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Interdisciplinary studies on *Coxiella burnetii*: From molecular to cellular, to host, to one health research

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ABSTRACT

The Q-GAPS (Q fever GermAn interdisciplinary Program for reSearch) consortium was launched in 2017 as a German consortium of more than 20 scientists with exceptional expertise, competence, and substantial knowledge in the field of the Q fever pathogen *Coxiella* (*C.*) *burnetii*. *C. burnetii* exemplifies as a zoonotic pathogen the challenges of zoonotic disease control and prophylaxis in human, animal, and environmental settings in a One Health approach. An interdisciplinary approach to studying the pathogen is essential to address unresolved questions about the epidemiology, immunology, pathogenesis, surveillance, and control of *C. burnetii*. In more than five years, Q-GAPS has provided new insights into pathogenicity and interaction with host defense mechanisms. The consortium has also investigated vaccine efficacy and application in animal reservoirs and identified expanded phenotypic and genotypic characteristics of *C. burnetii* and their epidemiological significance. In addition, conceptual principles for controlling, surveilling, and preventing zoonotic Q fever infections were developed and prepared for specific target groups. All findings have been continuously integrated into a Web-based, interactive, freely accessible knowledge and information platform (www.q-gaps.de), which also contains Q fever guidelines to support public health institutions in controlling and preventing Q fever. In this review, we will summarize our results and show an example of how an interdisciplinary consortium provides knowledge and better tools to control a zoonotic pathogen at the national level.

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1. Introduction

Q fever is a worldwide zoonotic disease, except in New Zealand and Antarctica. The disease is caused by the Gram-negative, obligate intracellular bacterium *Coxiella burnetii*. In humans, the disease can either be acute or chronic. While acute Q fever is often a mild and self-limiting flu-like illness, it can progress to an atypical pneumonia or a hepatitis (Maurin and Raoult, 1999). Although adequate treatment options exist for acute Q fever, 10–15% of the patients might develop the Q fever fatigue syndrome (Ayres et al., 1998), which can last more than five years (Marmion et al., 1996). Currently, there is no evidence-based recommendation for treating Q fever fatigue syndrome (Morroy et al., 2016). Furthermore, the acute infection can lead to chronic Q fever, typically characterized by endocarditis, hepatitis, osteomyelitis, and potential fatality (Maurin and Raoult, 1999). Chronic Q fever can develop years after the primary infection and results from the ability of *C. burnetii* to persist within the host. To treat chronic Q fever in humans, doxycycline in combination with chloroquine for at least 18 months is recommended (Ullah et al., 2022). This long-term treatment already illustrates that a much more effective therapy for chronic Q fever is urgently needed, also because of poor patient compliance.

Different animal species, especially mammals, birds, or even ticks, can be infected with *C. burnetii* (Angelakis and Raoult, 2010). Particularly the latter has been controversially discussed as vector for *C. burnetii* since its first isolation from a *Dermacentor* (*D.*) *andersoni* tick in 1938. *C. burnetii* is thought to multiply within the tick gut and is then shed with tick feces, which contaminates hides and wool and might transmit the infection to livestock (Stoker and Marmion, 1955). Human Q fever cases have been described after exposure to ticks, but a direct association as a source of infection remains unclear (Nett et al., 2012). The lack of reports on *C. burnetii*-positive ticks during Q fever outbreaks in ruminants and the missing discrimination of *Coxiella*-like tick endosymbionts and *C. burnetii* by using molecular detection methods in the past have contributed to an overestimation of ticks as vectors. Humans become infected primarily by contact with infectious material derived from domestic livestock, notably by inhalation of dust and aerosols from, e.g., amniotic fluid, placenta, or contaminated sheep wool (Maurin and Raoult, 1999). Clinical signs of coxiellosis in ruminants are quite diverse (Agerholm, 2013). Reproduction disorders such as placenta retention and a decline in fertility have been described in cattle (Garcia-Ispierto et al., 2014). High abortion rates due to *C. burnetii* are reported in goats, whereas the impact on sheep health seems minor (Bauer et al., 2020a; Bauer et al., 2022b). These findings indicate species-specific epidemiology and disease severity. Especially infected, and not as shedders identified asymptomatic livestock pose a risk to humans. We need to increase our knowledge concerning this situation in domestic livestock to reduce spillovers to local communities. Moreover, information dealing with disease prevention in livestock will decrease economic losses and ensure sustainable food production for a growing world population. Consequently, an effective information exchange platform connecting human and animal public health services would be highly desirable to confine or eradicate *C. burnetii* infection in livestock and to prevent human infection.

Genotyping methods (Multispacer Sequence Typing – MST, Multiple Locus Variable-Number Tandem Repeat Analysis – MLVA/VNTR, and single nucleotide polymorphism – SNP-based) often allow grouping of *C. burnetii* isolates according to their species or disease manifestation (Arricau-Bouvery et al., 2006; Glazunova et al., 2005; Hornstra et al., 2011). This indicates that the pathogenic potential of an individual *C. burnetii* isolate seems to depend on its genome sequence. However, attempts to identify genes, that can be used as biomarkers to classify the pathogenic potential of an isolate have not been made so far. This points to vast gaps in our understanding of many aspects of *C. burnetii* biology, such as epidemiology, transmission, host-pathogen interactions, pathogenesis, and immune subversion and defense, all affecting public and veterinary health decisions regarding surveillance, vaccination, and treatment. Multi- and interdisciplinary research collaborations present the most suitable platforms to fill these gaps. Therefore, the Q-GAPS consortium was established in 2017 with funding from the German BMBF (Bundesministerium für Bildung und Forschung - Federal Ministry of Education and Research). This review article aims to summarize the accumulated findings of the Q-GAPS network on different scientific aspects of *C. burnetii*, from the molecular to the cellular, to the host, and the One Health levels (Fig. 1), and to demonstrate the need for an interdisciplinary approach to investigate and control a critical bacterial zoonosis. The epidemiological data and control measures were developed under German guidelines and conditions but may be a suitable template for other countries to implement control strategies. This is important because the European Food Safety Authority recently classified *C. burnetii* as a transboundary pathogen threatening European Union countries and requiring coordinated surveillance under the One Health approach (EFSA, 2023). This multifaceted article does not take the form of a conventional review but instead provides an overview of interdisciplinary research on *C. burnetii* in Germany. Furthermore, it highlights current research, crucial gaps, and scientific challenges that must be addressed. This will help to improve our knowledge of Q fever and to develop strategies and essential measures to control and prevent *C. burnetii* infections in animals and humans.

2. A German perspective on the epidemiology of coxiellosis in small ruminants

Germany has a long history of human Q fever outbreaks mainly associated with lambing sheep (Bauer et al., 2020b; Hellenbrand et al., 2001). Data on the occurrence of *C. burnetii* in sheep flocks are crucial for risk assessment, but limited to individual studies at the federal state level, e.g., 8.7% (n = 3460) seropositive animals in Baden-Wuerttemberg (Sting et al., 2004) and 2.7% (n = 1714) positive animals from Lower Saxony (Runge et al., 2012) (Fig. 2). To receive more insight into the complex issue of coxiellosis in small ruminant flocks, new epidemiological data have been generated within the Q-GAPS framework in recent years. Therefore, investigations were performed in flocks from the five German federal states with the largest sheep populations: Baden-Wuerttemberg (BW), Bavaria (BAV), Lower Saxony (LS), North Rhine-Westphalia (NRW), and Schleswig-Holstein (SH). The proportion of *C. burnetii*-positive flocks was much higher in the southern federal states, with 78.6% in BW compared to 16.7% in NRW and SH (Wolf et al., 2020a). Primary risk factors for acquiring coxiellosis were the purchase of animals and the year-round lambing (Wolf et al., 2020b), which is common in southern Germany, based on the husbandry of the non-seasonal Merino sheep.

In the past, goats played a minor role in the epidemiology of *C. burnetii* in Germany. However, more cases of coxiellosis in goats have been reported in recent years, probably due to an increasing number of large dairy goat farms (Bauer et al., 2022c; Sting et al., 2013) and the greater presence of goats in sheep flocks due to higher subsidies for goats used in landscape protection. Moreover, recent reports provided evidence that cattle on mixed farms must be considered as a potential reservoir of *C. burnetii* (Bauer et al., 2020a; Bauer et al., 2021a; Jodełko et al., 2021; Rodolakis, 2009). Pregnant goats seem highly susceptible to *C. burnetii* (Roest et al., 2020), and they shed larger quantities of bacteria for more extended periods than sheep (Bauer et al., 2020a). *C. burnetii* infections resulted in high abortion rates of up to 90% in goat herds, whereas sheep flocks suffered only minor losses (< 5%) (Bauer et al., 2020a; Joulie et al., 2017; Van den Brom and Vellema, 2009). In German sheep flocks, co-infections with *C. burnetii* and *Chlamydia abortus* are frequent, possibly contributing to a higher abortion rate (Bauer et al., 2022b; Eibach et al., 2013). Consequently, species-specific differences seem to exist between sheep and goats. It is debatable whether sheep are less susceptible to *C. burnetii*, thereby less likely to be a reservoir for *C. burnetii* (Bauer et al., 2022a; Lang, 1990). Nevertheless, the zoonotic risk for humans during lambing and shearing of infected sheep flocks is

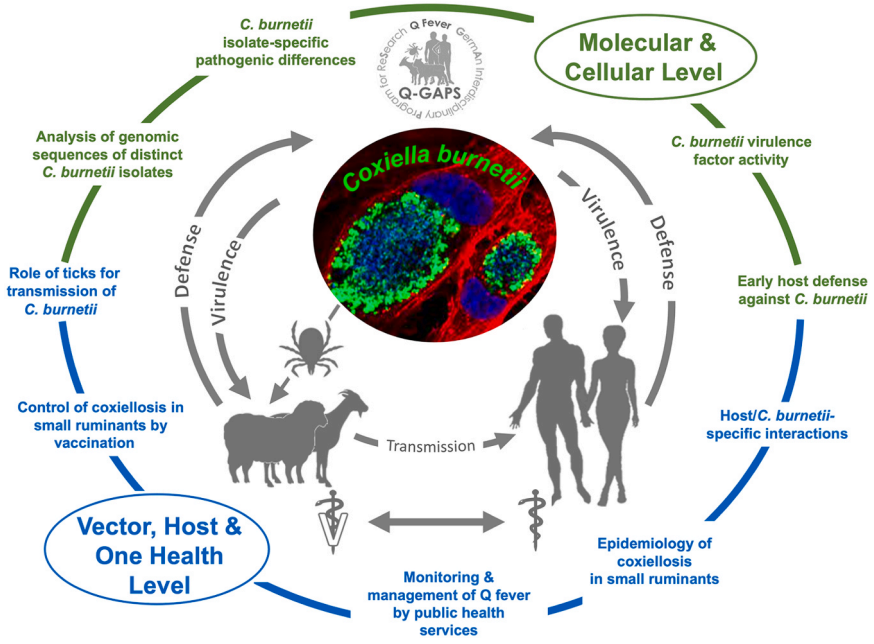


Fig. 1. Schematic representation of tasks, goals, and networking of the interdisciplinary Q-GAPS (Q fever GermAn interdisciplinary Program for reSearch) consortium. Q-GAPS is a multidisciplinary network of scientists dedicated to implementing the One Health approach. Q-GAPS aims to support the public health and veterinary communities in preventing and controlling Q fever.

still high due to the large number of bacteria released within a short period of time (Bauer et al., 2020a; Schulz et al., 2005).

In the past, dry and windy weather conditions favored *C. burnetii* transmission, probably by increasing the formation of contaminated dust particles (Boden et al., 2014; Roest et al., 2011b; Tissot-Dupont et al., 2004). Indeed, a 10% increase in humidity reduced the risk of detecting *C. burnetii* in sheep flocks by half (Wolf et al., 2020b), and precipitation appears to affect the spread of *C. burnetii* between ruminant herds negatively (Bauer et al., 2022a; Nusinovici et al., 2015). Therefore, it is suspected that climate change leading to drier and hotter summers may increase the likelihood of *C. burnetii* transmission among small ruminant husbandries. Consequently, future epidemiological studies should consider including meteorological data to evaluate their influence on *C. burnetii* transmission among livestock. In addition, molecular analyses can help identify the source of infection and clarify a possible transmission route, as specific *C. burnetii* isolates are associated with either a single livestock species or humans. Therefore, information on the genetic diversity of *C. burnetii*, as described below, improves our epidemiological understanding.

3. Genomic sequences of distinct *C. burnetii* isolates

Exploiting genetic biomarkers for classification, known as genotyping (Frangoulidis et al., 2022), is essential for identifying and distinguishing between pathogenic species and is a vital aspect of epidemiology. For instance, genotyping can help improve our understanding of the source and distribution of individual *C. burnetii* genotypes, e.g., during the Dutch Q fever epidemic or outbreaks on smaller farms with different ruminant species (Bauer et al., 2020a; Bauer et al., 2021a; Roest et al., 2011a). Platforms addressing genomic analysis, such as genotyping, can help increase the speed and reduce the total analysis cost (compared to wet lab procedures). They address reproducibility issues and provide sustained access to research data and analysis tools. To transfer genotyping research data to suit a broad range of Q fever-involved stakeholders, it is mandatory to guarantee curated data for further analysis and comparison.

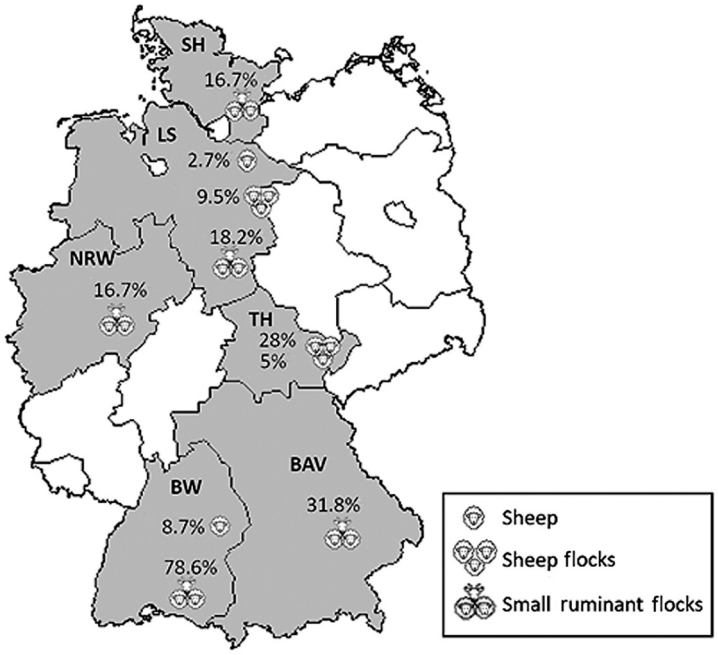
During our Q-GAPS research, we successfully established the new platform CoxBase (https://coxbase.q-gaps.de) (Fasemore et al., 2021). It was designed to address several aspects of *C. burnetii* genomic analysis, such as epidemiological surveillance, metadata summarization via visualization, in silico implementation of multiple genotyping systems, genotyping data query and submission, and genome annotation.

This is not the first instance of an online resource for genomic analysis of *C. burnetii* sequences. There are two existing resources: the Multiple Locus Variable-Number Tandem Repeat Analysis (MLVA) Bank (https://microbesgenotyping.i2bc.paris-saclay.fr/) and the Multispacer Sequence Typing (MST) webpage (https://ifr48.timone.univ-mrs.fr/mst/coxiella_burnetii/groups.html), both with inherent drawbacks that have been addressed by the CoxBase platform. To highlight some improvements, both resources only support single genotyping methods, MLVA genotyping for MLVABank and MST genotyping for the MST page. In contrast, CoxBase can be used for seven different genotyping methods with extra tools to combine multiple markers from other genotyping methods. The CoxBase platform also provides numerous query interfaces to isolate-based metadata, MLVA data (Arricau-Bouvery et al., 2006; Frangoulidis et al., 2014), and MST data (Glazunova et al., 2005). The platform can retrieve plasmid primers for genotyping analysis and houses more strain data and metadata than the two existing resources. The platform also implements features that remove barriers to data exchange via implementing bookmarkable result pages and ensure that analyses carried out on the platform are reproducible.

The platform application is implemented in Python using the Pyramid framework with an MySQL server for storage purposes; the entire application resides in the de.NBI cloud infrastructure.

Applications of the CoxBase platform:

I. Genome typing: The in silico genotyping implementation accepts as inputs either contigs or complete assemblies of sequences as an FASTA file. Multiple methods can be combined for a comprehensive analysis, or a single genotyping method can be used. Results can be exported into a PDF document for storage or retrieved later from the platform with a unique ID.
II. Genotyping data query and submission: The platform contains about 700 isolates with corresponding genotyping data, usually MST and MLVA data. Users can query the database using



Federal state	Species	Percentage of positive animals (number of sampled animals)	Percentage of positive flocks (number of sampled flocks)	Type of samples (assay)	Source
Baden-Wuerttemberg **BW**	Sheep	8.7% (3460)	n.a.	Blood (ELISA)	Sting et al. 2004
	Sheep & goats	N/A	78.6% (14)	Blood (ELISA) & genital swabs (PCR)	Wolf et al. 2020a
Bavaria **BAV**	Sheep & goats	N/A	31.8% (22)	Blood (ELISA) & genital swabs (PCR)	Wolf et al. 2020a
Lower Saxony **LS**	Sheep	2.7% (1714)	9.5% (95)	Blood (ELISA)	Runge et al. 2012
	Sheep & goats	N/A	18.2% (11)	Blood (ELISA) & genital swabs (PCR)	Wolf et al. 2020a
North Rhine-Westphalia **NRW**	Sheep & goats	N/A	16.7% (12)	Blood (ELISA) & genital swabs (PCR)	Wolf et al. 2020a
Schleswig-Holstein **SH**	Sheep & goats	N/A	16.7% (12)	Blood (ELISA) & genital swabs (PCR)	Wolf et al. 2020a
Thuringia **TH**	Sheep	4.3% (1158)	28% (39)	Blood (ELISA)	Hilbert et al. 2012
		2.7% (440)	5% (39)	Vaginal swabs (PCR)	

Fig. 2. Epidemiology of coxiellosis in small ruminants in different German federal states. The upper panel shows the detection of *C. burnetii* in sheep or mixed small ruminant flocks in six German federal states: Baden-Wuerttemberg (BW), Bavaria (BAV), Lower Saxony (LS), North Rhine-Westphalia (NRW), Schleswig-Holstein (SH), and Thuringia (TH) by serological investigations and/or PCR (Hilbert et al., 2012; Runge et al., 2012; Sting et al., 2004; Wolf et al., 2020a). The lower panel shows an overview of the detection of *C. burnetii* in sheep and goats in six German federal states. n.a. = not applicable. Genital swabs = vaginal and preputial swabs.

complete MLVA or MST profiles for isolates with detailed profiles, or specify the degree of disparity they are willing to tolerate.

III. Isolate discovery: The platform can be used to discover and compare *C. burnetii* isolates via approaches such as metadata query, with the possibility to aggregate multiple metadata fields and faceted search of isolates.
IV. Dynamic phylogenetic tree plots: CoxBase is suitable for generating a phylogenetic tree based on the Shriver distance for MLVA genotype comparison.
V. Metadata summarization: The database includes the service to summarize the metadata of the isolates at country level via visualization of host, location, genotype, and year of isolation.
VI. Genome annotation: The platform offers a web-based genome browser (sequence viewer) with a total of 17 integrated genomes of different *C. burnetii* isolates. Other whole genome sequences can also be uploaded and annotated with this feature.
VII. Surveillance Map: The surveillance map on CoxBase features a world map with details of the isolates obtained from each country, as shown in Fig. 3. This feature can provide a holistic view of the global status of available *C. burnetii* isolates.

This new database was developed as one of the contributions of the Q-GAPS consortium. We hope its existence will contribute to unrestricted access to organized and curated research data and provide the opportunity to investigate the genomes of *C. burnetii* for further research. We will continue to manage and update the database with new and current information to improve the comparison of different isolates obtained worldwide and to support *C. burnetii* surveillance. Finally,

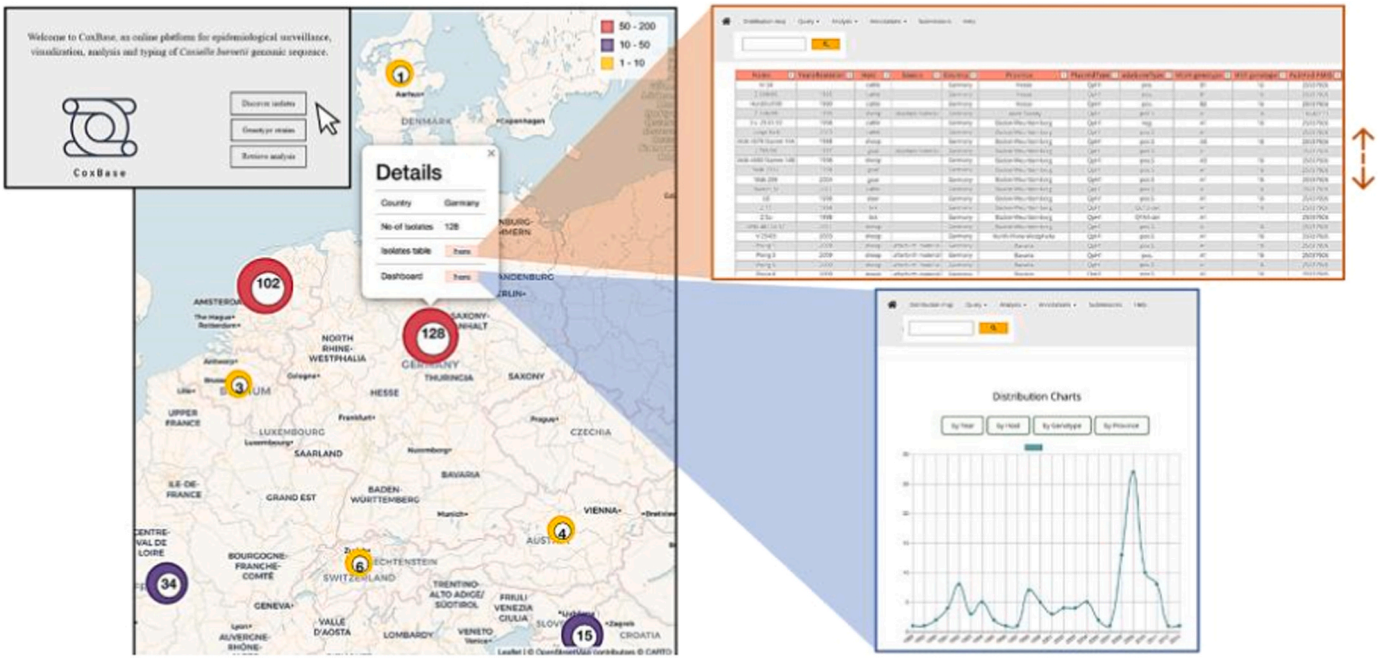


Fig. 3. Illustration and screenshots of the surveillance map feature of the CoxBase platform (https://q-gaps.de/coxbase.html) with Germany as the country selected. Depicted are the welcome window (upper left) with the Coxbase icon and selection menu, the chosen map of Germany (center) as well as the isolate table (upper right), and the corresponding dashboard (lower right).

genotyping methods could contribute to a better understanding of the source of infection of *C. burnetii* and the pathogen's distribution, which is of significant importance due to its easy transmission by wind. In addition to airborne dissemination, ticks probably also play a role as vectors of *C. burnetii*.

4. Ticks as vectors in the transmission of *C. burnetii*

Transmission of *C. burnetii* among animals and entry into a naïve herd are still not fully understood and require further elucidation. Along with airborne dissemination, Q fever is also postulated to be a ruminant-associated tick-borne disease since discovering the infectious agent *C. burnetii* in a *D. andersoni* tick in 1938 (Davis and Cox, 1938; Körner et al., 2021). Since then, field studies of ticks have often included *C. burnetii* amongst other intracellular tick-transmitted bacteria as a target (Del Cerro et al., 2022; Grochowska et al., 2022). Estimated prevalences are usually very low, but can reach high levels similar to those observed for strictly tick-transmitted bacteria. This variability seems to be related to the abundance of various tick species in different geographical areas (Duron et al., 2015b; Körner et al., 2021). Besides pathogenic bacteria, ticks harbor various endosymbionts, including the closely related *Coxiella*-like endosymbionts (CLE) (Zhong, 2012). Many genomic markers for their molecular detection are also present in *C. burnetii*, making it difficult to clearly distinguish between the two pathogens without isolating the pathogen. This led to the hypothesis that results are often misinterpreted and that the role of ticks in the transmission of *C. burnetii* is overrated (Duron, 2015; Duron et al., 2015b; Elsa et al., 2015).

Vector competence describes the ability of a vector to vertically (transstadial, transovarial) or horizontally (to the host) transmit a pathogen and is a crucial factor of vector capacity. It can be analyzed under laboratory conditions, and early experiments showed transmission of *C. burnetii* by several tick species as well as transstadial transmission, as summarized by Duron and colleagues (Duron et al., 2015b). Central Europe's most common tick species are *Ixodes ricinus*, *D. reticulatus,* and *D. marginatus* (Rubel et al., 2014). It has been proposed since 1977 that *D. marginatus* plays a crucial role in transmitting Q fever. Still, until today, only one validated case of a human infection via a tick bite has been reported (Graves et al., 2020). In Q fever outbreaks, *C. burnetii*-positive ticks have not been found (Sprong et al., 2012). Thus, the significance of ticks in Q fever transmission is still unclear, even 80 years after the first discovery of the causative agent.

Using a feeding system based on artificial membranes, we recently demonstrated the uptake and excretion of *C. burnetii* in *I. ricinus* and *D. marginatus* (Körner et al., 2020). Ticks fed on *C. burnetii* (10^6 genome equivalents (GE)/mL) inoculated whole blood shed viable bacteria continuously via feces with significantly higher excretion between days 10–13 (up to 10^5 GE/mg feces). Transstadial transmission from nymphs to adults was detected in 25% of *I. ricinus* ticks. These adult ticks, infected during their nymph stage, shed viable bacteria with their feces while feeding on bacteria-free blood. Throughout feeding, the blood remained negative for *C. burnetii*, which makes the excretion of the bacteria via the saliva unlikely. Nevertheless, the transmission of *C. burnetii* via a tick bite was demonstrated for *Hyalomma lusitanicum* using an artificial tick-feeding system and *H. aegypticum* in a guinea pig model (Duron et al., 2015a; Široký et al., 2010). From all clutches of eggs assessed (n = 37), only one was positive for *C. burnetii*, which makes transovarial transmission in *I. ricinus* unlikely (unpublished observation from our group). Additionally, localization of *C. burnetii* in adult *I. ricinus* ticks (Fig. 4) infected as nymphs is restricted to the midgut. This might indicate that *C. burnetii* replicates mainly within the gut and supports excretion via the feces as the main route. No bacteria were detected within the salivary glands or ovaries. This supports the notion that *C. burnetii* is not transmitted via the tick bite and is unlikely to be transovarially transmitted in *I. ricinus*. This study demonstrated the vector competence of *I. ricinus* and *D. marginatus*. They can excrete infectious *C. burnetii* within the feces, posing the risk of infection by inhalation. However, a high pathogen concentration must be present in the host's blood to infect a biting tick.

That ticks are competent for transmitting *C. burnetii* was suggested in the 1940s, but the transmission route could not be determined (Smith, 1940, 1941, 1942). The artificial feeding system as used by us (Körner et al., 2020), is suitable for studying the vector competence of ticks under controlled laboratory conditions. However, vector competence

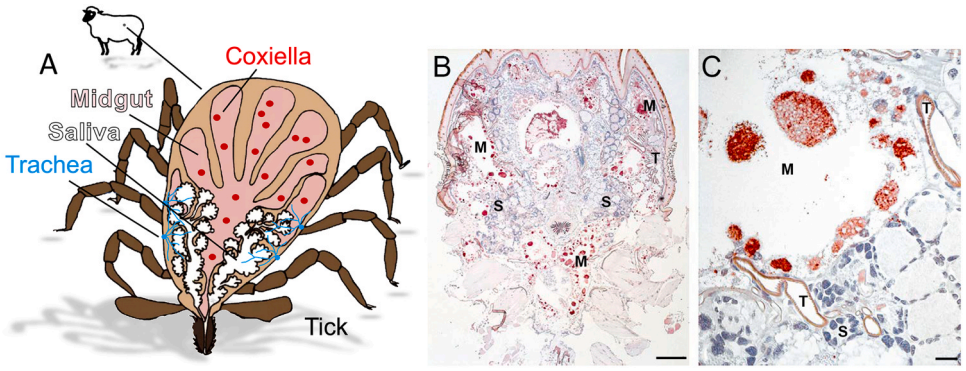


Fig. 4. Localization of *C. burnetii* in unfed *I. ricinus* ticks infected as nymphs. **A**. Schematic representation of a *C. burnetii*-infected tick (midgut, saliva, trachea, and *C. burnetii* are graphically displayed). **B**. Molted adult ticks were fixed in 4% neutral buffered formalin and embedded in paraffin. *C. burnetii* was detected by indirect immunoperoxidase using a polyclonal antiserum and AEC as chromogen (red). Horizontal sections through the entire tick: parasitophorous vacuoles with *C. burnetii* (red) in several sections of the midgut (M), but not in the trachea (T) and salivary glands (S). Size bar = 200 μm. **C**. Higher magnification: numerous parasitophorous vacuoles with *C. burnetii* (red) in epithelial cells of the wall and the midgut (M) lumen. Adjacent salivary glands (S) and trachea (T) do not contain *C. burnetii*. Size bar = 20 μm.

varies between tick species and must be determined individually. It contributes to vector capacity, which is relevant for determining the infection risk.

In summary, our recent work demonstrated the vector competence of *I. ricinus* and *D. marginatus*. Both are capable of excreting infectious *C. burnetii* within the feces, which possess the risk of infection by inhalation, especially during sheep shearing due to wool contaminated with tick feces (Schulz et al., 2005). However, a high pathogen concentration must be present in the host's blood to infect a biting tick. Hence, further investigations are necessary to elucidate the transmission of *C. burnetii* from ticks to mammals and vice versa. Infection of mammalian hosts with *C. burnetii* is not only influenced by extrinsic factors such as environmental conditions and vector-mediated transmission routes. The host's susceptibility, its pregnancy and immune status, as well as the *C. burnetii* genomic properties are essential not only for establishing an infection, but also affect disease severity and excretion. This critical aspect of *C. burnetii*-host-interaction was also intensively investigated within the Q-GAPS consortium.

5. Host-*C. burnetii*-specific interactions

Host contributions to the progression and outcome of an infection with *C. burnetii* can express themselves in very different ways: (i) the host biology can exert species-specific selective pressure leading to differences in the encoded and expressed protein repertoires of the pathogen isolate infecting the respective host, and (ii) in a host-specific organismic and/or cellular response to an infection.

There is some evidence of a potential host tropism among *C. burnetii* isolates from genotyping and genome sequence data (for a recent summary, see: (Hemsley et al., 2021)). Human disease isolates appear over-represented in genome groups IIb, IVb, V, and X, but not in genome groups III and VI (Fig. 5). In contrast, cattle isolates are over-represented in genome group III (Hemsley et al., 2021). In agreement with this observation, the allele numbers of six loci (ms23, ms24, ms27, ms28, ms33, ms34) identified by MLVA/VNTR in the recently described cattle-associated novel genotype C16 (Bauer et al., 2021a) fit best to the allele number profile most frequently identified in isolates from genome group III (Hemsley et al., 2021). If and how these differences at the nucleotide sequence level correlate with differences in effector protein profiles or metabolic activity has only been analyzed systematically for effector proteins in five strains, one of each from the genome groups I, IIa, IV, V, and VI (Larson et al., 2016). This comparison of the effector protein pool revealed considerable heterogeneity, with only 44 of approximately 150 genes being intact in all five strains. Sequence comparison of the *ankF* and *ankG* effector protein genes in approximately 50 isolates suggests that genome groups differ in allele

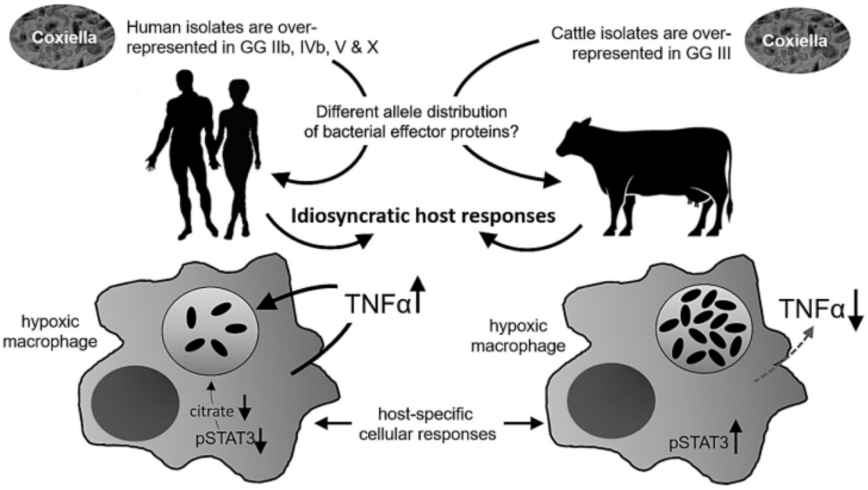


Fig. 5. Host specificity of *C. burnetii* in pathogen interactions. The figure summarizes the differences between humans and cattle regarding infection with different *C. burnetii* isolates, the possible differential allelic distribution of bacterial effector proteins in host-specific infections, and the differential host response to *C. burnetii*, exemplified by the response of infected macrophages under hypoxia.

distributions (Pechstein et al., 2020; Schäfer et al., 2020). This could lead to preferred host associations in individual isolates, and ultimately may lead to subspecies or even "novel" species. For example, we showed that genomic groups IV - VI encode different alleles of the anti-apoptotic effector protein AnkG than groups I, IIa/b, and III (Schäfer et al., 2020). Similarly, for AnkF, an effector protein essential for establishing the replicative *C. burnetii*-containing vacuole (CCV), genome groups IV - VI and group IIa (in three of four sequences analyzed) contain different alleles than the Nine Mile reference strain (Pechstein et al., 2020). Clearly, a systematic analysis of more isolates and more genes will be required to detect host-associated allele profiles.

There is also evidence that host responses to infection with *C. burnetii* differ between host species. Differences in the patterns of bacterial shedding in vaginal mucus, feces, urine, and milk between cattle, goats, and sheep (Bauer et al., 2020a; Rodolakis, 2009) suggest an idiosyncratic host response to the infection process (Fig. 5). The individual reactions could differ at many steps and sites, – for example, in the immune response to the pathogen. Still, they could also affect bacterial transmission, host cell tropism, or replication within the host and in individual infected cell types. We have also observed host-specific cellular responses within the Q-GAPS project. *C. burnetii* replication is controlled in hypoxic murine bone marrow-derived and hypoxic human monocyte-derived macrophages but not in hypoxic bovine monocyte-derived macrophages (Hayek et al., 2019; Mauermeir et al., 2023) (Fig. 5). This lack of control of *C. burnetii* replication in hypoxic bovine macrophages might be due to continued STAT3 activation, since it is required for *C. burnetii* replication in murine macrophages under normoxia (Hayek et al., 2019). Another critical difference between infected human and bovine macrophages was that the latter did not secrete TNF-α despite the expression of the gene (Mauermeir et al., 2023) (Fig. 5). TNF-α is essential for restricting *C. burnetii* in murine macrophages, and adding bTNF-α to infected bovine macrophages led to reduced bacterial counts. In contrast, different genome groups (I, IIa, V, VI, and X) elicited only marginal differences in responses between human and bovine monocyte-derived macrophages concerning invasion, replication, up-regulation of several cytokines, up-regulation of selected activation markers, and macrophage polarization (Sobotta et al., 2017) (Fig. 5). As these experiments were performed under normoxia, it is unclear if and to which extent oxygen availability would affect the responses detected. Further investigations will be needed to unravel the molecular mechanisms leading to the observed differences, and their consequences for the respective host's infection process. Based on these new findings, the differences in species tropism, immune response, and replication in infected cells and hosts of the different *C. burnetii* genome groups raise questions about the phenotypic impact on pathogenicity, ranging from acute to chronic disease patterns. This critical point is taken up in the following section.

6. *C. burnetii* isolate-specific differences in pathogenicity

The hypothesis of isolate-specific virulence or pathotype was generated by early comparative studies of *C. burnetii* isolates regarding plasmid content (Samuel et al., 1985) and restriction fragment length polymorphism (RFLP) analyses (Hendrix et al., 1991). These investigations revealed a phylogenetic separation of isolates originating from acute or chronic human cases. Six genomic groups were distinguished with well-established distinct pathotypes in mouse and guinea pig infection models. Group I - III isolates, associated with human acute Q fever, ticks, cow's milk, and abortion in goats, cause more severe clinical signs and have higher bacterial burdens than isolates associated with chronic Q fever (groups IV and V) or the severely attenuated isolates of group VI (Fig. 6). The induction of high levels of tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and interleukin (IL)−6 by the group I isolates in immunocompetent mice corresponds with cytokine patterns observed in humans with acute Q fever (Capo et al., 1999; Russell-Lodrigue et al., 2009; Tesfamariam et al., 2022). Similar clinical outcomes are described for the infection of guinea pigs with group I - III isolates causing severe disease, including fever (Fig. 6).

When infected with group V isolates, guinea pigs display moderate symptoms and no acute disease or fever when inoculated with group IV and VI isolates (Long et al., 2019; Russell-Lodrigue et al., 2009; Russell-Lodrigue et al., 2006; Scott et al., 1978) (Fig. 6). While these studies used similar sets of isolates established over 40 years ago, recent findings with circulating *C. burnetii* isolates in the US or Australia support the hypothesis of pathotypes or isolate-specific virulence (Islam et al., 2019; Priestley et al., 2021). Interestingly, mice challenged by aerosol developed hemothorax, a clinical sign of acute pneumonia, when infected with isolates carrying the QpH1 plasmid found in group I to III isolates, but not with isolates harboring the QpRS plasmid, which is present in group IV isolates (Priestley et al., 2021) (Fig. 6). This correlation was recently suggested by a pan-genomic analysis of 75 European *C. burnetii* isolates, where plasmid-less isolates or isolates harboring the QpRS plasmid were not associated with human acute Q fever cases (Abou Abdallah et al., 2022) (Fig. 6). However, several Australian *C. burnetii* strains from acute human Q fever cases harbor the QpRS plasmid but lack the acute disease gene A (adaA) (Vincent et al., 2016;

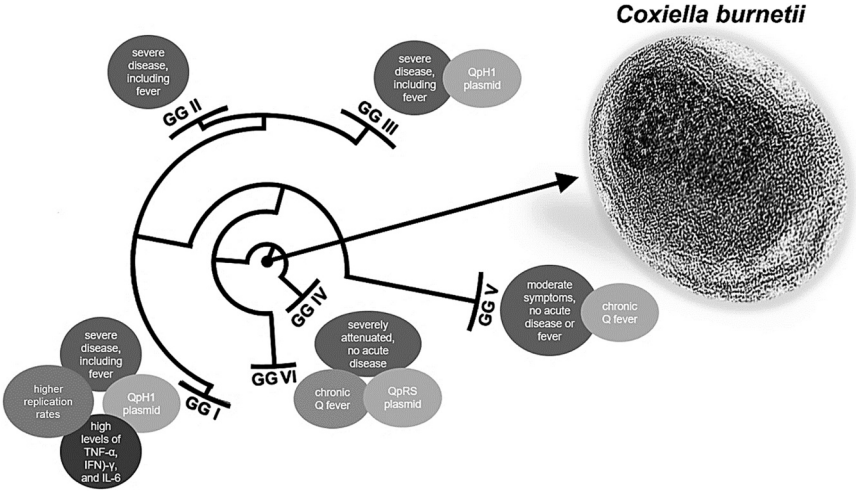
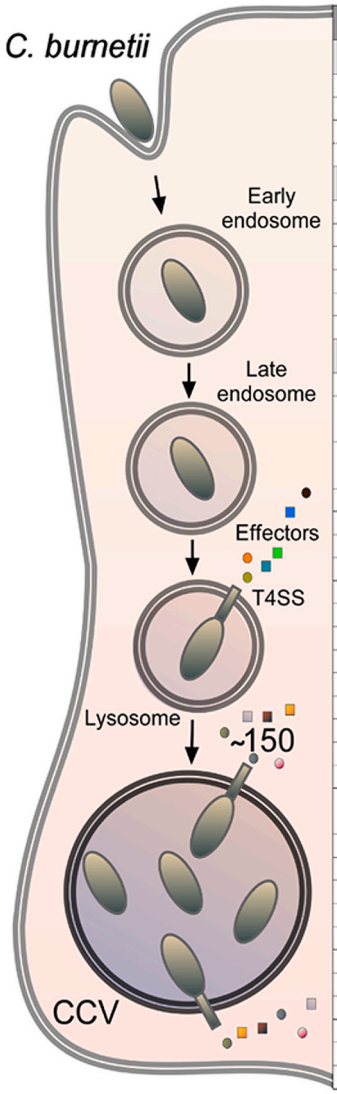


Fig. 6. Radial SNP tree of *C. burnetii* genomic genotypes (GG) complemented by the characteristic traits and specific features of the different GGs from I to VI. The depicted radial tree is rooted along the branch leading to GG IV. The image on the right shows the electron micrograph of an intracellular small cell variant of *C. burnetii*. The graphical GG tree representation in the figure is based on the results and the publication of Hemsley et al., 2019.

Walter et al., 2014). Only one of these isolates was analyzed in a mice infection model and characterized as low virulence (Priestley et al., 2021). This fosters the hypothesis that specific genomic traits contribute to the virulence of isolates. These genotype-specific differences are also displayed at cellular level. Group I isolates showed higher replication rates than group V and VI isolates in primary bovine and monocyte-derived human macrophages. Likewise, the expression of pro-inflammatory cytokines following infection by the group I isolates was higher than if infected by group V or VI isolates (Sobotta et al., 2017). This resembled the results observed upon infection of immunocompetent mice and during acute human Q fever. Interestingly, in bovine mammary-gland epithelial cells, replication efficiencies were similar when comparing isolates according to their genomic grouping, but differed for isolates clustered by their host species. None of the isolates tested in these cells induced a pro-inflammatory response except one sheep isolate, which caused a potent IL-1β and TNF-α reaction (Sobotta et al., 2022).

Comparative genomics revealed that *C. burnetii* isolates differ in novel gene content and that IS element-mediated genome rearrangements may lead to pseudogene formation (Beare et al., 2009). Such genome reduction was suggested as a driver of pathoadaptation, with the severely attenuated isolate Dugway having the largest genome. In contrast, highly virulent isolates, such as the hypervirulent *C. burnetii* Guyana CB175 from French Guiana, or *C. burnetii* isolates from the largest ever reported Q fever outbreak in the Netherlands, contain large deletions or mutations in critical genes, respectively (D'Amato et al., 2015; D'Amato et al., 2014; Kuley et al., 2017). The varying numbers of unique genes among isolates may contribute to isolate-specific virulence (Zhang et al., 2005). For instance, the repertoire of genes encoding effector proteins transported by the type IVB secretion system (T4SS) is of great interest since these effectors interfere with host cell signaling and are essential for the intracellular survival of *C. burnetii*. The lowest



Gene	Protein	Interactor
		Host Cell Death
CBU0781	AnkG	p32, importin-alpha1, DDX21, 7SK RNA
CBU1524	CaeA	HMGB1
CBU1532	CaeB	N/A
CBU1823	IcaA	N/A
		Host Immunity and Signaling
CBU0388	CetCb2	MAPK
CBU0513	CinF	IkappaBalpha
CBU0885	CetCb4	MAPKKK,GATAD2B
CBU1217	NopA	GTPase Ran
CBU1314	N/A	Host chromatin, CSNK2A1, PAF1C
CBU1676	Cem9	MAPK
		CCV Biogenesis, Trafficking and Replication
CBUA0013	CpeB	Rab11a
CBUA0024	CpeL	LC3 on autophagosomes, ubiquitinated proteins on CCV
CBU0021	CvpB/ Cig2	Phosphatidylinositol-3-Phosphatase, Phosphatidylserine on early endosomes
CBU0041	CirA	RhoA
CBU0077	MceA	MceA (multimeric complex)
CBU0175	CstK	TBC1D5 (Rab7 activating protein)
CBU0414	coxH1	N/A
CBU0425	CirB	20S host proteasome (PSMB5)
CBU0447	AnkF	Vimentin
CBU0626	CvpF	GTPase Rab26
CBU0635	N/A	Golgi, vesicles
CBU0665	CvpA	Clathrin adaptor AP2, GDI2, cofilin 1
CBU0937	MceB/ CirC	N/A
CBU0978	Cem3	N/A
CBU1387	Cem6	N/A
CBU1425	MceC	N/A
CBU1556	CvpC/ Cig50	CCV membrane, recycling endosomes (Transferrin receptor)
CBU1751	Cig57	FCHO2
CBU1780	N/A	N/A
CBU1818	CvpD	CCV membrane
CBU1825	N/A	N/A
CBU1863	CvpE	CCV membrane
CBU2007	Cem12	CHP1
CBU2013	Cem13	UBE2T
CBU2028	N/A	BAG2
CBU2052	CirD	N/A
CBU2059	CirE	N/A
CBU2072	EirA	N/A

Fig. 7. List of the *C. burnetii* T4SS effector proteins so far characterized. The respective effector gene number and protein name and its host cell binding partner(s) are given. N/A - not analyzed. The bacterial effectors studied interfere with **cell death** (Berens et al., 2015; Bisle et al., 2016; Carey et al., 2011; Cordsmeier et al., 2022; Crabill et al., 2018; Cunha et al., 2015; Eckart et al., 2014; Friedrich et al., 2021; Fu et al., 2022a; Klingenbeck et al., 2013; Lührmann et al., 2010; Newton et al., 2013; Pan et al., 2008; Rodriguez-Escudero et al., 2016; Schäfer et al., 2017; Schäfer et al., 2020; Voth et al., 2009), **immunity and signaling** (Burette et al., 2020; Carey et al., 2011; Chen et al., 2010; Crabill et al., 2018; Fu et al., 2022a; Lifshitz et al., 2014; Patrick et al., 2018; Weber et al., 2013; Weber et al., 2016; Zhang et al., 2022), and **CCV biogenesis and vesicular trafficking** (Abou Abdallah et al., 2022; Bacon and D'Orso, 2019; Beare et al., 2009; Capo et al., 1999; Cordsmeier et al., 2019; D'Amato et al., 2015; D'Amato et al., 2014; Fu et al., 2022a; Fu et al., 2022b; Hendrix et al., 1991; Islam et al., 2019; Kovacs-Simon et al., 2020; Larson et al., 2016; Long et al., 2019; Maturana et al., 2013; Newton et al., 2013; Pechstein et al., 2020; Priestley et al., 2021; Russell-Lodrigue et al., 2009; Russell-Lodrigue et al., 2006; Scott et al., 1978; Sobotta et al., 2022; Sobotta et al., 2017; Tesfamariam et al., 2022; Weber et al., 2013).

number of intact ankyrin repeat domain encoding genes is found in the highly virulent *C. burnetii* isolate Nine Mile in contrast to their number in the severely attenuated isolate Dugway. This is also true for plasmid-encoded effector proteins with four additional effector protein-encoding genes located on the Dugway-specific QpDG plasmid, implying a correlation between effector repertoire composition and virulence (Graham et al., 2015; Maturana et al., 2013; Schäfer et al., 2020; Voth et al., 2009). Given the evidence of isolate-specific virulence or pathotyping by adaptation of *C. burnetii* at genomic level, a more detailed knowledge of the complex function and activity of bacterial virulence factors is crucial for understanding the infection strategy of *C. burnetii* as an obligate intracellular pathogen. This was a further key point of scientific work in the Q-GAPS consortium.

7. *C. burnetii* virulence factors - function and activity

To ensure intracellular survival by blocking recognition and elimination by host cell defenses, *C. burnetii* encodes a large number of virulence factors, including a type 4B secretion system (T4SS). This multi-protein complex translocates numerous effector proteins into the host cell cytoplasm to ensure bacterial survival and replication (Beare et al., 2011; Carey et al., 2011). So far, approximately 150 different *C. burnetii* T4SS effector proteins have been identified (Burette and Bonazzi, 2020; Lührmann et al., 2017). For most of them, a molecular function has not been elucidated. The ones studied in more detail interfere with cell death, immunity, signaling, *C. burnetii*-containing vacuole (CCV) biogenesis, and vesicular trafficking (summarized with the corresponding literature in Fig. 7). Comprehensive information about the biological function and the methods used to analyze the effector proteins are shown in supplemental table 1 (Table S1).

Interestingly, there is considerable diversity in the repertoire of effector proteins in different *C. burnetii* isolates (Larson et al., 2016). Understanding the functions of these effector proteins will allow the prediction of both pathogenic profile and virulence in the individual *C. burnetii* isolates, paving the way to develop countermeasures against infection. Our Q-GAPS network investigated one conserved and two non-conserved T4SS effector proteins. The conserved effector protein might have global and essential pathogenic potential, while the non-conserved effector proteins might predict context- or species-specific virulence activity.

The conserved T4SS effector protein AnkF is a 21 kDa protein with three predicted ankyrin-repeats (Larson et al., 2016; Voth et al., 2009). Translocated AnkF seems to have a short half-life during infection due to proteasome-mediated degradation (Pan et al., 2008). Although AnkF is not involved in invasion and the development of a phagolysosomal-like compartment, it is crucial for the successful replication of *C. burnetii*. This is achieved by interfering with the biogenesis of the replicative niche, at least in part, by recruiting the intermediate filament vimentin to the CCV (Pechstein et al., 2020). Further research will be required to unravel the molecular activity of AnkF in detail.

The two non-conserved effector proteins are AnkG and CaeB. Both effector proteins interfere with the intrinsic apoptotic cell death pathway (Klingenbeck et al., 2013; Lührmann et al., 2010). AnkG is one of the first identified (Pan et al., 2008) and functionally best-described anti-apoptotic T4SS effector protein (Cordsmeier et al., 2019). It is localized to the hosts mitochondria. AnkG binds the host cell protein p32, and both are translocated to the nucleus upon sensing a cell death signal (Eckart et al., 2014). Nuclear import of AnkG is then mediated via binding to Importin-α1 (Schäfer et al., 2017).

CaeB inhibits mitochondrial outer membrane permeabilization (MOMP) and thus efficiently prevents intrinsic apoptosis (Klingenbeck et al., 2013). However, the molecular mechanisms of these effector proteins were not fully understood. Therefore, we aimed to dissect the molecular functions of AnkG and CaeB in modulating apoptosis, which is part of innate host immunity. AnkG was shown to modulate host cell transcription (Cordsmeier et al., 2022). Thus, AnkG binds to the DEAD-box RNA helicase DDX21 and the small nuclear RNA 7SK. Both influence the 7SK small nuclear ribonucleoprotein (snRNP) complex and thereby the activity of the positive transcription elongation factor (P-TEF) b (Bacon and D'Orso, 2019). In agreement, we demonstrated that AnkG alters host cell transcription. Mainly anti-apoptotic genes were up-regulated, while pro-apoptotic genes were down-regulated. In addition, AnkG influenced the expression of genes involved in signaling and trafficking (Cordsmeier et al., 2022).

During infection, translocated CaeB is associated with the endoplasmic reticulum (ER) and inhibits ER stress-induced cell death by modulating IRE1 signaling (Friedrich et al., 2021). Significantly, this activity is conserved in plant and mammalian cells, making CaeB a cross-kingdom effector protein. However, how an ER-localized effector protein can interfere with MOMP (Klingenbeck et al., 2013) must still be clarified.

AnkG and CaeB are essential for *C. burnetii*-mediated apoptosis inhibition and full pathogenicity in the *Galleria mellonella* infection model (Cordsmeier et al., 2022; Friedrich et al., 2021; Schäfer et al., 2020), suggesting that they are critical virulence factors. Nevertheless, both are only expressed as potential T4SS effector proteins in a subset of *C. burnetii* isolates, indicating specificity.

Taken together, we detailed the molecular activity of one conserved and two non-conserved T4SS effector proteins. Further research on the function of additional effector proteins is required to predict the pathogenic profile and the virulence of individual *C. burnetii* isolates. It is important to note that *C. burnetii* virulence factors critically influence the host immune response to the pathogen at multiple levels, and *C. burnetii* has unique, sophisticated strategies to evade and subvert host defenses, thereby ensuring its intracellular proliferation and host cell-to-host cell transmission. This is described below for the early immune response to *C. burnetii* involving specific innate immune cells.

8. Early host defense against *C. burnetii* - the basis for an effective downstream response

During infection, natural killer (NK) cells are the first line of defense of the innate immune system that makes early contact with *C. burnetii* (Calverley et al., 2010). These early processes functionally direct downstream responses and initiate the development of T cell immunity (Iwasaki and Medzhitov, 2015), which is critical for defense against *C. burnetii* (Read et al., 2010). Despite this central role in immune function, the cellular processes involving NK cells during *C. burnetii* infections are not understood. NK cells belong to the subgroup of lymphocytes of the white blood cells and play an essential role in the early detection and lysis of pathogen-infected cells (Topham and Hewitt, 2009). NK cells constitute about 5–10% of the recirculating lymphocyte population, and their induced IFN-γ secretion (Paolini et al., 2015) is crucial in controlling various immunological events during infections with different microbes. The recognition of target cells is achieved via antibody-dependent cell-mediated cytotoxicity (ADCC), based on recognizing antigen-specific antibodies on the target cells by Fc receptors (Topham and Hewitt, 2009). In addition, NK cells recognize infected target cells with specifically down-regulated major histocompatibility complex class I (MHC I) (Topham and Hewitt, 2009). The lysis of target cells is recognized by NK cells via the degranulation of cytoplasmic granules, which represent secretory lysosomes (Trapani and Smyth, 2002). This leads to the release of perforins and granzymes into the extracellular space by targeted exocytosis (Trapani and Smyth, 2002). NK cells are essential for the immune defense against intracellular bacterial pathogens (Radomski et al., 2019). Indeed, we previously demonstrated that *Chlamydia*-infected NK cells are functionally mature and prevent bacterial intracellular establishment and growth (Radomski et al., 2019). Bacteria taken up by NK cells are killed and released via degranulation in a noninfectious, highly immunogenic form, triggering a solid adaptive anti-chlamydial immune response (Radomski et al., 2019). Based on these observations, we wanted to determine whether

this NK cell-mediated defense mechanism also fights *C. burnetii* that localizes in acidic cell compartments during their intracellular life cycle. Cellular immunity is critical for fighting *Coxiella* infections (Andoh et al., 2007). Key Th1 cytokines like TNF-α and IFN-γ directly activate professional antigen-presenting cells (APCs) to control the intracellular growth of *C. burnetii* (Shannon and Heinzen, 2009). Although *C. burnetii* (like chlamydia) infects a wide range of host cell types (Voth and Heinzen, 2007), it is unknown whether it can also infect NK cells and, if so, whether the pathogen can escape NK cell-mediated killing. Our studies revealed for the first time that NK cells are indeed infectable with *C. burnetii* (Matthiesen et al., 2020). However, the infected NK cells prevent the establishment and replication of internalized bacteria by expelling them into the extracellular environment (Matthiesen et al., 2020). This process is accompanied by functional NK cell activation, characterized by phospho-activation of protein kinase C (PKC) Θ as well as IFN-γ and granzyme (Grzm) B release (Matthiesen et al., 2020). Microscopic analyses showed that intracellular bacteria colocalize with secretory granules and that *C. burnetii* release occurs via degranulation of the infected NK cells. Nevertheless, killing within the secretory granules of infected NK cells appears to be evaded by *C. burnetii* due to its acid and protease resistance (Matthiesen et al., 2020) (Fig. 8). Thus, released *C. burnetii* largely maintain their integrity and ability to infect neighboring host cells after degranulation (Fig. 8). Interestingly, it seems that perforin from the granules is physically attached to the released extracellular bacteria (Matthiesen et al., 2020). This may have functional importance because perforin is known to activate clathrin-dependent endocytosis, which is required to uptake *C. burnetii* by the respective target cells (Matthiesen et al., 2020). Hence, it is conceivable that this might even support the spread of bacteria from infected NK cells to neighboring target cells. Other cells of the innate immune system might also be involved in spreading the infection. These include *C. burnetii*-infected macrophages and dendritic cells (DC), which are known to be impaired in their proper maturation and function and thus may contribute to tolerance (and/or ignorance) of bacterial antigens (Shannon and Heinzen, 2009). Nonetheless, although *C. burnetii* escapes elimination by secretory granules, representing a critical Achilles heel of the innate immune response, the release of IFN-γ by infected NK cells still positively affects the anti-bacterial activity of infected cells (Turco et al., 1984). *C. burnetii*-infected DCs show a block of maturation and MHC-mediated antigen presentation by the autocrine release of immunosuppressive transforming growth factor ß (TGF-β) (Fig. 8). However, our recent studies have shown that IFN-γ reverses the TGF-β-mediated impairment of infected DCs and activates host cell survival, antigen presentation, and bacterial elimination, mainly through the induction of iNOS/NO (Matthiesen et al., 2023) (Fig. 8). Further unpublished experiments by us in normoxic and hypoxic infection models of DCs suggest that hypoxic culture conditions induce *C. burnetii* to form spore-like particles without productive vacuole formation. This survival form of intracellular *C. burnetii* is characterized by IFN-γ resistance, enhanced oxygen binding, and radical detoxification due to altered gene expression and retention of infectivity. This suggests that hypoxia provides intracellular *C. burnetii* with additional opportunities for efficient immune evasion, favoring undetected bacterial presence within infected host cells and presumably promoting chronic *C. burnetii* infections.

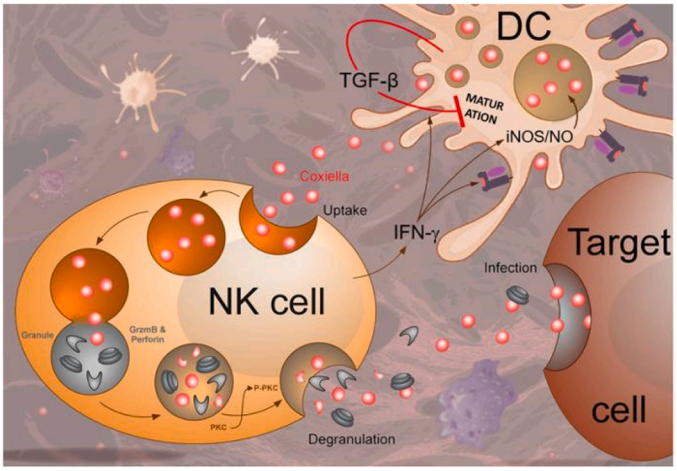


Fig. 8. Subversion and cellular self-defense in *C. burnetii*-infected NK cells and DCs. Upon uptake into NK cells, the infection activates the host cells via PKCΘ, driving increased IFN-γ secretion. During this activation process, *C. burnetii* structures fuse with lytic granules/secretory lysosomes that are then released via degranulation. Most invading organisms survive their temporary stay in these acidic and degrading compartments and largely retain their infectivity after release. *C. burnetii* infection in DCs leads to a subversion of DC function via autocrine TGF-β release. Both iNOS/NO-mediated defense and MHC I presentation are impaired. This creates conditions that promote the growth of *C. burnetii*. However, IFN-γ released by activated NK cells attenuates autocrine TGF-β action on infected DCs. Consequently, it enables cellular self-defense against *C. burnetii* via iNOS/NO, which then blocks bacterial growth.

Combined, *C. burnetii* infections induce innate and adaptive immune responses (Andoh et al., 2007). Our results contribute to a better and more in-depth understanding of *C. burnetii* infection in NK cells and DCs and provide new insights into the contributions of IFN-γ-based immune reactions during the early phase of anti-*C. burnetii* defense. This might have important implications for future therapies and vaccination strategies, which are paramount for preventing and controlling *C. burnetii* infections in animals and humans. A robust and protective cellular/IFN-γ immune response against bacterial pathogens involves the processing and presentation of pathogen-derived antigens by APCs for an enhanced downstream T-cell response in conjunction with the humoral defense. This way, the different components of a combined immune response triggered by a pathogen or vaccine could work together to maximize their effector mechanism. Investigating the activation and interaction of cellular and humoral immunity during the vaccination of small ruminants against *C. burnetii* was another focus of the Q-GAPS research network.

9. Control of coxiellosis in small ruminants

Currently, no antimicrobial substance is sufficiently adequate to treat coxiellosis in ruminants. In the past, oxytetracycline was unsuccessfully used to control the disease (Astobiza et al., 2013; Byeon et al., 2022). *C. burnetii* multiplies in the phagolysosomes of host cells with an acidic pH value, leading to an inactivation of tetracyclines through epimerization at the C4 atom (Rogalski, 1985). Therefore, prevention of abortion and pathogen shedding in small ruminants can be achieved best by vaccination. For several decades, vaccines have been developed to control *C. burnetii* in ruminants (Arricau-Bouvery et al., 2005; Avbersek et al., 2019; Muleme et al., 2021; Sádecký et al., 1975; Schmeer et al., 1987). Many of them were based on formaldehyde-inactivated whole-cell antigens. Vaccines based on the phase I antigen were more protective than vaccines containing phase II antigens (Arricau-Bouvery et al., 2005; Ormsbee et al., 1964). These results suggest that phase I LPS is an essential protective component, but it remains unclear if there are specific protective antigenic epitopes in phase I LPS (Zhang et al., 2012). However, new findings demonstrated that a formalin-inactivated *C. burnetii* Nine Mile (NM) phase II strain, together with a Quil-A® adjuvant, induced significant protection in sheep (Williams-Macdonald et al., 2023).

Since 2010, an inactivated *C. burnetii* phase I vaccine (Coxevac®, Ceva Santé Animale, Libourne, France) has been licensed in many European countries for cattle and goats. According to former product information, a dose of 1 mL is sufficient to vaccinate sheep, which is half the recommended volume for goats. The manufacturer no longer maintains this recommendation, but the vaccine is widely used to immunize sheep throughout Europe (Bauer et al., 2021b; Vellema et al.,

2021). Our present studies observed that the humoral and cell-mediated responses did not differ in naïve sheep when 1 mL or 2 mL were administered (Bauer et al., 2023). However, a third vaccination (booster) seems necessary to stimulate IFN-γ release. In contrast to these findings, the inactivated *C. burnetii* vaccine enhances the longevity of IgG immunity in naturally pre-infected sheep for at least two years (Bauer et al., 2021b; Bauer et al., 2022b). Therefore, in our opinion, the annual revaccination of sheep with coxiellosis seems to be unnecessary. Moreover, the exclusive vaccination of gimmers successfully prevented *C. burnetii* shedding long-term (Böttcher et al., 2022). This is in agreement with studies in goats, which suggested that primiparous animals benefit the most from immunization (De Cremoux et al., 2012; Hogerwerf et al., 2011; Rousset et al., 2009). In an acute case of coxiellosis, vaccination may reduce the bacterial load of pre-infected animals but does not prevent pathogen excretion (Bauer et al., 2021b; Bauer et al., 2022c; Hogerwerf et al., 2011). Nevertheless, our studies suggest significantly reduced *C. burnetii* shedding in pre-infected immunized sheep during the subsequent lambing season (Bauer et al., 2022b) (Fig. 9). The insufficient stimulation of a T cell-mediated immune response in naïve sheep after primary vaccination (Bauer et al., 2023) and the onset of side effects such as swellings and abscesses as well as a significant drop in milk yield after repeated application of *C. burnetii* phase I vaccines in goats and cattle support the need for a long-lasting and safer vaccine. In recent years, significant efforts have been made to develop Q fever subunit vaccines based on recombinant proteins and epitopes, with promising results in the rodent model (Fratzke et al., 2021; Gilkes et al., 2020; Scholzen et al., 2019). Although vaccination is an excellent prophylaxis tool, there are yet no sufficient medical treatment options to control an acute Q fever outbreak in livestock. This can only be achieved through intensive cooperation between all stakeholders in the sense of the One Health approach, as described below.

10. Management and monitoring of Q fever under German conditions - a potential blueprint and a critical revision

Communication and collaboration are essential to control Q fever in animals and humans. Therefore, different groups of people and institutions, which are listed in Fig. 10, must be considered in Q fever management.

In most cases, Q fever outbreaks are investigated retrospectively. A *C. burnetii* infection is primarily diagnosed in diseased people by primary care physicians, clinicians, and laboratories. Acute Q fever is a notifiable human disease in many European countries, including Germany. Therefore, local health authorities must be informed after diagnostic tests confirm the diagnosis. Local veterinary authorities must also be involved in identifying the origin of human Q fever cases (Fig. 11).

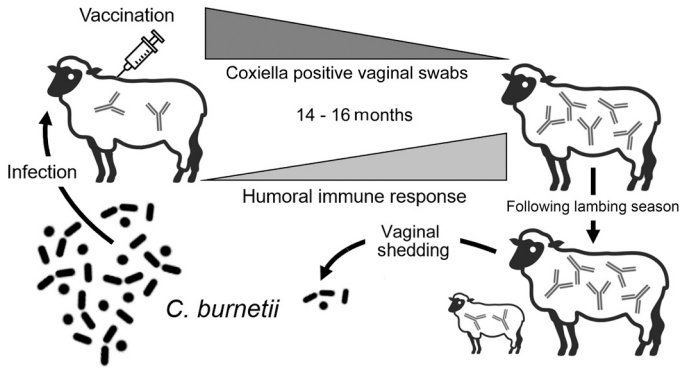


Fig. 9. Graphical overview of the results obtained from our field studies (Bauer et al., 2021a and Bauer et al., 2022b) with vaccinated pre-infected sheep (phase I vaccine). The small sheep in the bottom row represents lambs born by pre-infected and vaccinated ewes. These lambs acquired antibodies by intake of maternal colostrum, and the ewe still shed *C. burnetii* in the following lambing season despite vaccination.

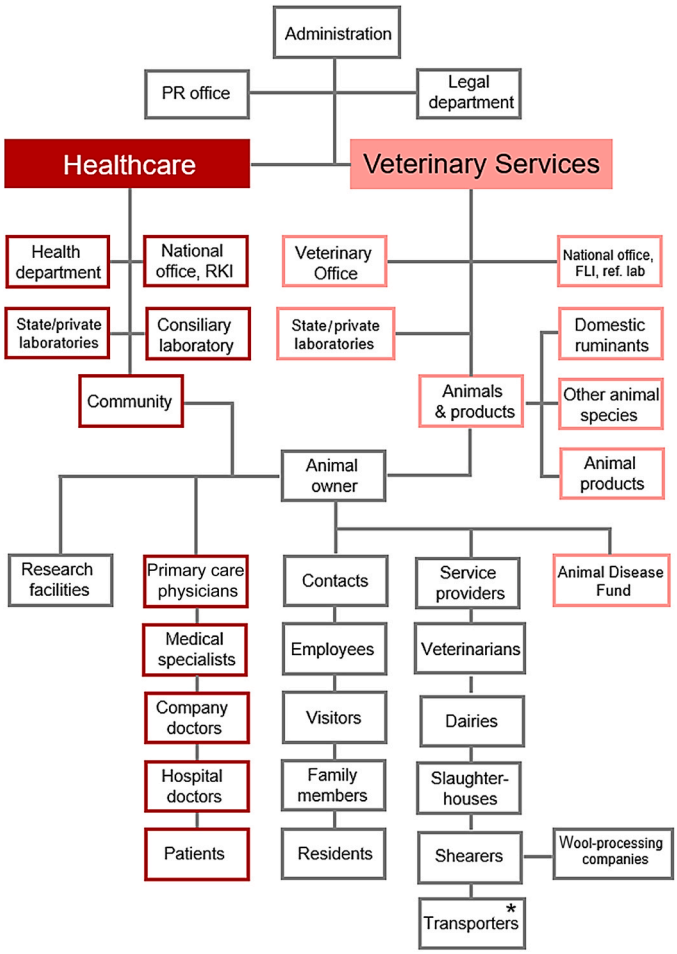


Fig. 10. Q fever management - Groups/institutions to be considered in Germany. The flowchart is based on the Q fever guidelines (ww.q-gaps.de). The dark and light red boxes represent the human and veterinary areas, respectively. The gray color boxes cover both areas. The FLI (Friedrich-Loeffler-Institut) is the German federal research institute for animal health, and the RKI (Robert Koch Institute) is the German federal government agency and research institute responsible for disease control and prevention. PR office: Public Relations office. *Live animal transport, processing plants, animal by-products, transport of manure, etc.

Tracing is essential to detect the *C. burnetii*-infected animal population. However, this can be difficult because the pathogen may spread over long distances as dry and windy weather conditions favor dissemination (Clark and Soares Magalhaes, 2018). Small ruminant flocks are among the most common sources of Q fever epidemics worldwide. Affected flocks, including neighboring ruminant husbandries, must be examined, and control measures, e.g., vaccination, indoor lambing and shearing, must be implemented to prevent further spreading to animals and residents. Livestock owners are often unaware of a *C. burnetii* infection within their flock. Therefore, appropriate information must be shared with farmers and other persons and institutions involved, e.g., family members, farm employees, neighbors, slaughterhouse employees, and veterinarians. Human and veterinary health authorities should coordinate control measures considering the One Health approach. They are responsible for mutual communication between all parties involved, such as the farmer, the physicians, the laboratory, and the general public.

To meet these requirements, we initially generated Q fever guidelines (available at www.q-gaps.de) to control Q fever in Germany. The guideline addresses veterinary and public health authorities and

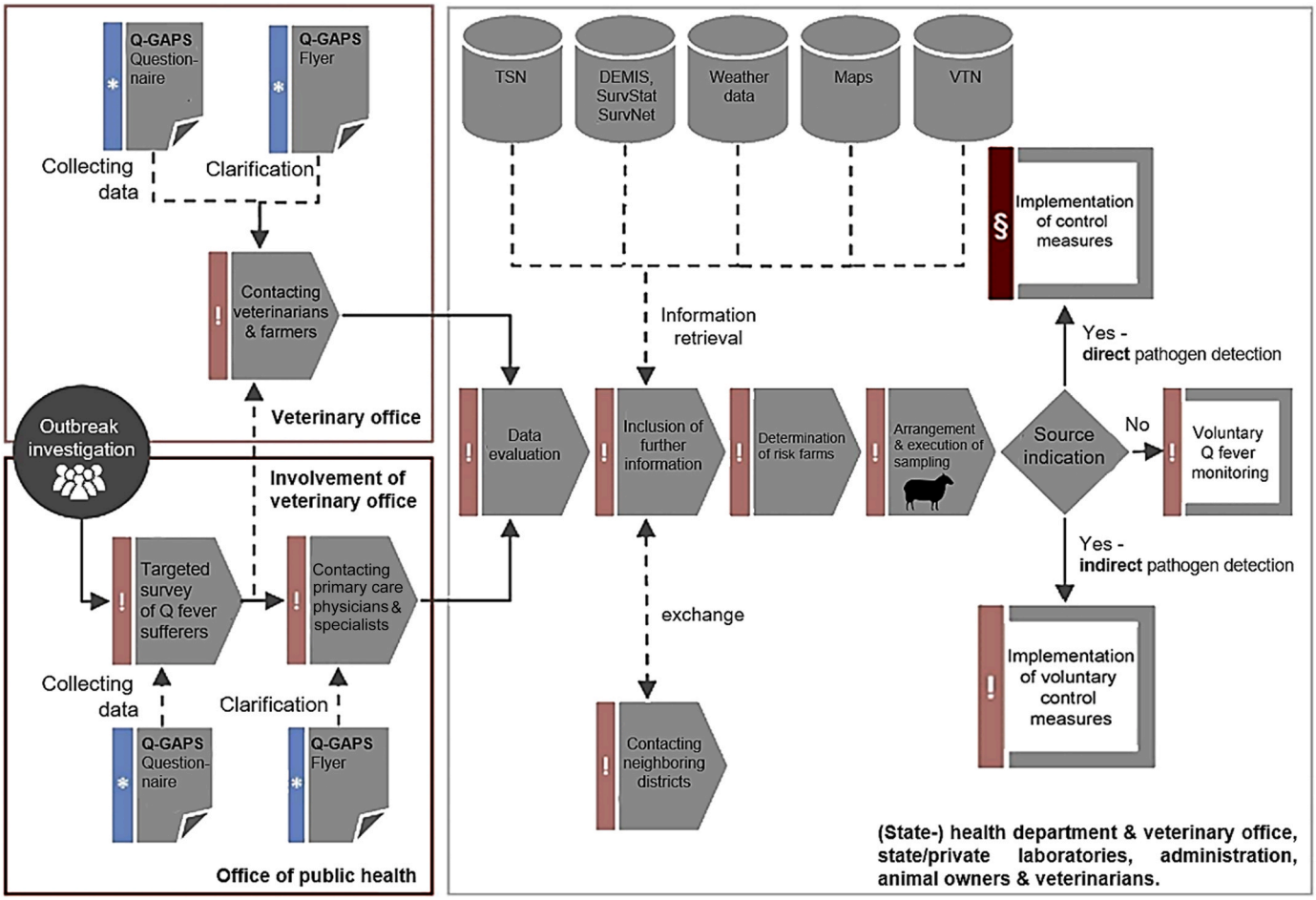


Fig. 11. Process Flow Scenario: Q fever outbreak investigation following reports in the population. The flowchart is based on the Q fever guidelines (www.q-gaps.de). TSN – German animal disease messaging system, VTN – German animal by-products processing plant, DEMIS – German electronic reporting and information system for infectious disease protection. SurvStat - Query of reporting data according to the German Infection Protection Act via the Web. SurvNet – Official software that collects, analyzes, and transmits reporting data for public health departments and state agencies in Germany. The paragraph symbol (§) stands for legally binding. The asterisk (*) stands for offer. The exclamation mark (!) stands for strongly recommended.

includes background information on Q fever in humans and animals. Moreover, procedures for various Q fever scenarios are graphically presented and described, recommendations for action are given, and supplementary material, such as questionnaires for back-tracing, flyers for information, or drafts for a press release, are available at the Web-based information platform (www.q-gaps.de). The aim is to act quickly and effectively to reduce or prevent the further spread of Q fever. For sustainable success in Q fever control, developing diagnostic and action plans considering local conditions is essential (risk groups, type and management of animal holdings, geography, etc.). Therefore, outbreak investigations and subsequent measures must be individually adapted to the particular situation on site.

Education is a critical instrument in disease prevention. According to the One Health approach, the authors conducted a public health seminar focusing on Q fever to inform all veterinary and public health sector members, e.g., human and veterinary health professionals from government agencies, laboratories, and livestock practices (Winter et al., 2020). The participants emphasized the need for practice-oriented training about zoonotic diseases, e-learning tools, and easy access to information. Moreover, it was suggested that networking among human and veterinary medicine students should be established at the university level to promote the One Health approach as early as possible. The need for a joint zoonosis reporting system was emphasized to support interdisciplinary work between human and veterinary authorities. Such a system can provide direct access to current reporting data on humans and animals. This will ease the exchange of disease notifications from the animal and human populations, which is mandatory according to German regulations. In addition, communication between human and veterinary practitioners, animal owners, other risk groups, and the media was repeatedly emphasized. Early education of all stakeholders was seen as a critical function in preventing new infections and avoiding hysteria. Consequently, we have to expand the concept of "One Health" to "One Health Communication" to enhance health care and to find standard solutions to prevent zoonotic diseases (Cipolla et al., 2015).

11. Revision of Q fever monitoring in Germany – lessons we have learned

In Germany, monitoring of Q fever cases occurs passively for humans and animals, respectively (Bauer et al., 2020b), as detailed in the German Infection Protection Act (IfSG) - in its current version (https://www.gesetze-im-internet.de/ifsg/); the German Animal Health Law (TierGesG) - in its current version (http://www.gesetze-im-internet.de/tiergesg/) and the Regulation on Reportable Animal Diseases (TKrMeldpflV) - in its current version (www.gesetze-im-internet.de/tkrmeldpflv_1983/BJNR010950983.html). Hence, the current aim of monitoring is to collect information about *C. burnetii* infections in humans and animals. However, there is no standardized systematic nationwide approach to determine prevalence. Within the past 20 years, the number of reported cases in the human population fluctuated around

a median of 196 (mean: 211) and has been comparably low since 2019 (Robert Koch-Institut: SurvStat@RKI 2.0, https://survstat.rki.de, date: 2023/01/20). Notably, the total number of notified cases per year is highly dependent on more extensive outbreaks, which probably caused a higher awareness among all responsible parties during the respective time period and within the affected region. Consequently, it has to be anticipated that many cases remain undetected and underreported (Doherr and Audige, 2001; Hilbert et al., 2012; Winter et al., 2021). These circumstances make it difficult to estimate prevalence and incidence reliably and to conduct epidemiological investigations to trace back and control the source of the infectious agent. Due to this low reporting rate and the mostly non-specific and infrequent severe health events in the human population, the impression could be mistakenly created that monitoring or surveillance measures are unnecessary.

As a consequence of our epidemiological studies and interdisciplinary collaboration, we propose the following revision of Q fever monitoring in Germany:

First, epidemiological studies to determine the prevalence in the German ruminant population, especially in sheep and goats, and in the human population, as well as reflections on the (aerogenic) transmission route are essential. First attempts to assess the prevalence of Q fever in small ruminants showed, for example, an increasing prevalence from northern to southern federal states within Germany (Bauer et al., 2020b; Wolf et al., 2020a). We recommend constantly assessing the current Q fever status in all ruminant populations (cattle, sheep, goats; prevalence estimation) to obtain the most recent numbers of *C. burnetii*-positive livestock. This may be accomplished by an epidemiological field study (with a cross-sectional approach) that could be performed for several consecutive years.

Second, if one decides to implement a continuous monitoring and surveillance system (MOSS), it should be directed to prevent (i) Q fever outbreaks in the human population and (ii) economic losses from Q fever. Current passive monitoring cannot ensure this. In many cases, only (larger) outbreaks among humans may be recognized and traced back to sources like animal husbandries (Fischer et al., 2016; Gilsdorf et al., 2008; Porten et al., 2006). Consequently, control measures to prevent the further spreading of the pathogen are implemented too late or not at all. However, up to 15% of patients can develop chronic fatigue, up to 1% develop serious health problems (chronic Q fever) with a high mortality rate, and pregnant women and their unborn babies can also be affected (Angelakis and Raoult, 2010; Morroy et al., 2016; Tissot-Dupont, 2007). Hence, a MOSS should support active control measures in the population concerned. Thus, we need an early warning system for animals that are be suspected of playing a vital role in triggering human infections (through inhalation), namely prenatal female small ruminants (Winter et al., 2021).

Moreover, people with an increased occupational risk of infection (e.g. farmers, agricultural and slaughterhouse workers) or with lung and/or heart disease have not been able to be protected by a vaccine, yet. Although a protective vaccine from CSL (Q-VAX®) exist, it is only available and licensed in Australia (Sam et al., 2023). Therefore, regular testing for the presence of *C. burnetii* antibodies in the above-mentioned groups of persons could alternatively reduce the risk of undetected cases and thus possible complicated courses of infection. This approach also makes sense from the point of view that many physicians are unaware of Q fever as a differential diagnosis for respiratory diseases and are also insufficiently informed about the corresponding risk factors (Winter and Campe, 2022).

To complete the claim of the One Health approach, the environmental impact on pathogen dissemination needs further elucidation, as weather conditions play a crucial role in distributing *C. burnetii* to humans and animals (Bauer et al., 2022a; Tissot-Dupont et al., 2004). For instance, advanced atmospheric dispersion models can be a valuable tool to assess the risk from airborne pathogens (Van Leuken et al., 2016).

Subsequently, with this scientific knowledge and MOSS in place, it would be possible to conduct a qualitative risk assessment for the main risk pathway, namely the inhalation of an infectious agent through dust particles and aerosols originating from small ruminants.

To implement all of these requirements, it is necessary to secure funding. In this context, it has to be considered that Q fever is a human and animal disease. However, the impact of *C. burnetii* on sheep and cattle health is minor and still under debate (Agerholm, 2013; Bauer et al., 2022a). Therefore, it would be unreasonable to place sole responsibility of disease prevention on the livestock sector. In contrast, *C. burnetii* can cause severe human illness, as observed during the Dutch Q fever epidemic and small-scale outbreaks throughout Germany (Ankert et al., 2022; Fischer et al., 2016; van Asseldonk et al., 2013). Finally, the public health and agricultural sectors should share the costs for implementing an active MOSS, vaccination programs for ruminants, and further advanced investigations within the animal and human populations.

All of these ideas will remain wishful thinking if they lack the political will for financial and legal support. With passive monitoring in the human and animal population, and consequent underreporting of cases, we will never determine the true impact of Q fever infections on the human population (as in disability-adjusted life years (DALYs) and quality-adjusted life years (QALYs), and economic damage). We cannot tackle and control this zoonotic disease without knowing important critical data from animal and human populations.

12. Conclusions

The importance of combating zoonoses is internationally recognized and has been highlighted by the World Health Organization (WHO) through the One Health approach. Engaging experts from various fields and fostering collaboration among different disciplines is crucial to fully understand all zoonotic aspects. A consortium of specialists in human and veterinary medicine, immunology, infection biology, genomics, and epidemiology is required to address critical unanswered questions and comprehend the zoonotic infection's complexity. However, successful containment of a zoonosis also relies on effective communication of research findings. Implementing the acquired information and transferring the newly gained insights to the practical world is essential, albeit presenting significant challenges. This review presents and discusses findings from the German Q-GAPS network, which focused on diverse research fields, as described earlier. To disseminate knowledge and raise awareness of Q fever in Germany, we have established a central knowledge and information platform accessible to everyone, including research groups, affected individuals, the interested public, and stakeholders involved in prevention and control efforts. Recognizing the pivotal role of public health services in improving health and preventing diseases, we have provided a Q fever guideline (available at www.q-gaps.de) to assist public health authorities in the prevention and/or control of Q fever in Germany. All information provided can be regularly updated and thus contribute comprehensively to the enhanced detection, prevention, and control of this zoonosis.

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CRediT authorship contribution statement

BUB, MRK, DF, and AL conceived, structured, and finalized the review article and provided critical inputs to the manuscript. MRK made graphical representations, adaptations, and schemes of all figures shown. All authors participated in preparing and revising the manuscript text and read and approved the submitted published version of the manuscript.

Conflict of Interest

The authors are members of the Zoonosis Consortium Q-GAPS (www.q-gaps.de) and declare no conflict of interest. The funders had no role in the study's design; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Data Availability

No data was used for the research described in the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ijmm.2023.151590.

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