported (Ohlson *et al.* 2014). Furthermore, the detection of antibodies and shedding the pathogen is not necessarily correlated (Berri *et al.* 2001; Joulié *et al.* 2017; de Cremoux *et al.* 2012). These circumstances may lead to an unrecognised contamination of the environment. The pathogen is able to stay for a long time in the environment and transmission to humans most commonly occurs through the inhalation of aerosolised bacteria (Eldin *et al.* 2017; Todkill *et al.* 2018). The use of PCR associated with ELISA is described as suitable strategy for the detection of *C. burnetii* infections (Arricau-Bouvery and Rodolakis 2005; Sidi-Boumedine *et al.* 2010). However, this approach is very time-consuming and expensive at herd-level. Sampling of bulk tank milk (BTM) and testing with PCR on the

other hand is less expensive and is suggested to be a proper test matrix for monitoring Q fever in dairy goat farms (van den Brom *et al.* 2012). The disadvantage of this approach is that the detection of the pathogen is only possible after its excretion and thus the contamination of the environment and the spread of the pathogen has already occurred and an active surveillance is not available especially for non-dairy sheep and goat flocks (Bauer *et al.* 2020). A novel approach, evaluated in this study, is the examination of preputial swabs of the breeding sires during or after the mating season by qPCR. The pathogen could be transmitted via the contact of the vaginal and preputial mucosa during mating or from semen containing *C. burnetii*. Other bacteria such as *Brucella* spp. or *Listeria* spp. have already been detected in samples of the preputial mucosa (Keplan *et al.* 2009; Xavier *et al.* 2010). The examination of the preputial swab could enable an early detection of the pathogen at herd-level with a small number of samples before the main shedding at lambing occurs and thereby allows the implementation of preventive measures that protect humans and animals alike.

C. burnetii has a widespread distribution and in many countries numerous studies were conducted to determine the occurrence of the pathogen in domestic ruminants (Guatteo *et al.* 2011; EFSA 2010). Overall, prevalence varies between countries (Guatteo *et al.* 2011). In southern and central Europe, Q fever is endemic (Kampen *et al.* 2012). Moreover, it is considered to be endemic in humans in several Mediterranean countries (Villari S. *et al.* 2018) and sheep are described to pose a risk for human infection in these regions (Ruiz-Fons *et al.* 2010). In northern Europe there are few investigations on the distribution of *C. burnetii* in comparison to southern Europe, but an increase in the occurrence of the infection was observed (Kampen *et al.* 2012). In addition, some studies identified risk factors for an infection at herd or animal-level such as flock size (Lambton *et al.* 2016; Rizzo *et al.* 2016; Anastácio *et al.* 2013; Villari S. *et al.* 2018; Barlozzari *et al.* 2020), goat density in proximity to the farm (Lambton *et al.* 2016; Schimmer *et al.* 2014) and the animals`age (Rizzo *et al.* 2016; Anastácio *et al.* 2013; Ruiz-Fons *et al.* 2010; García-Pérez *et al.* 2009). Especially farm-specific factors appear to facilitate infection at the animal-level and the spread within the flock (Schimmer *et al.* 2014). Furthermore, the management system is suggested to

influence the degree of exposure to infection and thus the local productive characteristics will have a major effect on *C. burnetii* prevalence in livestock from different geographical areas (Rizzo *et al.* 2016).

In Germany, Q fever is a notifiable disease in humans (German Protection Against Infection Act, Infektionsschutzgesetz IfSG) (IfSG 2020) and animals (Regulation on notifiable animal diseases, Verordnung über meldepflichtige Tierkrankheiten TKrMeldepflV) (TKrMeldepflV 2020) alike. Despite human infections and small scale epidemics in several regions of Germany (Hellenbrand *et al.* 2001; Bauer *et al.* 2020), data on the prevalence, epidemiology and prevention of *C. burnetii* in small ruminants, especially in the northern federal states are rare (Bauer *et al.* 2020; Runge *et al.* 2012). There is no standardised concept for the detection of *C. burnetii* infections in small ruminants and a nationwide active monitoring and surveillance programme (MOSS) should be established (Bauer *et al.* 2020; Runge *et al.* 2012; Winter *et al.* 2021).

In general, the primary aim of the preventive measures is to minimise the risk of human exposure to animal and environmental contamination (Arricau-Bouvery and Rodolakis 2005). However, despite the presence of proposals on how to prevent or proceed Q fever in small ruminant flocks in Germany (BMEL 2014; Sting *et al.* 2017; Bauer *et al.* 2020; Q-GAPS 2020; RKI 2018), the management is regulated in each federal state at district level individually whereby a consistent approach cannot be achieved.

Overall, there remain many open questions about epidemiology and prevention of *C. burnetii* infection in humans and small ruminants (Bauer *et al.* 2020). Within the framework of the zoonosis network Q-GAPS (Q fever GermAn Interdisciplinary Program for reSearch), studies are being conducted to gain new insights into *C. burnetii* in the sense of One Health (Bauer *et al.* 2020).

The results from all subprojects of the Q-GAPS network will be summarised on a Q fever information platform (www.q-gaps.de) and in a Q fever guideline. Both are intended to support Public Health Service employees with the detection, monitoring and control of *C. burnetii* infections (Bauer *et al.* 2020). The Q fever guideline will be developed on the basis and as an extension to the guideline of Q fever in small ruminants in Baden-Wuerttemberg (Sting *et al.* 2017). In addition, a Q fever risk barometer will be designed to identify sheep and goat flocks that could pose an

increased risk of pathogen transmission and thus contribute to an optimised infection surveillance.

The Clinic for Swine, Small Ruminants, Forensic Medicine and Ambulatory Service, University of Veterinary Medicine Hannover Foundation, will conduct several subprojects to investigate *C. burnetii* in small ruminants. The results will be used to develop new diagnostic, therapeutic and preventive concepts for sheep and goats.

The aim of the present study was to determine herd and within-herd prevalence and risk factors for a *C. burnetii* infection across five federal states with a representative number of sheep: Schleswig-Holstein (SH), Lower Saxony (LS), North Rhine-Westphalia (NRW), Bavaria (BAV) and Baden-Wuerttemberg (BW) (Destatis 2016). In addition, an approach to detect an infection at herd-level using preputial swabs was evaluated.

2. Publications

2.1 Prevalence study

Prevalence of *Coxiella burnetii* in German sheep flocks and evaluation of a novel approach to detect an infection via preputial swabs at herd-level

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Abstract

A prevalence study was conducted on German sheep flocks including goats if they cohabitated with sheep. In addition, a novel approach was applied to identify an infection at the herd-level before lambing season with preputial swabs, suspecting venereal transmission and ensuing colonisation of preputial mucosa with *Coxiella (C.) burnetii*. Blood samples and genital swabs were collected from breeding males and females after the mating season and were analysed by enzyme-linked immunosorbent assay (ELISA) and quantitative polymerase chain reaction (qPCR) respectively. In total, 3367 animals were sampled across 71 flocks. The true herd-level prevalence adjusted for misclassification probabilities of the applied diagnostic tests using the Rogan-Gladen estimator for the prevalence estimate and a formula by Lang and Reiczigel (2014) for the confidence limits, ranged between 31.3% and 33% (95% confidence interval [95% CI] 17.3–45.5) detected by the ELISA and/or qPCR. Overall 26–36.6% (95% CI 13–56.8) were detected by ELISA, 13.9% (95% CI 4.5–23.2) by

the qPCR and 7.9–11.2% (95% CI 0.08–22.3) by both tests simultaneously. The range of results is due to data obtained from literature with different specifications for test quality for ELISA. Among eight farms with females shedding *C. burnetii*, three farms (37.5%) could also be identified by preputial swabs from breeding sires. This indicates less reliability of preputial swabs if used as a single diagnostic tool to detect *C. burnetii* infection at the herd-level.

Prevalence of *Coxiella burnetii* in German sheep flocks and evaluation of a novel approach to detect an infection via preputial swabs at herd-level ERRATUM

A. Wolf[1], T.L. Prüfer[2], C. Schoneberg[3], A. Campe[3], M. Runge[2], M. Ganter[1] and B.U. Bauer[1] (2020)

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During the proofing stage for the above article, Figures 2 and 3 were inadvertently switched.

2.2 Risk factor analysis

Risk factors for an infection with *Coxiella burnetii* in German sheep flocks

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Abstract

In Germany, sheep are the main source of human Q fever epidemics, but data on *Coxiella burnetii* (*C. burnetii*) infections and related risk factors in the German sheep population remain scarce. In this cross-sectional study, a standardised interview was conducted across 71 exclusively sheep as well as mixed (sheep and goat) farms to identify animal and herd level risk factors associated with the detection of *C. burnetii* antibodies or pathogen-specific gene fragments via univariable and multivariable logistic regression analysis. Serum samples and genital swabs from adult males and females of 3367 small ruminants from 71 farms were collected and analysed using ELISA and qPCR, respectively. On animal level, univariable analysis identified young animals (<2 years of age; odds ratio (OR) 0.33; 95% confidence interval (CI) 0.13–0.83) to reduce the risk for seropositivity significantly (p <0.05). The final multivariable logistic models identified lambing all year-round (OR 3.46/3.65; 95% CI 0.80–15.06/0.41–32.06) and purchases of sheep and goats (OR 13.61/22.99; 95% CI 2.86–64.64/2.21–239.42) as risk factors on herd level for *C. burnetii* infection detected via ELISA and qPCR, respectively.

3. General discussion

In Germany, sheep play an important role with regards to human infections and small-scale epidemics with *C. burnetii* (Bauer *et al.* 2020; Hellenbrand *et al.* 2001; Runge *et al.* 2012). However, data on the occurrence of *C. burnetii* in the German sheep population is scarce and an appropriate sampling strategy for active surveillance is necessary (Bauer *et al.* 2020).

The first part of this study (manuscript I) estimated the prevalence of *C. burnetii* in flocks of small ruminants across five federal states of Germany with a representative number of sheep and flocks (Destatis 2016). In addition, the number and proportion of infected flocks were determined in each federal state and among sheep and mixed sheep and goat flocks. Moreover, a novel approach to detect an infection with *C. burnetii* at herd-level with preputial swabs of breeding sires during or after mating was evaluated.

In the second part of this study (manuscript II), data obtained from a standardised questionnaire were analysed to identify risk factors at animal and herd-level for an infection with *C. burnetii* detected by ELISA and qPCR, respectively.

Domestic ruminants constitute the most common reservoir for *C. burnetii* and are usually the source of human infections (Eldin *et al.* 2017). The largest human Q fever outbreak with over 4,000 notified human infections between 2007-2010 (Schneeberger *et al.* 2014) was traced back to abortions in dairy goat flocks in the Netherlands (Roest *et al.* 2011; Steenbergen *et al.* 2007; Vellema and van den Brom 2013). Therefore, the EFSA Panel on Animal Health and Welfare has recommended further research on *C. burnetii* infections in domestic ruminant population, especially in small ruminants (EFSA 2010). Prevalence studies were conducted in several countries (Guatteo *et al.* 2011). These studies determined the seroprevalence at animal, herd and within-herd level in different species (cattle/sheep/goats), while direct detection of the pathogen was less frequent (Guatteo *et al.* 2011).

However, differences in the study design and applied diagnostic methods must be taken into account and make a direct comparison difficult (Guatteo *et al.* 2011; Schimmer *et al.* 2014; Bauer *et al.* 2020). In addition, timing and purpose should be considered. For example, some studies were not carried out originally to determine

prevalence, but rather to verify whether the pathogen is present in the region or to evaluate the impact of *C. burnetii* in the context of clinical symptoms (Guatteo *et al.* 2011), so that there is a preselection of the samples. Comparability is further hampered by the use of different detection systems and their test quality and whether the results are presented as apparent prevalence or corrected for misclassification probabilities (true prevalence) (Guatteo *et al.* 2011).

Nevertheless, comparing data of European countries is useful to classify the results of this study, considering that several studies describe the prevalence for a particular region and the results are not transferable to the whole population of a country as in this study.

This study determined an overall apparent prevalence (ap) of 17.1%/43.3% in sheep/mixed flocks (sheep and goat) at herd-level using ELISA, which is in the middle range compared to the data from other European countries. Higher for sheep flocks, but similar values for mixed flocks were obtained in a study in Central Portugal (estimated apparent prevalence: 37.5/38.5%) (Anastácio *et al.* 2013). Higher results were also recorded in sheep/mixed flocks in Northwest Italy (raw prevalence: 40.5%/56.4%) (Rizzo *et al.* 2016) and in sheep flocks in Sardinia (38%) (Masala *et al.* 2004). In Mediterranean countries, higher herd prevalences (67.6-87.2%) were observed compared to our results (Villari S. *et al.* 2018; Barlozzari *et al.* 2020; Ruiz-Fons *et al.* 2010; García-Pérez *et al.* 2009). Lower herd-level prevalences have been reported particularly in northern European countries/regions. For instance in Norway, no antibodies were detected in samples from the examined farms (Kampen *et al.* 2012) and only a seroprevalence of 0.4% (apparent prevalence) at herd-level was determined in Sweden (Ohlson *et al.* 2014). Moreover, low herd-level prevalences of 9.7% (crude prevalence) (Lambton *et al.* 2016) and 8.4% (Ryan *et al.* 2011) were identified in Great Britain and Ireland, respectively.

Neighbouring countries to Germany revealed a wide range of prevalences. A study conducted in Switzerland estimated a lower herd-level seroprevalence of 5% compared to Germany among samples from 100 sheep farms (Magouras *et al.* 2017). In the Netherlands, the herd-level seroprevalence was 14.5% in sheep flocks in 2008 (van den Brom *et al.* 2013). Later, Schimmer et al. (Schimmer *et al.* 2014) presented

a herd-level seroprevalence of 30.5% in non-dairy sheep flocks and 78.6% in dairy sheep flocks in a cross-sectional study in 2009-2011. A higher herd-level seroprevalence compared to our results were revealed in French sheep flocks with an average prevalence of 55.7% (Gache *et al.* 2017).

In Germany, prevalence studies in sheep were most commonly conducted in a few federal states or on district level by serology (Bauer *et al.* 2020). An apparent seroprevalence of 9.5% from 95 examined sheep flocks was reported in 2004 in Lower Saxony (Runge *et al.* 2012). Despite insufficient power to interpret findings on a federal state level, the present study revealed a similar result (9.1%) and might indicate that the rate of *C. burnetii* infection in Lower Saxony was constant over the past years. In North Rhine-Westphalia, the numbers of positive farms are low, but in 2003 one of the largest human Q fever outbreaks occurred in the district of Soest located in North Rhine-Westphalia with around 299 reported human cases (Porten *et al.* 2006). Evidently, even in areas with low *C. burnetii* infection levels, there is still a risk for large-scale human epidemics under special circumstances. Schleswig-Holstein has a high density of sheep population (Bauer *et al.* 2020), especially on the West coast, but the number of affected flocks is low in the present study. Overall, in this federal state, there is a low level for the detection of a *C. burnetii* infection in human and veterinary medicine alike (Bauer *et al.* 2020). In southern Germany, 8.7% infected sheep were identified by the examination of serum samples in the four districts of BW collected in 2001 (Sting *et al.* 2004). Data on the herd-level are unavailable. In Bavaria, prevalences in sheep were identified using phase-specific ELISA during an outbreak in 2008 (phase I 9.8%, phase II 17.4%) and two follow up investigations in 2009 (phase I 0.5%, phase II 0.8%) and 2010 (phase I 0.6%, phase II 2%), respectively (Böttcher *et al.* 2011).

In comparison to serological findings, studies estimating prevalence by PCR are rare and usually based on the examination of vaginal swabs or bulk tank milk in dairy farms. Among 39 sheep farms in Thuringia, Germany, an apparent herd-level prevalence of 5% by the examination of vaginal, rectal and foetal swabs and afterbirths with PCR was identified (Hilbert *et al.* 2012). In a study conducted from 2012 to 2015 in France, Gache et al. (Gache *et al.* 2017) detected 7.2% of 1,450 investigated vaginal or

endocervical swabs from sheep by qPCR above the clinical threshold (abortive episodes potentially related to *C. burnetii*) of 10^4 bacteria per swab.

The reasons for the varying prevalences between different countries and even between regions within the countries may be related to different factors. Firstly, climatic conditions due to geographical location may have an influence on the possibility to get infected with *C. burnetii*. Local environmental and weather conditions (e.g. wind, vegetation, soil moisture, precipitation, temperature) have been identified to influence the risk of infection, transmission and spreading in ruminants and from infected flocks to humans (Nusinovici *et al.* 2015; van der Hoek *et al.* 2011; van Leuken *et al.* 2016; Tissot-Dupont *et al.* 2004; Gilsdorf *et al.* 2008). Secondly, the type of production and the farm-specific management were intended to influence the risk of infection (Schimmer *et al.* 2014; Rizzo *et al.* 2016). Rizzo et al. (Rizzo *et al.* 2016) suggested local productive characteristics have a major effect on *C. burnetii* prevalence in livestock from different geographic areas. Some type of production might be associated with a higher occurrence of the pathogen. For example, Schimmer et al. (Schimmer *et al.* 2014) presented a herd-level prevalence of 78.6% in dairy and 30.5% in non-dairy sheep farms in the Netherlands. Gache et al. (Gache *et al.* 2017) made the same observation with 75.6% average herd-level prevalence in dairy and 39.8% in meat sheep flocks. *C. burnetii* is excreted in the milk of small ruminants (Eldin *et al.* 2017). The pathogen was suspected to persist in the mammary glands and the excretion may lead to a contamination of the environment in experimentally infected goats (Roest *et al.* 2020). In addition, compared to other types of farms, dairy sheep are usually kept in the barn for a longer period of time and cleaning and disinfection of the milking parlour is not common practice in small ruminant farms, in contrast to cattle farming. However, compared to other ruminants, sheep shed *C. burnetii* to a lesser extent in milk but they shed the pathogen mainly via other routes (vaginal mucus, faeces) (Rodolakis *et al.* 2007). Furthermore, Schimmer et al. (Schimmer *et al.* 2014) found that breeding lambs as the main farm purpose increases the risk for seropositivity at individual sheep level. This may be correlated with an increased risk for seropositivity at farm level with the number of females that lambed in 2009 in the same study. A higher rate of lambing females could increase the total population at

risk and subsequently the risk of pathogen introduction and transmission (Rizzo *et al.* 2016), as shedding of *C. burnetii* occurs mainly during parturition (Maurin and Raoult 1999; Berri *et al.* 2001). However, the most common production type in Germany as well as in this study is landscape conservation and/or lamb production. The number of examined dairy sheep flocks is very low and therefore, the results are not comparable to other production systems. In addition, the variable production type was not included in the risk factor analysis.

However, the production type is associated with the husbandry system established on the farm. For example, in the Netherlands, the dairy goat industry is very concentrated in the South with farms keeping between 300-7,000 goats often located close to villages and cities (Roest *et al.* 2011). The increase of goat density in some areas and the extension of the farms may have affected in-herd and between-herd dynamics of Q fever and their proximity to highly populated areas is suspected as an explanation for human infections (Roest *et al.* 2011).

On the other hand, sheep kept in extensive systems are possibly more likely to come into contact with the pathogen as outdoor lambing is supposed to lead to the contamination of the environment with *C. burnetii* and to a higher incidence in humans in spring (Maurin and Raoult 1999) and lambing in the pasture is described to increase the risk for seropositivity for sheep at animal-level (Schimmer *et al.* 2014). Furthermore, preventive biosecurity measures can be established more easily in intensive farming systems. In a study conducted in Lower Saxony, Runge et al. (Runge *et al.* 2012) described a higher seroprevalence of migrating flocks. This is in accordance to our results, where this type of husbandry seems to increase the risk for infections detected by ELISA, although this influence is not significant. Migrating flocks are mainly used for landscape protection and move over long distances and thus, may have a higher probability to get in contact with the pathogen than flocks kept on one location. In addition, they constitute a high risk for spreading the pathogen due to a large radius of grazing (Runge *et al.* 2012). Furthermore, flocks kept on pasture have a higher probability to get in contact with other livestock which were supposed to increase the risk of introduction and transmission (Rizzo *et al.* 2016; Schimmer *et al.* 2014). In addition, contact to wildlife animals on pastures in extensive grazing systems are

supposed to increase the transmission of a huge variety of pathogens (Barlozzari *et al.* 2020). However, except of poultry, the presence of other livestock and game does not have a significant influence in this study. In Germany, usually a combination of husbandry systems can be observed due to the combination of production types. This study includes no farm which keeps the animals only indoors, but rather a combination of extensive grazing with housing at lambing time. In addition, due to the small number of examined farms for risk factor analysis and the variability of production type and husbandry, it is difficult to evaluate their influence on a *C. burnetii* infection.

Furthermore, husbandry and production type also require a different composition in terms of flock size, age structure, species and gender distribution within the flocks. An increase in the total flock size or larger herds were associated with seropositivity (Lambton *et al.* 2016; Anastácio *et al.* 2013; Rizzo *et al.* 2016; Villari S. *et al.* 2018; Barlozzari *et al.* 2020). A higher likelihood to get infected for animals in bigger flocks may be through the contact in overcrowded livestock buildings facilitating the introduction of *C. burnetii* or the decreased chance of clearing the infection because of the higher number of susceptible individuals as high animal density causing impaired flock welfare (Villari S. *et al.* 2018). This effect was not observed in this study. Furthermore, older animals were described to have an increased risk being seropositive (García-Pérez *et al.* 2009; Rizzo *et al.* 2016; Anastácio *et al.* 2013; Ruiz-Fons *et al.* 2010) which could indicate horizontal transmission mainly during lambing season and the maintenance of *C. burnetii* within adult population (García-Pérez *et al.* 2009; Anastácio *et al.* 2013) and a higher probability of contact with lifetime (Ruiz-Fons *et al.* 2010; Rizzo *et al.* 2016). This is in accordance with the results of our study, where animals <2 years of age decrease the risk to be seropositive at animal-level.

An increasing number and a high density of goats nearby the farm were identified as risk factor associated with seropositivity (Lambton *et al.* 2016; Schimmer *et al.* 2014). Moreover, similar to other studies (Rizzo *et al.* 2016; Anastácio *et al.* 2013), the proportion of positive mixed flocks is higher compared to pure sheep flocks in the present study. However, the number of mixed flocks is overrepresented and this fact must be taken into account. One reason for a higher number of mixed flocks could be an increased use of goats in flocks of sheep for landscape conservation in recent years

(Bauer *et al.* 2020), as the nature conservation authorities in the districts have been paying increased subsidies if goats are also kept in the sheep flock. Comparing the prevalences at species level, Rizzo et al. (Rizzo *et al.* 2016) and Anastácio et al. (Anastácio *et al.* 2013) found a lower rate in goat flocks than in sheep flocks, while at an overall individual animal-level, the detection rate among the goats was slightly higher than among the sheep (Anastácio *et al.* 2013), especially in mixed flocks (Rizzo *et al.* 2016). However, other studies observed a higher prevalence for sheep (Ruiz-Fons *et al.* 2010) and sheep in pure sheep farms presented a slightly higher prevalence at animal-level compared to goats in pure goat flocks (Rizzo *et al.* 2016). Regarding the flock type for individual animal prevalence especially in sheep, Rizzo et al. (Rizzo *et al.* 2016) reported that sheep in mixed flocks showed a higher prevalence than sheep in pure sheep flocks. Comparing the species, the rate of clinical symptoms seems to be higher in goats (Sidi-Boumedine *et al.* 2010). Moreover, goats were suspected to have a higher risk for *C. burnetii* associated abortion (Agerholm 2013) and may have a higher susceptibility to the infection (Rizzo *et al.* 2016). In general, abortion might be associated with seropositivity as high seroprevalence was found in flocks with previous history of abortion (García-Pérez *et al.* 2009). Schimmer et al. (Schimmer *et al.* 2014) identified the presence of more than six stillborn lambs in 2009 and Rizzo et al. (Rizzo *et al.* 2016) infertility during the last 12 months as risk factors. However, in this study, the majority of farms had not indicated specified reproductive disorders, so that their role to *C. burnetii* infection should be evaluated in further studies. Further research is necessary to investigate the epidemiology of the pathogen in different ruminant species and their interspecies interaction (transmission routes). Moreover, the influence of husbandry, production type and environmental conditions on an infection should be examined in order to understand the different prevalences and corresponding risk factors across European countries.

Our results reflect even varying detection rates of *C. burnetii* between different regions within Germany. Sheep farming in Germany is not as homogeneous compared to swine, cattle or poultry farming systems. The majority of the small ruminants are kept extensively, while in other livestock, husbandry tends to be intensive with automatised production processes. Farm location, differences in sheep husbandry and farm

management within Germany might imply a variability of risk factors for the introduction and transmission of *C. burnetii* into the flock. Therefore, the occurrence of *C. burnetii* and identified risk factors cannot easily be compared across different regions, husbandry and farm management. Nevertheless, the results have shown that the occurrence of the pathogen varies greatly from region to region and is higher especially in the southern federal states (BAV, BW), where the area is known for their high occurrence of *C. burnetii* in sheep (Sting *et al.* 2004; Hellenbrand *et al.* 2001; Bauer *et al.* 2020). In these federal states, notified infections in small ruminants and humans are more frequent and small-scale epidemics occur sporadically (Bauer *et al.* 2020; Hellenbrand *et al.* 2001). In addition, some identified risk factors (infestation with ticks, aseasonal lambing and lambing on pasture) and the presence of mixed flocks (sheep and goat) are more common in the south (Bauer *et al.* 2020) and are associated with an increased risk for an infection or a higher prevalence. However, the prevalence estimation at the federal state level is precluded due to the sample size and differences might on the one hand occur by chance, but they might otherwise have substantiated reasons.

Firstly, ticks - and especially *Dermacentor marginatus* - is considered to be a vector for *C. burnetii* in Germany (Sting *et al.* 2004). This tick species does not occur in all parts of Germany (Pluta *et al.* 2010). In this study, the infestation with ticks was identified to increase the risk for seropositivity. However, *C. burnetii* was scarcely found in *Dermacentor spp.* in endemic areas in southern Germany (Sting *et al.* 2004; Pluta *et al.* 2010). Therefore, ticks are considered to have a minor influence of an infection in Germany (Bauer *et al.* 2020). Nevertheless, infected ticks excrete large amounts of the pathogen in their faeces (Körner *et al.* 2020), which can remain infectious on the wool for a long time (Bauer *et al.* 2020). Therefore, the excrements seem to pose a risk for infection (Bauer *et al.* 2020; Sting *et al.* 2004) which should be evaluated in further studies.

A higher temperature and a decreasing mean humidity were identified to increase the risk for an infection detected by qPCR. Dry weather conditions favours the spread of *C. burnetii* (Tissot-Dupont *et al.* 2004; Hellenbrand *et al.* 2001; Nusinovici *et al.* 2015). In comparison, the North Sea causes high humidity throughout the year and rainy

weather conditions may hamper the spread of *C. burnetii* (Gilsdorf *et al.* 2008). It is likely for these reasons that *C. burnetii* infection was not found on farms located predominantly along coastal areas.

Another plausible explanation might be seasonal lambing behaviour. Aseasonal lambing was identified to increase the risk for an infection with *C. burnetii* in this study and is a common practice in the south due to a high proportion of sheep farms keeping the aseasonal breed Merino Landrace (Bauer *et al.* 2020). With aseasonal lambing, susceptible sheep are available all year round and the infection may circulate within the flock. Year-round lambing may indicate a continuous shedding of the pathogen with favourable conditions for the survival and transmission of *C. burnetii*. Aseasonal lambing with warm and dry climate in the south is suggested to increase infections in comparison to indoor lambing of seasonal breeds usually in winter and early spring with cold and humid climate in the northern parts of Germany (Bauer *et al.* 2020). Schimmer (Schimmer *et al.* 2014) reported a higher risk for seropositivity for sheep lambing in the pasture. However, in a study from Meadows et al. (Meadows *et al.* 2015) outdoor lambing had no significant influence on seropositivity in sheep and it has been suggested that it could reduce the likelihood of exposure.

Our results indicate regional differences in the occurrence of *C. burnetii* and corresponding risk factors in German sheep flocks and therefore varying risk for human infection.

Overall, a higher detection rate was observed for ELISA compared to qPCR at herd-level, while the proportion of seropositive adults within the positive farms detected by ELISA is lower compared to the proportion of infected adults detected by qPCR within qPCR positive farms. Moreover, the identified risk factors differed between the two applied diagnostic test systems. A higher herd seroprevalence could be explained by antibodies being detectable for several weeks after infection (Berri *et al.* 2001). In comparison, shedding the pathogen is usually associated with parturition or abortion (Bauer *et al.* 2020; Maurin and Raoult 1999; Angelakis and Raoult 2010; Berri *et al.* 2001) and sampling took place before lambing season in order to evaluate a novel approach of an early detection of an infection at herd-level by the examination of preputial swabs of the breeding sires during or after mating.

On the other hand, a high proportion of animals shedding the pathogen within the positive flocks may be due to the high sensitivity of the applied qPCR compared to the ELISA. In general, PCR is described as a rapid and sensitive method for the detection of *C. burnetii,* whereas ELISA can present different sensitivities (Sidi-Boumedine *et al.* 2010). Moreover, as in this study, the presence of the pathogen and shedding *C. burnetii* does not lead inevitably to the formation of antibodies (Joulié *et al.* 2017; Berri *et al.* 2001; de Cremoux *et al.* 2012), which may be an explanation for different detection rates. Thus, serological surveys can be applied to estimate population-level exposure, but are less reliable at an individual level (Ryan *et al.* 2011). Therefore, both test procedures should be considered in the context and separately, especially on the individual animal-level. These circumstances could explain some differences of prevalence estimates and identified risk factors regarding the applied test system in the current study.

It should be taken into account that we investigated a convenient sample and that the results depended on the selection of the farms/animals and of the applied test system (ELISA and qPCR) and their misclassification probabilities. Estimates for sensitivity and specificity may vary among population and/or subpopulations due to the distribution of the influential covariates (Greiner and Gardner 2000). Furthermore, no sample size was determined for risk factor analyses and the limited number of 71 examined farms and the variability of sheep husbandry may explain why the impact of some risk factors is not significant. Therefore, the results are inconclusive, but they provide an indication of possible influences on an infection with *C. burnetii* (Glaser and Kreienbrock 2011).

Further research is necessary to obtain more data on the prevalence and risk factors of the individual federal states, districts and regions.

In the second part of this study (manuscript I) the use of preputial swabs of the breeding sires as a low-cost and time-saving method for the detection of an infection at herd-level was evaluated. This novel approach could detect the presence of *C. burnetii* before the main shedding at lambing occurs and enables the implementation of measures on the farm to prevent human infections. However, not all flocks in which the excretion of the pathogen was detected on the vaginal swabs by qPCR have also

been identified by the examination of the preputial swabs. The successful detection may depend on a high within-herd prevalence and a high shedding rate. However, main shedding of *C. burnetii* occurs during birth or abortion (Angelakis and Raoult 2010; Bauer *et al.* 2020; Berri *et al.* 2001; Maurin and Raoult 1999) and the flocks were tested before lambing season. The results indicate that positive flocks may be underestimated if preputial swabs were used as single detection matrix before parturition. Moreover, the number of rams per ewe, as well as the duration and number of the mating periods depends on the production type, the husbandry system and the reproductive behaviour of the animals and varies regionally. Therefore, the application of preputial swabs has to be adapted to different husbandry and management systems. At individual animal-level, breeding sires with positive preputial swabs were all seronegative. This indicates that the presence of *C. burnetii* does not induce inevitable the formation of antibodies as described for females (Joulié *et al.* 2017; de Cremoux *et al.* 2012; Berri *et al.* 2001) or the rams' prepuce was only contaminated with the pathogen which does not result in an infection. The detection of two farms by preputial swabs without females shedding the pathogen simultaneously could be an indication of contamination of the prepuce. A possible explanation could be the location of the prepuce at the upper abdomen exposed to the soil, while *C. burnetii* has a high resistance (Kazar 2005; Eldin *et al.* 2017) and has been detected ubiquitously after an infection (Kersh *et al.* 2013; Astobiza *et al.* 2011).

In the risk factor analysis, the gender was not identified to have a significant influence for an infection with *C. burnetii* at animal-level. However, purchases, which were in the majority breeding sires, turned out to be a significant risk factor. The purchase of breeding sires for the mating season may constitute a risk for the introduction and transmission of *C. burnetii*. Therefore, the examination of preputial swabs of the purchased breeding sires including quarantine should be conducted before mating season and introduction into the flock.

Overall, the preputial swabs should not be used as single detection matrix and the application have to be adapted to different husbandry and management systems. Nevertheless, it could be appropriate to identify flocks with high excretion and within-herd prevalence before the main shedding at lambing occurs and for a follow-up

monitoring in already identified positive flocks. Further research is necessary to obtain data on the epidemiology of *C. burnetii* related to the gender, on the transmission from females to males and vice versa and possible environmental contamination of the mucosa to evaluate the use of preputial swabs as a detection matrix in more detail.

The results of this study can be used for the establishment of a MOSS for small ruminants in Germany. The recommendations of hygienic requirements for keeping ruminants by the Federal Ministry of Food and Agriculture (Bundesministerium für Ernährung und Landwirtschaft BMEL) (BMEL 2014) determines a flock as suspect, if there is an increased incidence of abortion and/or serological results indicating the occurrence of *C. burnetii*. After bacteriological detection, the flock is considered to be infected (BMEL 2014). In this study, abortions in positive farms had rarely been reported, which would have led to an underestimation of suspected flocks and subsequently to an increased risk for the spread of *C. burnetii* and for human infections. In general, all farms have a potential risk of being infected and of transmission of *C. burnetii* to humans and should therefore be investigated (Winter *et al.* 2021). However, the results of this study showed that herd prevalence varies between flocks and that there are risk factors for an infection at herd/animal-level. Therefore, especially these flocks with a possibly increased risk or prevalence (e.g. aseasonal lambing, high number of purchases, mixed farms) should be monitored. An active monitoring is necessary due to *C. burnetii* infection in sheep may occur without clinical symptoms (Runge *et al.* 2012; Winter *et al.* 2021). The advantage of an active monitoring and surveillance system is that it does not depend on the presence of an infection with typical symptoms (Winter *et al.* 2021). Moreover, the varying occurrence of *C. burnetii* between the federal states observed in this study may require a regionally adapted MOSS, which considers differences in husbandry, management and production type. Early control and hygiene measures and subsequent surveillance should be implemented in all flocks having tested positive in order to reduce and prevent human infection (Winter *et al.* 2021; Bauer *et al.* 2020). Although there are recommendations for the management of Q fever in ruminants (Sting *et al.* 2017; BMEL 2014), measures for surveillance and control vary between the districts and federal states due to an inhomogeneous management in case of an infection with the

pathogen. This hampers a transparent and effective approach and causes uncertainty, especially on affected farms. Furthermore, it became clear that the cooperation between veterinary and human health authorities and between veterinarians and physicians should be facilitated. Close cooperation between human and veterinary medicine is important to prevent and counter pathogen transmission (Bauer *et al.* 2020; Winter *et al.* 2021). Therefore, it is necessary to provide further information about epidemiology, pathogenesis, control and prevention of *C. burnetii* to improve Q fever management in Germany (Bauer *et al.* 2020). These circumstances support the objectives of Q-GAPS developing a Q fever guideline, risk barometer and information platform in order to control and prevent infections and support cooperation in the sense of One Health.

Annika Wolf: Prävalenz und Risikofaktoren von *Coxiella burnetii* in deutschen Schafherden und Evaluierung eines neuen Ansatzes zum Nachweis einer Infektion mittels Präputialtupfer auf Herdenebene.

4. Zusammenfassung

Schafe stellen in Deutschland die Hauptquelle humaner *C. burnetii*-Infektionen dar. Ziel dieser Arbeit war es deshalb, die Verbreitung von Coxiellen-Infektionen in deutschen Schafherden zu bestimmen und zeitgleich die Anwendung von Präputialtupfern der Deckböcke während oder nach der Decksaison als Detektionsmethode auf Herdenebene zu evaluieren (Manuskript I). Weiterhin wurden mögliche Risikofaktoren für eine *C. burnetii*-Infektion auf Herden- und Tierebene identifiziert (Manuskript II). Zu diesem Zweck wurden insgesamt 3367 Tiere (2920 Schafe und 447 Ziegen) in 71 Herden in fünf der 16 Bundesländer zwischen November 2017 und Juni 2018 beprobt. Die wahre Herdenprävalenz lag bei 31,3-33 % für den Antikörper-ELISA und/oder den Erregernachweis mittels qPCR. Der wahre Anteil infizierter Tiere lag bei 11-12 % in 19-24 ELISA- und/oder qPCR-positiven Betrieben. Während der serologische Nachweis auf Herdenebene häufiger auftrat (26-36,6 % ELISA vs.13,9 % qPCR), lag innerhalb der positiven Herden eine höhere Detektionsrate mittels qPCR vor (8-13 % ELISA vs. 18 % qPCR). In Bayern (31,8 %) und Baden-Württemberg (78,6 %) zeigte sich eine höhere Nachweisrate für *C. burnetii* auf Herdenebene als in den nord-westlichen Bundesländern (SH: 16,7 %; NDS: 18,2 %; NRW: 16,7 %). Des Weiteren wurden *C. burnetii*-Infektionen häufiger in Mischbetrieben (Schaf und Ziege) (50 %) als in reinen Schafherden (22 %) nachgewiesen. Von acht mittels Vaginaltupfer positiv getesteten Betrieben, konnte das Pathogen nur in drei Betrieben mit einer höheren Ausscheidung ebenfalls bei der Untersuchung der Präputialtupfer nachgewiesen werden (37,5 %). Entsprechend könnten Präputialtupfer als Untersuchungsmaterial eher für Herden mit einer höheren Ausscheidung geeignet sein. Die Risikofaktorenanalyse ergab in der univariablen Analyse auf Tierebene, dass junge Tiere (<2 Jahren) das Risiko für das Vorhandensein von Antikörpern signifikant (*p*<0.05) vermindern, während gemäß finaler multivariabler Analysen auf Herdenebene Zukäufe und asaisonale Lammung das Risiko für Infektionen erhöhen. Im Hinblick auf die Ergebnisse und das zoonotische Potential von

infizierten Schafen für die Übertragung auf den Menschen in Deutschland sollte zukünftig ein aktives, regional adaptiertes, die Unterschiede in Haltung, Betriebsmanagement und Produktionsart berücksichtigendes Monitoring und Surveillance System (MOSS) bei kleinen Wiederkäuern etabliert werden, um das Risiko für humane Infektionen zu reduzieren.

Annika Wolf: Prevalence and risk factors of *Coxiella burnetii* in German sheep flocks and evaluation of a novel approach to detect an infection via preputial swabs at herd-level

5. Summary

Sheep represent the main source of human *C. burnetii* infections in Germany. Therefore, the aim of this study was to determine the spread of *C. burnetii* in German sheep flocks and, at the same time, to evaluate the use of preputial swabs of breeding sires during or after mating season as a detection method at herd-level (manuscript I). Furthermore, potential risk factors were identified for a *C. burnetii* infection at herd and animal-level (manuscript II). For this purpose, a total of 3,367 animals (2,920 sheep and 447 goats) in 71 flocks across five of the 16 federal states were sampled between November 2017 and June 2018. The true herd prevalence was 31.3-33% for antibody ELISA and/or pathogen detection by qPCR. The true proportion of infected animals was 11-12% in 19-24 ELISA and/or qPCR positive farms. While serological detection was more frequent at herd-level (26-36.6% ELISA vs. 13.9% qPCR), a higher detection rate by qPCR was present within the positive flocks (8-13% ELISA vs. 18% qPCR). In Bavaria (31.8%) and Baden-Wuerttemberg (78.6%) a higher detection rate for *C. burnetii* at herd-level was found than in north-western states (SH: 16.7%; LS: 18.2%; NRW: 16.7%). Furthermore, *C. burnetii* infections were detected more frequently in mixed flocks (sheep and goat) (50%) than in pure sheep flocks (22%). Of eight farms tested positive by vaginal swabs, the pathogen could also be detected by the examination of preputial swabs in only three farms with higher excretion (37.5%). Accordingly, preputial swabs could be more suitable as examination material for flocks with a higher excretion. The risk factor analysis revealed in the univariable analysis at the animal-level that young animals (<2 years) significantly ($p<0.05$) reduce the risk for the presence of antibodies, whereas according to final multivariable analyses at herd-level purchases and aseasonal lambing increase the risk for infections. In light of these results and the zoonotic potential of infected sheep for transmission to humans in Germany, an active, regionally adapted monitoring and surveillance system (MOSS), regarding differences in husbandry, farm management and production type, should be established in small ruminants in future to reduce the risk for human infections.

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7. Acknowledgements