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Review

Chlamydiaceae in cattle: Commensals, trigger organisms, or pathogens?

Petra Reinhold [a,*], Konrad Sachse [a,b], Bernhard Kaltenboeck [c]

[a] Institute of Molecular Pathogenesis at The Friedrich-Loeffler-Institut (Federal Research Institute for Animal Health), Naumburger Str. 96a, 07743 Jena, Germany
[b] OIE Reference Laboratory for Chlamydiosis at The Friedrich-Loeffler-Institut (Federal Research Institute for Animal Health), Naumburger Str. 96a, 07743 Jena, Germany
[c] Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL 36849-5519, USA

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ABSTRACT

Epidemiological data indicate that infection of cattle with chlamydiae such as *Chlamydophila* (*C.*) *pecorum*, *C. abortus*, *C. psittaci* and *Chlamydia suis*, is ubiquitous with mixed infections occurring frequently. The apparent lack of association between infection and clinical disease has resulted in debate as to the pathogenic significance of these organisms, and their tendency to sub-clinical and/or persistent infection presents a challenge to the study of their potential effects. However, recent evidence indicates that chlamydial infections have a substantial and quantifiable impact on livestock productivity with chronic, recurrent infections associated with pulmonary disease in calves and with infertility and sub-clinical mastitis in dairy cows. Data also suggest these infections manifest clinically when they coincide with a number of epidemiological risk factors. Future research should: (1) use relevant animal models to clarify the pathogenesis of bovine chlamydioses; (2) quantify the impact of chlamydial infection at a herd level and identify strategies for its control, including sub-unit vaccine development; and (3) evaluate the zoonotic risk of bovine chlamydial infections which will require the development of species-specific serodiagnostics.

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Introduction

Chlamydiae are obligate intra-cellular bacteria that cause a wide range of diseases in animals and humans (Storz and Kaltenboeck, 1993). Chlamydial infections in cattle cause abortion (Borel et al., 2006), polyarthritis (Storz et al., 1966; Twomey et al., 2003), encephalomyelitis (McNutt and Waller, 1940), keratoconjunctivitis (Otter et al., 2003), pneumonia (Wilson and Thomson, 1968), enteritis (Doughri et al., 1974), hepatitis (Reggiardo et al., 1989), vaginitis and endometritis (Wittenbrink et al., 1993a,b), infertility (DeGraves et al., 2004; Wehrend et al., 2005) and chronic mastitis (Biesenkamp-Uhe et al., 2007).

With the introduction of PCR-based detection methods came the recognition that chlamydial infection could also be sub-clinical (Shewen, 1980; Reggiardo et al., 1989; Bodetti et al., 2003; DeGraves et al., 2003a; Borel et al., 2004; Jee et al., 2004; Reinhold et al., 2008; Kemmerling et al., 2009) which resulted in the ongoing debate as to whether these organisms were genuine pathogens or commensals. Until recently, the potential of sub-clinical chlamydial infection on animal health has not been addressed, although field observations of dairy herds where infection is endemic, have identified sub-optimal production in the absence of other significant pathogens. These observations have suggested a role for chlamydiae in multi-factorial diseases involving interactions between low-virulence pathogens, nutritional deficiencies, poor management and hygiene, and host genetics.

Given the ubiquitous nature of sub-clinical chlamydial infection in cattle, it is important that the potential pathogenic effects of these organisms on animal health and production are investigated. This review summarizes our current knowledge of bovine chlamydial infection, with particular emphasis on studies reported in the past decade, and assesses the epidemiological peculiarities and economic impacts of infection.

Chlamydial species found in cattle

Taxonomy

The order *Chlamydiales* has been re-classified several times over the last decades. The latest revision led to the separation of the family *Chlamydiaceae* into the genera *Chlamydophila* and *Chlamydia*, with a total of nine species, namely, *Chlamydophila* (*C.*) *abortus*, *C. pecorum*, *C. psittaci*, *C. pneumoniae*, *C. felis*, *C. caviae*, *Chlamydia trachomatis*, *Chlamydia suis* and *Chlamydia muridarum*. Three new families were added, *Parachlamydiaceae*, *Waddliaceae*, and *Simkaniaceae* (Everett et al., 1999). However, this division into two *Chlamydiaceae* genera has been widely criticized, and efforts are underway to combine all nine *Chlamydiaceae* spp. into a single *Chlamydia* genus (Stephens et al., 2009).

* Corresponding author. Tel.: +49 3641 804 2269; fax: +49 3641 804 2228.
E-mail address: petra.reinhold@fli.bund.de (P. Reinhold).

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The use of highly specific and sensitive diagnostic methods, such as standard and real-time (RT) PCR and DNA microarray technology, has identified a spectrum of chlamydial agents occurring in cattle including: *C. pecorum*; *C. abortus*; *C. psittaci*; and *Chlamydia suis* (Table 1). Of these species, the presence of *C. pecorum*, *C. abortus*, and *C. psittaci* has been confirmed in multiple, independent studies, while the detection of *Chlamydia suis* appears to be extremely rare in cattle. However, it must be noted that since highly specific and sensitive detection methods were only introduced in the 1990s, it is not always possible to clearly ascertain the full identity of chlamydial species reported in previous decades.

Basic biology and genomics

Chlamydiae replicate as approximately 1 μm diameter non-infectious reticulate bodies (RB) within host cytoplasmic vacuoles (termed inclusions) and survive in the environment as infectious, but non-replicating spore-like elementary bodies (EB) of approximately 0.3 μm diameter. EBs contain electron-dense structures comprising tightly coiled genomic DNA covered by histone-like proteins. Spikes in the organism's membrane are thought to be the tubular apparatus of a type III secretion system (Matsumoto, 1982). RBs are metabolically active, with a cytoplasm rich in ribosomes. As RBs begin to differentiate into EBs, sites of re-condensation of nucleic acid appear in their cytoplasm. In the maturing inclusion, chlamydial particles appear to be packed tightly within the inclusion membrane.

Development of chlamydiae is highly dependent on nutrient supply and on the metabolic status of the host cell (Harper et al., 2000). Varying infectivity and replication efficiencies determine consistently, but not absolutely, the virulence of the chlamydial strain. *Chlamydiaceae* produce minimally or non-toxic products and regulate their growth based on the availability of host cytoplasmic nutrients. These features result in chlamydiae being minimally cytotoxic, with disease occurring when an inefficient immune response results in persistent infection in the host (Wang et al., 2009).

The chlamydial genome is one of the smallest of all bacteria, some 1.1–1.25 Mb in size. Genome sequence comparisons have been used to identify functional differences between chlamydiae based on 'niche-specific' genes, as genetic manipulation systems are not available for these organisms (Read et al., 2000). Only a single putative toxin gene *tox* has been found in chlamydiae that is very similar to the cytotoxic enterobacterial *Efa1* gene found in enterohemorrhagic *E. coli* or to clostridial large cytotoxins (LCT).

The cytotoxicity of these gene products is related to their effect on the actin cytoskeleton (Belland et al., 2001). *C. abortus* (Thomson et al., 2005) does not contain the 'niche-specific' *tox* orthologs, or tryptophan synthesis or nucleotide-salvaging genes that characterize other species such as *C. caviae* (Read et al., 2003) and *C. felis* (Azuma et al., 2006). This may explain why *C. abortus* can: (1) easily be propagated to higher yields in cultured cells than other chlamydiae; (2) be found in high numbers in infected host tissue; (3) colonize macrophages effectively; and (4) rapidly spread systemically after mucosal inoculation (Huang et al., 2002; Bouakane et al., 2003; Kerr et al., 2005; Li et al., 2005).

Genome sequencing of the remaining bovine-associated chlamydial species – *C. pecorum*, *C. psittaci*, and *Chlamydia suis* – is currently underway. Initial data indicate that *C. pecorum* carries multiple copies of the *tox* gene homolog, consistent with its high cytotoxicity (Myers et al., 2005).

Methods of diagnosis

Although a presumptive diagnosis of chlamydial infection can be made based on clinical history, confirmation requires robust and sensitive laboratory-based assays. A comprehensive review of novel developments in the laboratory diagnosis of chlamydial infections has been published recently (Sachse et al., 2009).

Serology

Serological detection is generally only suitable for prevalence surveys, rather than for the retrospective diagnosis of infection. Most chlamydial infections do not elicit sufficiently high antibody responses to facilitate unambiguous diagnosis of recent infection, the exception being the diagnosis of chlamydial abortion in ruminants (Perez-Martinez et al., 1986; Griffiths et al., 1996).

The standard method for detecting antibodies to *Chlamydiaceae* spp. in animals is still the complement fixation test (CFT) using

Table 1
Chlamydiaceae spp. found in cattle and their clinical significance.

Species	Main host	Other animal hosts	Clinical significance in cattle
Chlamydophila pecorum	Cattle (Jee et al., 2004; Jaeger et al., 2007; Kauffold et al., 2007; Reinhold et al., 2008; Kemmerling et al., 2009) Sheep Goats	Pigs Koalas	Polyarthritis, enteritis, pneumonia, keratoconjunctivitis, urogenital infections, encephalomyelitis and sub-clinical, chronic pulmonary inflammation and growth retardation
Chlamydophila abortus	Sheep Goats	Cattle (Jee et al., 2004; Twomey et al., 2006; Jaeger et al., 2007; Kauffold et al., 2007; Reinhold et al., 2008; Pantchev et al., 2009a) Swine Horses Birds	Reproductive disorders, mastitis and sub-clinical, chronic pulmonary inflammation and growth retardation
Chlamydophila psittaci	Psittacine birds Poultry Wild birds	Cattle (Borel et al., 2006; Twomey et al., 2006; Kauffold et al., 2007; Pantchev et al., 2009a; Kemmerling et al., 2009) Swine Horses Dogs Laboratory rats	Reproductive disorders and airway inflammation
Chlamydia suis	Swine	Cattle (rarely) (Teankum et al., 2007; Pantchev et al., 2009a) Goats Horses Sheep	No reports

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crude or partially purified preparations of *Chlamydiaceae*-specific lipopolysaccharide (LPS). The CFT depends on the binding of anti-*Chlamydiaceae* host antibodies to guinea pig complement, and the assay has a highly variable sensitivity depending on the host species and antibody isotype (Perez-Martinez et al., 1986). Due to very poor binding of guinea pig complement to bovine immunoglobulins, this test results in a very high proportion of false-negative diagnoses and is not suitable for use in cattle (Kaltenboeck et al., 1997a).

Numerous ELISA methods have also been used, but are all highly cross-reactive between chlamydial species, so that no commercially available assay is able to differentiate antibodies at the species level (Sachse et al., 2003, 2009). Another confounding factor in chlamydial serodiagnosis is the fact that it is difficult to find chlamydia antibody-negative bovine serum and the serum of gnotobiotic calves should be used as a negative control in serological assays (Kaltenboeck et al., 1997a).

Organism detection

Initially, propagation of the organisms in the yolk sac of chicken embryos and demonstration of the characteristic intracellular inclusions was the standard method of confirming chlamydial infections (Storz, 1971). This technique was superseded by the direct isolation of chlamydiae in confluent cover-slip cell culture monolayers (Croy et al., 1975), a method which is now the preferred isolation procedure. Buffalo green monkey kidney (BGMK) cells support chlamydial replication effectively, particularly when cultivated in Iscove's modified Dulbecco's medium (Li et al., 2005).

A key advance in the laboratory diagnosis of chlamydial infections has been the development of tests that do not require the agent to be viable. The detection of chlamydial EBs and inclusions in clinical material relies on either fluorochrome-labelled chlamydia-specific monoclonal antibodies or on the capture and detection of antigen by enzyme immunoassay. The 'PATHFINDER' enzyme immunoassay (EIA, Sanofi/Kallestad), the IMAGEN chlamydia kit (Dako), the IDEIA (Boots-Celltech) and others use *Chlamydiaceae* family-specific monoclonal antibodies against chlamydial LPS, and have therefore a wide detection spectrum suited for use in diagnosis. While LPS detection in tissue sections or monolayers by fluorescent staining is highly specific and accurate, solution-based capture assays using antibodies against LPS result in a large number of false positives and are not suitable for diagnosis.

Whereas direct antigen detection remains in widespread use, such methods have been superseded by assays detecting chlamydial nucleic acid by PCR, and more recently, by PCR combined with differentiation of DNA by microarray. Nucleic acid amplification is now central to the diagnosis of chlamydial infections (Kaltenboeck et al., 1991, 1997b; Sachse and Hotzel, 2003; Sachse et al., 2009), amplifying either target DNA or RNA, and such tests are at least 15-fold more sensitive than culture or antigen detection (Ostergaard, 1999; Huang et al., 2001; Sachse et al., 2003). RT-PCR techniques not only allow determination of target DNA copy numbers, but are also better suited for routine high-throughput testing than is conventional PCR. The RT method reduces the risk of false positives through 'carry-over' contamination and results in an increased sensitivity of detection.

Validated and widely used RT-PCR (LightCycler and TaqMan) assays for the family *Chlamydiaceae* (DeGraves et al., 2003b; Ehricht et al., 2006) and for all chlamydiae relevant in cattle (DeGraves et al., 2003a; Pantchev et al., 2009a,b) have been reported. These methods are undergoing continuous improvement through use of enhanced nucleic acid preservation and extraction techniques (DeGraves et al., 2003a,b). Finally, the use of combined PCR and low-density single-tube microarrays has resulted in a rapid, accurate, and flexible typing format for the detection and multiplex typing of chlamydial amplicons (Sachse et al., 2005; Borel et al., 2008).

Chlamydial infection of cattle

Prevalence

Data published in the last two decades suggest a high seroprevalence of chlamydial infection in herds worldwide, with seropositivity at a herd level ranging from 45% to 100% (Table 2). There appears to be no difference in seroprevalence between randomly selected farms and those pre-selected on the basis of specific health problems. The generally high seroprevalence has led to the assumption that infections with *Chlamydophila* spp. are ubiquitous in cattle (Kaltenboeck et al., 2005).

Given this high prevalence, there is a basic problem in interpreting the significance of serological findings. Individual titers are difficult to interpret and, even if paired serum samples are collected, sharp increases after clinical disease are rarely observed. Presumably, cellular rather than humoral host immunity is the predominant host response (Stephens, 2003), a hypothesis supported by the lack of correlation between PCR and antibody data. A recent survey of stud bulls demonstrated a seropositivity of 51% compared to PCR detection rates in semen, preputial washes and feces of between 9% and 18%. Furthermore, serology failed to identify bulls shedding chlamydiae in their semen (Kauffold et al., 2007). In contrast, a study monitoring the course of natural chlamydial infection in calves found that only approximately 60% of animals became serologically positive up to 7 months of age, whereas all were 'shedders' of the agent (Reinhold et al., 2008). This discrepancy appears to be a consequence of a continuous accumulation of chlamydial antibodies in young animals, while ongoing colonization by the agent creates recurrent exposure of the host.

An alternative, simpler explanation for the seronegativity of the animals in some studies may be the technical limitations of the current ELISA methods for antibody detection, where there may be too high a cut-off point due to the presence of antibody in the negative control sera. Interestingly, in another study involving both young and adult cattle, antibody titers increased with age while the proportion of PCR-positive samples decreased (Vogel et al., 2006). Table 2 details the high prevalence rates for *Chlamydiaceae* in herds based on PCR. However, the picture regarding the distribution of infection with particular chlamydial spp. remains sketchy.

In Germany, a recent study identified *C. psittaci* as the most prevalent representative of the *Chlamydiaceae* (56% of positive vaginal swabs), followed by *C. abortus* (37%) and *C. pecorum* (8%) (Kemmerling et al., 2009). In a study of stud bulls, these three species were found in the semen and preputial washings, with *C. psittaci* found most frequently, while *C. pecorum* was found most frequently in fecal samples (Kauffold et al., 2007). A study of 72 dairy herds in Taiwan found 100% of the herds and approximately 40% of all cows positive for *C. abortus* in vaginal swabs and samples from abortions, although swabs from animals that had not aborted were also found to be positive (Wang et al., 2001). Although few cross-sectional studies have been carried out in cattle in the US, a high prevalence of both *C. pecorum* and *C. abortus* has been found, while *C. psittaci* has not been detected (DeGraves et al., 2003a; Jee et al., 2004). In an Austrian study, cervical or vaginal swabs from cows contained *C. pecorum* (9% of samples), while *C. abortus* was not found and the study did not look for *C. psittaci* (Petit et al., 2008). Recent surveys in Switzerland revealed *C. abortus* and *C. psittaci* in aborted material from cattle, but *Parachlamydia* spp. were more commonly associated with cases of abortion (Borel et al., 2006; Ruhl et al., 2009).

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Table 2
The prevalence of *Chlamydiaceae* spp. found in cattle – results of national surveys.

Country	Positive on serology		Positive on antigen detection			Reference
	Herds	Individual animals	Herds	Individual animals	Detection method	
Studies on randomly selected herds						
Austria	45% (*n* = 196)			8.9% (*n* = 644, cows)	PCR and restriction length polymorphism	Petit et al. (2008)
Germany			61% (*n* = 100)	13.5% (*n* = 1074, cows)	RT[c]-PCR	Kemmerling et al. (2009)
Germany	90% (*n* = 20)					Abd El-Rahim (2002)
Germany			60.8% (*n* = 97)	13.0% (*n* = 975, calves)	RT-PCR	Hollberg et al. (2005)
Germany		100% (*n* = 174, cows)		49% (*n* = 174, cows)	Nested PCR	Biesenkamp-Uhe et al. (2007)
Switzerland		47.1% (*n* = 104, bulls)		*Chlamydiaceae* – 6.6% (*n* = 304 semen samples) *Chlamydia*-like organisms – 4.9%	Conventional PCR	Teankum et al. (2007)
Studies on pre-selected herds[a]						
Germany		42% (*n* = 225, calves, heifers, cows)	100% (*n* = 15)	13–100% of animals tested/herd	RT-PCR	Vogel et al. (2006)
Germany	91% (*n* = 89)					Sting (1997)
Germany	54% (*n* = 59)					Wittenbrink et al. (1988)
Germany		50.8% (*n* = 122, bulls)		9.2% in semen (*n* = 120) 10.7% in preputial washings (*n* = 121) 18.0% in feces (*n* = 122)	Nested PCR (followed by DNA sequencing)	Kauffold et al. (2007)
Germany		61.5% of PCR-positive calves (*n* = 13)		100% (*n* = 13, calves [mainly dually-infected with *C. abortus* and *C. pecorum*])	RT-PCR (selected samples by DNA microarray assay)	Reinhold et al. (2008)
Italy		45% (*n* = 671, cows [post-abortion]) 24% (*n* = 600, control cows)				Cavirani et al. (2001)
Sweden	81% (*n* = 70)	31% (*n* = 179, cows [post-abortion]) 28% (*n* = 239, control cows)		4.7% (*n* = 43, cows [*C. pecorum*])	Nested PCR (followed by DNA sequencing)	Godin et al. (2008)
Switzerland				*Chlamydiaceae* – 5.1% of all abortions (*n* = 235) *Chlamydia*-like organisms – 18.3%	Conventional PCR	Borel et al. (2006) Ruhl et al. (2009)
Taiwan	100% (*n* = 72)	51.3% (*n* = 672 [clinically normal cows]) 71.4% (63 cows post-abortion)		*C. abortus* – 45.2% (*n* = 31 [clinically normal cows]) 34.9% (63 cows post-abortion)	Nested PCR and restriction length polymorphism	Wang et al. (2001)
USA (Alabama)	100% (*n* = 18)	100% (*n* = 40 [cows from herds where abortion had occurred])				Kaltenboeck et al. (1997a)
USA (Alabama)	100% (*n* = 1)	100% (*n* = 51)	100% (*n* = 1)	53% (*n* = 51, heifers [24% *C. abortus*, 39% *C. pecorum*])[b]	RT-PCR fluorescent probes	DeGraves et al. (2003a)
USA (Alabama)	100% (*n* = 1)	100% (*n* = 80)	100% (*n* = 1)	61% (*n* = 40, calves [12% *C. abortus*, 59% *C. pecorum*])[b] 20% (*n* = 40, cows [15% *C. abortus*, 8% *C. pecorum*])[b]	RT-PCR fluorescent probes	Jee et al. (2004)

[a] Herds with increased rates of infertility or abortion and/or respiratory problems in calves.
[b] Percentages add up to >100% because animals infected with both *C. abortus* and *C. pecorum*.
[c] RT, real time.

The carrier status of individual animals may vary over time because of the intermittent shedding of organisms (Jee et al., 2004; Reinhold et al., 2008), and as a result, false negative PCR findings occur. This is why the percentage of positive herds may be higher than the positivity rate among the individual animals (Table 2).

Whether chlamydia infecting cattle differ in their organ tropisms remains unclear. According to the data presented in Table 1, *C. abortus* is predominantly associated with the genital tract and mammary disease, *C. pecorum* with pulmonary, joint, intestinal, ocular and central nervous system disease (Kaltenboeck et al., 2009), and *C. psittaci* has been identified in samples from the respiratory and genital tracts (Kauffold et al., 2007; Kemmerling et al., 2009). In calves, naturally infected in their herd of origin, *C. pecorum* was mainly detected in the gastro-intestinal tract, whereas *C. abortus* was found in samples from the respiratory tract and conjunctiva (Reinhold et al., 2008).

Mixed infections within one host and within one organ are common (Borel et al. 2008). Pantchev et al. (2009a, b) recently found the following combinations of organisms in bovine samples: *C. psittaci* and *C. abortus*; *C. abortus* and *C. pecorum*; *C. abortus* and *Chlamydia suis*; and *C. pecorum* and *Chlamydia suis*. From a quantitative perspective, there were two categories of samples where either both chlamydial species were present in approximately equal concentrations, or where one species dominated at a ratio of approximately 100:1. These observations raise interesting questions on the interactions and synergies between the co-infecting agents and their combined impact on the course of the infection.

Routes of transmission

Fecal shedding of chlamydiae by carrier animals is thought to be the most important mode of transmission. In addition, chlamydiae

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may be shed in vaginal, ocular, and nasal discharges, in uterine fluid, placental tissue, urine, and semen (Perez-Martinez and Storz, 1985; Longbottom and Coulter, 2003; Kauffold et al., 2007; Reinhold et al., 2008; Kemmerling et al., 2009). Whether or not organisms are exhaled remains unclear. Infection can be acquired by direct contact between animals or by indirect means such as the fecal-oral route or via inhalation of contaminated air (Longbottom and Coulter, 2003). Venereal transmission from bulls is possible through both natural mating and by artificial insemination (Storz et al., 1976; Amin et al., 1999). In-utero transmission has not been reported.

Although there is little information on the environmental survival of chlamydiae, the organisms are remarkably resistant in cool, dry, and dark conditions. While elevated temperatures reduce survival rates, EBs survive in cool water for up to 17 days and in dry feces, dust, litter or feathers for several months (Krauss and Schiefer, 1983; Perez-Martinez and Storz, 1985). Cryopreservation of samples such as semen, fails to eliminate the pathogen (Storz et al., 1976).

Chlamydial infection of dairy cows

Impact on herd health

Unlike in sheep and birds, where outbreaks of disease affecting many animals can be caused by *C. abortus* or *C. psittaci*, respectively, the clinical significance and course of bovine chlamydial infection is usually less obvious. As a consequence, chlamydial infections in cattle have been widely regarded as insignificant and few systematic field studies of infection have been carried out. This attitude has changed somewhat in the last few years as it has become clear that chlamydial infections in cattle, although largely latent and sub-clinical, do have a measurable impact on herd health. The ability of chlamydiae to chronically infect multiple organs, combined with their ubiquitous distribution, facilitates long-standing, low-level infection of the udder, and of the respiratory, reproductive and intestinal tracts, which may negatively impact on milk production, fertility and growth rate. The tangible effects of these sub-clinical infections can be difficult to detect because there is often no straightforward 'cause-and-effect' relationship. Frequently only multivariate approaches reveal relevant 'infection-disease' correlations.

Vogel et al. (2006) found chlamydiae using PCR on 15 pre-selected dairy farms with a history of infertility, mastitis and/or respiratory disease in Germany, where detection rates were found to vary from 13% to 100% among farms (Table 2), and 42% of animals were seropositive on both samples taken. However, no correlation was found between the presence of the organisms and disease. Although this study did not assume that *Chlamydiaceae* spp. acted as a primary pathogen, a potential role for these organisms as part of multi-factorial disease processes must be considered. For instance, chlamydiae were frequently detected in milk samples from cows with ocular and respiratory problems and herds with poor hygiene and nutritional management typically had increased detection rates of these organisms in conjunctival swabs and milk samples.

A recent, cross-sectional study of 100 randomly-selected dairy farms specifically addressed the question as to whether or not dairy production parameters were affected by chlamydial infection (Kemmerling et al., 2009). Following collection of vaginal swabs at early lactation from 1074 non-pregnant animals (10% of the total number), RT-PCR found 13.5% of the cows positive, the herd prevalence was 61%, and comparison of a large number of performance parameters between positive and negative farms revealed significant differences. Average annual milk production/cow was significantly lower on infected farms (8681 vs. 9197 kg), and the number of lactations/animal was reduced (2.4 vs. 2.9). The presence of chlamydial infection also correlated with elevated rates of abortion, pre-mature calving and peri-natal calf deaths ($P < 0.001$). Significantly, hygiene was generally better on negative farms. While these results indicate chlamydial infections have an economic impact, even when sub-clinical, the study also indicates that appropriate herd management can reduce the risk of such infections.

Impact on fertility

Infection with *Chlamydophila* spp. appears to be widespread in male cattle as organisms have been detected in the semen of bulls with vesiculitis (Storz et al., 1968) as well as from clinically normal animals (Kauffold et al., 2007; Teankum et al., 2007). The species identified were *C. psittaci*, *C. pecorum*, and *C. abortus* (Kauffold et al., 2007) and, interestingly, standard ejaculate parameters did not differ between semen-positive or semen–negative animals (Kauffold et al., 2007). Cows inseminated with infected semen either naturally or through artificial insemination can develop genital chlamydiosis, although infection is more prevalent in herds using natural service (Kemmerling et al., 2009). Furthermore, negative bulls may become infected during siring and then function as a vector for infection. Since chlamydiae affect placental function, abortion or peri-natal deaths are well-described consequences of infection (Cavirani et al., 2001; Wang et al., 2001), and a 6.6-fold greater risk (odds ratio) of abortion has been reported for seropositive cows (Wehrend et al., 2005). A further recent PCR-based study also found that abortion, pre-mature calving and increased peri-natal calf loss were significantly associated with chlamydial infection (Kemmerling et al., 2009).

DeGraves et al. (2004) investigated the effects of controlled re-infection on the fertility of cattle naturally exposed to *C. abortus*. Twenty estrus-synchronized heifers were artificially inseminated and immediately challenged by intra-uterine inoculation. Ten more inseminated heifers were challenged after 2 weeks and these animals had also been indirectly exposed to *C. abortus* by contact with the previously challenged animals. This contact, along with low (below median) pre-challenge immunoglobulin (Ig) M, and inoculum dose were found to be significant, independent risk factors for infertility by logistic regression analysis. In addition, the risk of infertility was 17-fold higher for 'cohort-exposed' heifers and those with low pre-challenge IgM than for unexposed animals and those with high pre-challenge antibodies. The findings of this investigation demonstrated that sub-clinical, non-venereally transmitted infection with *C. abortus* has an influence on fertility (Kaltenboeck et al., 2005), and suggests that infertility caused by re-infection with *C. abortus* depends on the infecting dose, route of infection and the immune response to previous infection.

Impact on milk production

Infection with *C. abortus* and *C. pecorum* have been associated with bovine mastitis, including sub-clinical disease, where chlamydiae have been detected in the conjunctiva and vagina of dairy cows (Biesenkamp-Uhe et al., 2007). This prospective cohort study of a herd of 140 Holstein dairy cows, found that all animals had serum antibodies against chlamydiae, and 49% were positive for *C. abortus* by PCR on conjunctival or vaginal swabs. Sub-clinical disease was confirmed by somatic cell count (SCC) and perturbation of the immune response to *C. abortus* and/or *C. pecorum* was used to assess the effect on disease. All cows had anti-chlamydial serum antibodies and many were PCR positive. Animals received either a 'therapeutic vaccination' (two doses of inactivated *C. abortus*/*C. pecorum* EBs) or a mock vaccine on days 0 and 35 of the investiga-

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tion. Therapeutic vaccination increased the antibody response and significantly reduced the milk SCC (Fig. 1), but did not reduce the shedding of chlamydiae. Vaccination also resulted in improved body condition score of the cows after 10 weeks. The 'protective effect' of such vaccination was maximal at 10 weeks, and persisted for an additional 4 weeks. In contrast to co-relational studies, the interventional study by Biesenkamp-Uhe et al. (2007) demonstrated, for the first time, the causal involvement of ubiquitous chlamydial infections in sub-clinical mastitis.

Chlamydial infection of calves

Routes of infection

In chlamydia-positive dairy herds, carrier cows are the most likely source of infection for calves. Calves born free of infection acquire both *C. pecorum* and *C. abortus* within 2 weeks (Jee et al., 2004). Chlamydial DNA can be found in clinically normal animals in nasal, rectal, and conjunctival swabs over many months with detection following irregular and intermittent patterns (Reinhold et al., 2008). With increased calf population densities, both the prevalence and intensity of infection rise exponentially (Jee et al., 2004). Antibody responses lack uniformity of pattern or are not detectable (Reinhold et al., 2008). Although such infections do not typically result in disease, it is not inconceivable that, when recurrent and chronic, they ultimately impact on calf health and development.

Effects on calf health

In a study aimed at assessing the long-term effects of natural infection with *Chlamydophila* spp., calves were grouped according to their 'carrier' status and continuously monitored between 2 and 7 months of age (Reinhold et al., 2008). Despite the absence of clinical signs, infection with *C. abortus* and/or *C. pecorum* resulted in significantly higher (although sub-febrile) body temperature and significantly lower body-weight, when compared to chlamydia-free controls. Furthermore, chlamydial infection was associated with reduced serum iron concentrations and lower hematocrit. Overall, infected calves were leukopenic, mainly due to reduced absolute numbers of lymphocytes (Fig. 2). At necropsy, pharyngeal tonsil weight was significantly higher in infected calves relative to controls. These impacts of chlamydial infection were seen despite the fact that all animals were housed under optimal management conditions.

Chlamydial infections in cattle have been largely associated with 'pneumonia' characterized by fever, depression, nasal secretions, coughing and dyspnea (Storz and Kaltenboeck, 1993). However, infection has also been associated with keratoconjunctivitis (Otter et al., 2003), and acute respiratory disease (Twomey et al., 2006). Hollberg et al. (2005) investigated the potential role of chlamydial infection in calf respiratory disease in 975 animals between 1 and 8 weeks of age in 97 dairy herds. Although chlamydiae were found in 61% of the herds, the prevalence of these organisms in calves with respiratory disease was lower than in clinically normal animals. Only *Mannheimia haemolytica* was found more frequently in calves with disease than in controls. However, the finding that chlamydiae occurred in the respiratory tract of 13% of this population suggests a significant potential reservoir of infection.

Chronic infection of the calf respiratory tract with *C. abortus* and/or *C. pecorum* is associated with chronic inflammation that does not manifest clinically (Jaeger et al., 2007). However, lung function tests reveal significantly increased respiratory rates and elevated peripheral airway resistance, indicating peripheral airway obstruction in infected calves. Such sub-clinical pulmonary dysfunction was detectable at 2 months of age and persisted over at least 6 months. Follicular bronchiolitis and markedly activated bronchus-associated lymphoid tissue (BALT) causing partial obstruction of bronchiolar lumens were observed histologically in the lungs of these calves (Fig. 3). Furthermore, significantly elevated concentrations of total protein and 8-iso-prostane (8-IP), as well as increased matrix metalloprotease (MMP)-2 activity were detected in broncho-alveolar lavage fluid from infected calves suggesting pulmonary inflammation and tissue re-modeling (Fig. 4). Remarkably, the lungs of these calves appeared macroscopically normal despite these findings. Chlamydiae were not detected by immunohistochemistry in these lungs, arguing against their pathogenic significance, although low-level infection, as indicated by intermittent PCR positive nasal, conjunctival, and rectal swabs, remained for over 6 months and lung tissue was also positive by PCR at the termination of the study.

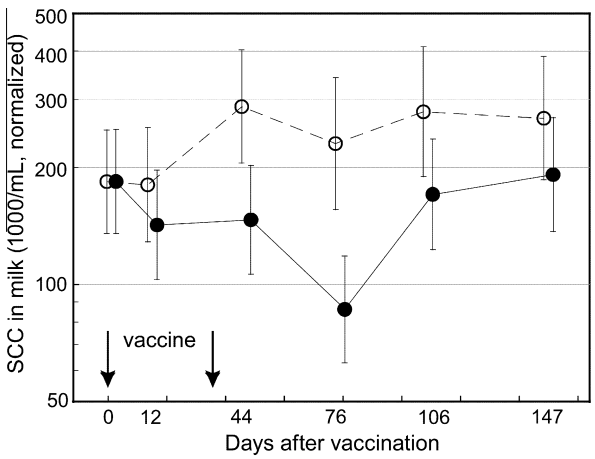

Fig. 1. Graph illustrating the effect of vaccination against chlamydiae on milk somatic cell counts (SCC) (●, chlamydia vaccine; ○, control vaccine). Data are normalized to identical 'day 0 means' of chlamydia- and control-vaccinated animals (means ± 95% CI). Chlamydia-vaccinated cows have significantly lower milk SCC than control-vaccinates for all combined time-points after day 0 ($P = 0.007$) (Biesenkamp-Uhe et al., 2007).

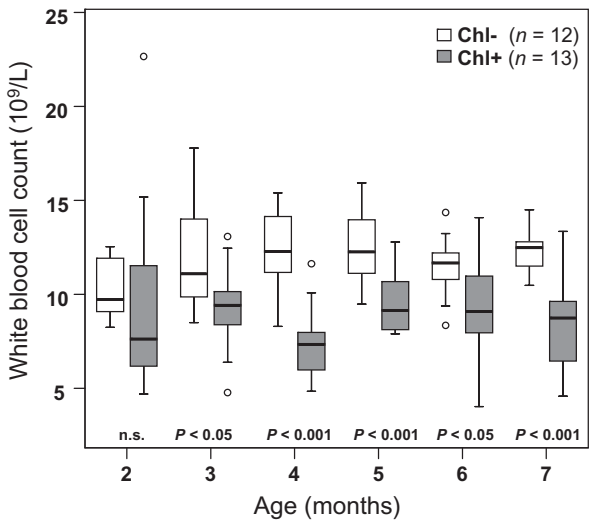

Fig. 2. A box-and-whisker plot illustrating the white blood cell counts in calves aged from two to six months old, with (Chl +; $n = 13$) or without (Chl −; $n = 12$) chlamydial infection. Plot represents median values, 25% and 75% percentiles (box), ranges, and outlier values (o). *P* values indicate significant differences between groups at given time-points as determined by a *W*-test. These data (adapted from Reinhold et al., 2008) demonstrate that the white blood cell counts are significantly reduced in calves with sub-clinical infection. The leukopenia persisted for >6 months.

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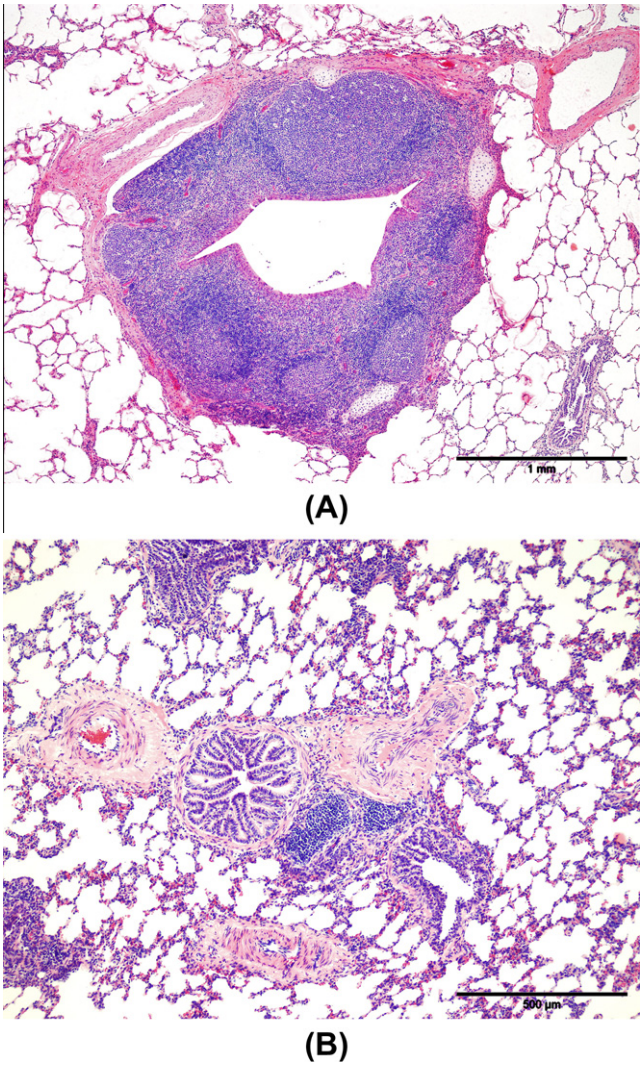

Fig. 3. Photomicrographs illustrating: (A) hyperplastic bronchus-associated lymphoid tissue (BALT) causing airway stenosis in a calf with sub-clinical pulmonary chlamydial infection; (B) normal BALT in an age-matched, clinically normal calf. Photos: Elisabeth Liebler-Tenorio (FLI, Jena, Germany).

Collectively, these data suggest that not only pulmonary infection, but also the total infection load at multiple sites including the intestine, may contribute to chlamydia-related lung disease (Jaeger et al., 2007; Reinhold et al., 2008), possibly mediated by systemic inflammatory mediators as observed in type 2 diabetes in a murine model (Wang et al., 2009). Chronic pulmonary dysfunction and inflammation demonstrates the persistent effect of sub-clinical chlamydial infection in the lungs which may negatively influence lung development and maturation that lasts from the post-natal period until one year of age (Lekeux et al., 1984; Lekeux, 1993). During this critical period, such respiratory infections are likely to contribute to growth retardation through impaired lung function.

Strategies for the control of chlamydial infections

Herd management

A higher prevalence of chlamydial infections in herds has been linked to unhygienic conditions and to animals being maintained on a poor plane of nutrition (Vogel et al., 2006). 'Scores' for the cleanliness of bedding, walkways, and animals are better on farms were infection is absent, and the purchase of replacements, natural siring and lack of separate calving pens are also major risk factors (Table 3) (Kemmerling et al., 2009).

Although there are statistical links between poor herd management and performance, and chlamydial infection, a definitive causal link between these events remains to be determined. Studies by Biesenkamp-Uhe et al. (2007) and DeGraves et al. (2004) demonstrated specific immunological intervention against chlamydiae, eliminated sub-clinical mastitis, and that experimental challenge with *C. abortus* reduced cow fertility, respectively. A recent study of infected and non-infected calves held under optimal, standardized conditions also suggested a causal link between infection and performance (Reinhold et al., 2008). Nevertheless, questions about the complex interactions between herd management and chlamydial infection remain.

Anti-microbial treatment

Antibiotics that specifically treat bovine chlamydial infection are not available, and the effectiveness of compounds such as macrolides, tetracyclines and quinolones, used therapeutically in a range of species, is the subject of ongoing debate. Besides the genetically determined resistance of *Chlamydia suis* to tetracycline (Dugan et al., 2004, 2007; Di Francesco et al., 2008), it is hypothesized that anti-microbial treatment may facilitate persistent, latent infection (Mårdh and Löwing, 1990; Belland et al., 2003; Hogan et al., 2004; Goellner et al., 2006; Ouellette et al., 2006). A recent in vivo study in mice by Wang et al. (2009) found that macrolides, while reducing the initial chlamydial burden, also delayed the subsequent elimination of the organism. Research in pigs found that a 3–5 day treatment with fluoroquinolone (enrofloxacin) initially reduced chlamydial shedding, but that shedding recurred within a week of terminating the treatment (Reinhold et al., 2010). Overall, there is no evidence pointing to the successful use of antimicrobials to eliminate bovine chlamydial infection. However, such treatments may be useful in suppressing chlamydial infection following insemination or during periods of stress (Wang et al., 2009).

Vaccination

Chlamydial diseases in animals that currently merit vaccination are: abortion in small ruminants; the respiratory disease complex of turkeys of which *C. psittaci* infection is an important component; and conjunctivitis and respiratory infection in cats. While both live attenuated and inactivated vaccines against *C. felis* are used in cats, there is little hard data relating to their efficacy. A significant reduction in chlamydial shedding, and virtually complete protection from disease following respiratory challenge with *C. psittaci* was achieved in turkeys vaccinated with *C. psittaci ompA* (Van Loock et al., 2004). While a live, attenuated, temperature-sensitive vaccine against *C. abortus* (Enzovax, Intervet) was shown to reduce abortion in small ruminants (Rodolakis and Bernard, 1984), convincing experimental and epidemiological evidence of the wider efficacy of such vaccines is required.

Justification for the development of vaccines against bovine chlamydial infection includes the reduced impact of sub-clinical infections on herd production parameters created by vaccine-induced immunity and the fact that prophylaxis in production animals is preferable to the widespread use of antibiotics. Animal models and epidemiological studies indicate that vaccination against chlamydiae does not induce sterile immunity or 100% protection from disease (Stemke-Hale et al., 2005; Wang et al., 2008). However, 'therapeutic' vaccination may provide substantial animal health and production benefits in ameliorating the effects of mastitis and infertility in herds (Stemke-Hale et al., 2005; Biesenkamp-Uhe et al., 2007). Although live and inactivated

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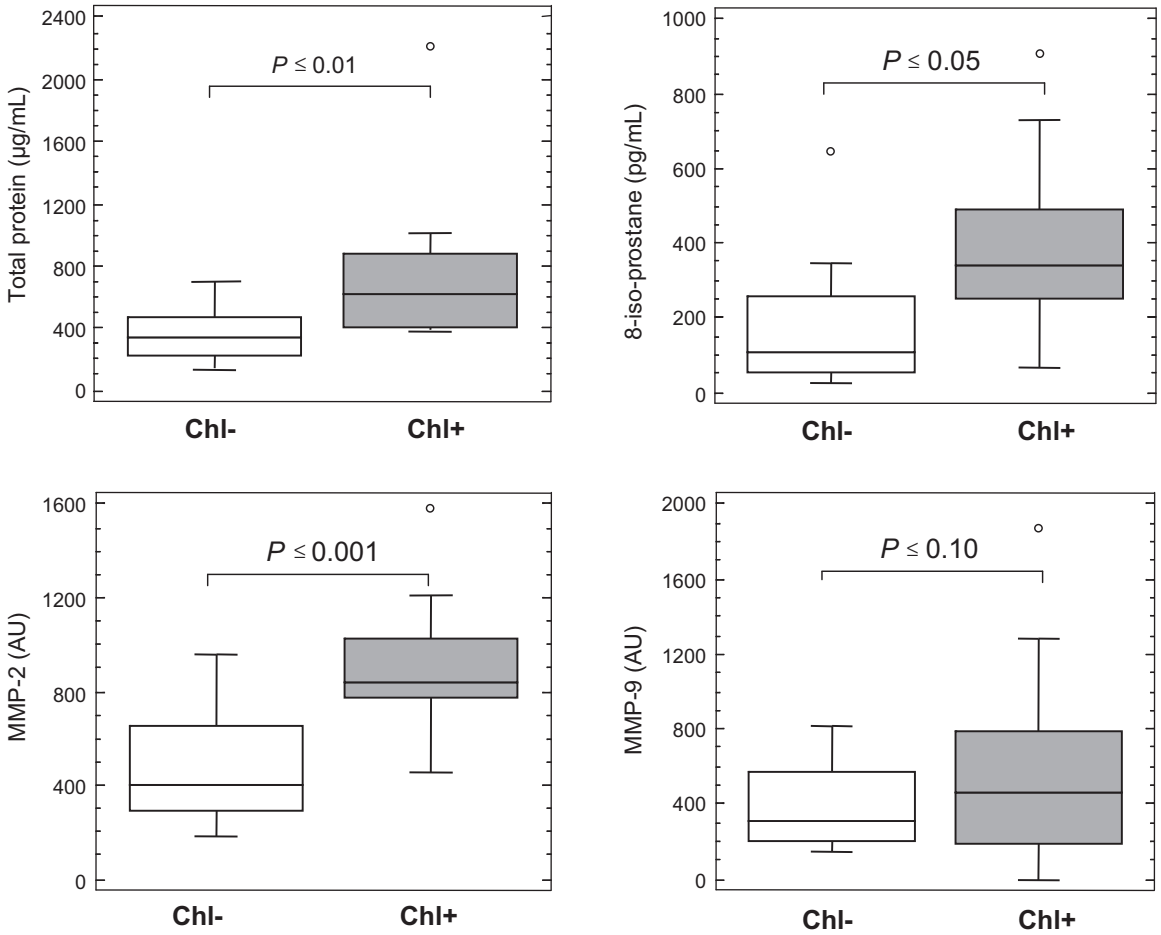

Fig. 4. Box-and-whisker plots illustrating inflammatory biomarkers in the broncho-alveolar lavage fluid (BALF) of calves with (Chl +) or without (Chl −) chlamydial infection. Plots represent median values, 25% and 75% percentiles (box), ranges, and outlier values (o). *P* values indicate significant differences between groups at given time-points as determined by a *W*-test. These data, adapted from Reinhold et al. (2008), demonstrate that all biomarkers were significantly increased in calves with sub-clinical chlamydial infection and indicate that a persistent neutrophilic response was associated with pulmonary oxidative stress (increased 8-iso-prostrane concentrations) and tissue remodeling (increased matrix metalloprotease [MMP]-2) activity. AU, arbitrary units.

Table 3
Husbandry factors significantly associated with chlamydial infection of dairy herds.

Husbandry factor		Level of increased risk	Reference
Management practices	• Use of external replacements	2.3-fold	Kemmerling et al. (2009)
	• Use of natural service rather than artificial insemination	2.6-fold	
	• No separate calving pens		
Housing and hygiene	• Reduced cleanliness of bedding		Kemmerling et al. (2009)
	• Reduced cleanliness of walkways		
	• Reduced cleanliness of animals (cows)		
	• Reduced vaginal cleanliness		
Animal health	• Feet and leg problems		Kemmerling et al. (2009)
	• Problems related to claws		
	• Problems related to joints		
Population density	• Crowding (calves)	4-fold increase for every 2-fold increase in group size	Jee et al. (2004)
	• Percentage of infected calves		
	• Chlamydial DNA detected		

vaccines against abortion in sheep and goats due to *C. abortus* are available, there is considerable interest in the development of vaccines of higher efficacy and that allow discrimination of vaccinated from naturally-infected animals. Synthetic, subunit vaccines may fit these criteria with the additional benefit of being inexpensive to produce (Caro et al., 2003; Héchard et al., 2003, 2004; Stemke-Hale et al., 2005).

Future research

The multifactorial pathogenesis of bovine chlamydial infection

In veterinary practice, it is difficult to definitively attribute the role of factors such as herd management, nutrition, and animal susceptibility in the pathogenesis of potentially chlamydia-related dis-

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ease. However in an experimental setting, a murine model of *C. pneumoniae* lung disease demonstrated that dietary protein deficiency delayed the T-helper cell immune response in inbred C57BL/6, but not in A/J mice, resulting in severe pulmonary disease in the former and no disease in the latter (Wang et al., 2008). Similarly, obese C57BL/6 mice on a high-fat diet developed an exacerbated insulin resistance following respiratory challenge with a low-dose of *C. pneumoniae*, while no such change was observed in A/J mice (Wang et al., 2009). In contrast, no strains developed insulin resistance on a low-fat diet. The most direct evidence for a role for host genetics in determining susceptibility to chlamydia-associated disease comes from genetic mapping experiments in murine models. Min-Oo et al. (2008) found that the level of infection of *C. pneumoniae* in the lungs of mice following intra-nasal challenge was partially regulated by a quantitative trait locus that maps to a region on chromosome 17 that also contains the major histocompatibility complex II. Taken together, these data clearly demonstrate the interactive and multi-factorial nature of chlamydial infection and the central roles played by the host's genetic background and environment. The multiplicity and complexity of such interactions likely results in a spectrum of disease outcomes with outbred cattle herds.

There is evidence from human medicine that chlamydiae are frequently part of mixed infections with other pathogens (Marrie et al., 2003). Mixed mycoplasma and chlamydial infections are well-recognized in humans with respiratory disease (Esposito et al., 2001; Martin et al., 2001; Johnston and Martin, 2005), as are *Chlamydia suis* and *Mycoplasma hyorhinis* infection in pigs (Reinhold et al., 2005). In turkeys, super-infection with *Escherichia coli* or avian pneumovirus exacerbate chlamydial disease (Van Loock et al., 2006a,b). Currently, such evidence of significant co-infection is lacking for cattle.

Zoonotic risk

Infection of humans with chlamydiae such as *C. psittaci* from birds or *C. abortus* from small ruminants, can result in acute, sometimes life-threatening illness (Walder et al., 2003, 2005; Haas et al., 2006; Janssen et al., 2006; Pandeli and Ernest, 2006; Gaede et al., 2008; Harkinezhad et al., 2009). Co-infection with the human pathogens *C. pneumoniae* and with *C. psittaci*, of unknown origin, was described in a human patient with fulminant myocarditis (Walder et al., 2007). Although genetic markers have not yet been identified that can distinguish avian from bovine strains of *C. psittaci*, or ovine from bovine strains of *C. abortus*, there appears to be wide variation in the capacity of such strains to cause acute disease in humans. It remains unclear whether or not the zoonotic potential of avian strains of *C. psittaci* or of ovine/caprine strains of *C. abortus*, are genetically determined, or whether other factors associated with transmission are involved.

Zoonotic chlamydial infection is a recognized occupational hazard (Bazala and Renda, 1992; Hinton et al., 1993; Wittenbrink, 2002), and persistent infection with *C. psittaci* has been identified in humans with pulmonary emphysema and/or chronic obstructive pulmonary disease (COPD) (Theegarten et al., 2004). The latter suggests a link between chronic inflammation and persistent infection with chlamydiae. Although there is anecdotal evidence linking atypical respiratory disorders or asthma-like symptoms in farmers working with chlamydia-infected herds, proof of cause and effect remains elusive. On current evidence, the zoonotic potential of the chlamydiae found in cattle would appear minimal.

Conclusions

Epidemiological surveys suggest chlamydial infection of cattle is endemic but low-grade, typically involving *C. abortus*, *C. pecorum*, or *C. psittaci*. Herd management factors and hygiene practices have an important impact on the risk of chlamydial infection which occurs in the respiratory or genital tracts and is only slowly eliminated by the host immune response. Ongoing infections only result in clinical signs if several risk factors, such as stress and high population densities, coincide. However, sub-clinical infections do cause chronic inflammation in host tissues, the pathogenesis of which is currently poorly understood, but that ultimately negatively impact on animal performance. Such chronic, inapparent infections in cattle are more likely to be economically significant than the rare outbreaks of clinical disease recorded. Future research should be targeted at dissecting the animal health and economic impacts of bovine chlamydial infection, and of devising strategies such as vaccination, to ameliorate their negative effects.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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