

Bovine *Chlamydophila* spp. Infection: Do We Underestimate the Impact on Fertility?

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ABSTRACT

Classical methods for detection of *Chlamydophila* species, and of antibodies against these agents, have indicated that these bacteria are highly prevalent in cattle and associated with numerous disease conditions. These methods demonstrated acute *Chlamydophila*-induced diseases such as epizootic bovine abortion, as well as worldwide variable, but generally high, *Chlamydophila* seroprevalence. However, it was impossible to consistently detect the low levels of these organisms which were suspected to be present in endemic infections. Application of highly sensitive real-time PCR and ELISA methods for detection of *Chlamydophila* spp. DNA and of antibodies against *Chlamydophila* spp., respectively, in a series of prospective cohort studies revealed a high prevalence of *Chlamydophila* spp. genital infections in female calves (61%) and adult heifers (53%). These infections were acquired by extragenital transmission in the first weeks of life, and infection frequency was increased by crowding of the animals. A challenge study demonstrated that infection with *C. abortus* resulted in decreased fertility of heifers. The experimental use of a *C. abortus* vaccine provided evidence for immunoprotection against *C. abortus*-induced suppression of bovine fertility. The results of these investigations suggest that bovine *Chlamydophila* infection should be viewed more as pervasive, low-level infection of cattle than as rare, severe disease. Such infections proceed without apparent disease or with only subtle expressions of disease, but potentially have a large impact on bovine herd health and fertility.

Keywords: bovine fertility, cattle herd health, *Chlamydophila abortus* vaccine, prevalence

Abbreviations: *C.*, *Chlamydophila*; CFT, complement fixation test; EB, elementary body; ELI, expression library immunization; ELISA, enzyme-linked immunosorbent assay; LPS, lipopolysaccharide; PCR, polymerase chain reaction

INTRODUCTION

Classical investigations of diseases induced in cattle by intracellular bacteria of the order *Chlamydiales* have revealed severe, but rare, disease manifestations such as pneumonia, enteritis, polyarthritis, sporadic encephalomyelitis, and abortion and fertility disorders (Shewen, 1980). Early investigators realized that these sporadic appearances of acute diseases represented only the ‘tip of the iceberg’ of a ubiquitous distribution of subclinical infections with these agents, and that specific circumstances precipitated the clinical manifestation of disease (Storz, 1971).

Today’s prevailing view of bovine chlamydial infection is that of a curiosity, significant in rare incidences of abortion and zoonosis, but not in animal health and production. This view has been created by inadequate sensitivity and inconsistent application of standard diagnostic methods such as complement fixation test and culture methods. Investigations using modern ELISA and PCR techniques show a very different picture of chlamydial infections in cattle. High seroprevalence, approaching 100%, and chlamydial genomic DNA prevalence as high as 50–60% has been associated with clinically inapparent bovine infection with *Chlamydophila abortus* and *Chlamydophila pecorum* (Cavirani *et al.*, 2001; DeGraves *et al.*, 2003a; Domeika *et al.*, 1994; Jee *et al.*, in press; Kaltenboeck *et al.*, 1997a,b; Wang *et al.*, 2001). The ubiquitous presence of two chlamydial species in cattle begs a number of questions, most importantly: what is their impact on herd health and production?

The authors have addressed this question in a series of epidemiological and heifer challenge experiments with *C. abortus*. These experiments demonstrated that previous infection and established immunity against *C. abortus* did not protect against decreased fertility following direct uterine challenge with *C. abortus* or following challenge through contact with herd mates. The results also indicated that pregnancy results after uterine *C. abortus* or mock inoculation were strongly influenced by simultaneous cohort challenge, as well as by pre-challenge immunity against *C. abortus*, expressed as anti-*C. abortus* IgM serum antibodies. The experimental use of a *C. abortus* vaccine provided evidence for immunoprotection against *C. abortus*-induced suppression of bovine fertility (DeGraves *et al.*, 2002). The results of these investigations suggest that bovine *Chlamydophila* spp. infections occur mainly without apparent disease, or only with subtle signs of disease. Nevertheless, these infections may impact on herd health and fertility more profoundly than overt clinical chlamydial disease.

THE HISTORICALLY RECOGNIZED DISEASE POTENTIAL OF CHLAMYDIAE FOR CATTLE

Bacteria of the order *Chlamydiales* are obligate intracellular parasites that are unable to replicate outside of eukaryotic host cells (Storz, 1971). They are ubiquitous, infect a wide range of vertebrate and invertebrate hosts, and cause a wide variety of diseases (Schachter, 1999). It is thought that diseases induced by chlamydial organisms are mainly the result of an immune reaction that is ineffective at clearing the organisms,

but results in collateral tissue damage (Ward, 1999). Vertebrate isolates typically have low host and cell type specificity, but certain chlamydial strains and species tend to associate with specific hosts and diseases (Shewen, 1980; Storz and Kaltenboeck, 1993).

Chlamydiae were first associated with disease of cattle (*Bos taurus*) when McNutt isolated intracellular organisms from cases of sporadic bovine encephalomyelitis in feedlot cattle (McNutt and Waller, 1940). After chicken embryo and cell culture methods for chlamydiae became widely used around 1955, a number of studies worldwide documented chlamydiae in many acute diseases of cattle. A prominent example is epizootic bovine abortion, similar to classic ovine abortion caused by *C. abortus*. This disease was found worldwide in many countries including France (Hidiroglou and Prevost, 1959), Germany (Schoop *et al.*, 1965; Wehner and Wehr, 1980), India (Nanda *et al.*, 1992), Japan (Kawakami *et al.*, 1955; Nabeya *et al.*, 1991), the United Kingdom (Griffiths *et al.*, 1995), and the USA (Storz *et al.*, 1960). The causal agent also caused epididymitis and seminal vesiculitis and was excreted in bull semen (Storz *et al.*, 1968). Chlamydial strains from ruminant abortion were identified as serotype 1 of ruminant chlamydiae (Schachter *et al.*, 1975), biotype 1 (Spears and Storz, 1979), immunotype 1 (Perez-Martinez and Storz, 1985), or *ompA* type B577 (Kaltenboeck *et al.*, 1993). Recently, reclassification as *Chlamydophila abortus* was proposed (Everett *et al.*, 1999). *C. abortus* has also been associated with bovine mastitis (Corner *et al.*, 1968; Kaltenboeck *et al.*, 1997b; Rønsholt and Basse, 1981; Wehnert *et al.*, 1980).

A second chlamydial agent was associated with clinically severe bovine chlamydial disease manifestations including abortion, sporadic bovine encephalomyelitis, pneumonia, enteritis, polyarthrititis, kerato-conjunctivitis, nephritis, or purulent endometritis (Bannister *et al.*, 1962; Dymal, 1965; French and Snowdon, 1960; Kölbl and Psota, 1968; Romvary, 1964; White, 1965; Wilson and Thompson, 1968; Wittenbrink *et al.*, 1988). This chlamydial strain was diagnosed as serotype 2 of ruminant chlamydiae (Schachter *et al.*, 1975), biotype 2 (Spears and Storz, 1979), immunotype 2 (Perez-Martinez and Storz, 1985), *ompA* type LW613 (Kaltenboeck *et al.*, 1993), or *C. pecorum* (Fukushi and Hirai, 1992). Recently, reclassification as *Chlamydophila pecorum* was proposed (Everett *et al.*, 1999).

Numerous studies have confirmed the disease potential of *C. abortus* and *C. pecorum* by experimentally reproducing acute diseases such as those listed above (Bowen *et al.*, 1978; Jones *et al.*, 1998; Storz *et al.*, 1976; Wittenbrink *et al.*, 1993). Shewen (1980) summarized the status of our understanding of chlamydial infections in animals: 'Exceptionally, some animals may experience severe or even fatal disease as a result of chlamydial exposure. A well balanced host-parasite relationship represents the common nature of chlamydial infection. This long-lasting inapparent or 'latent' state has been documented in several species: birds, cattle, guinea pigs, sheep and humans. Under circumstances of stress, 'carrier' animals may shed the organisms in large numbers or may in fact lapse into clinical disease.'

IMPROVED DIAGNOSTIC METHODS DETECT UBIQUITOUS SUBCLINICAL CHLAMYDIAL INFECTIONS IN CATTLE

Despite improvements in diagnostic techniques, most notably the introduction of the PCR, our understanding about the prevalence and pathogenetic significance of these infections has not substantially changed since Shewen's review in 1980. The major impediment has been the cumbersome nature and insensitivity of diagnostic procedures, particularly of the CFT for determination of seroprevalence of chlamydial infection in cattle (Kaltenboeck *et al.*, 1997a; Perez-Martinez *et al.*, 1986). Only recently, several investigations reported high prevalence of chlamydial infection or of antibodies against chlamydiae in cattle, and linked these data with increased diseases such as abortion, endometritis, and fertility disorders (Cavirani *et al.*, 2001; Domeika *et al.*, 1994; Wang *et al.*, 2001; Wittenbrink *et al.*, 1988). One of the main reasons that chlamydial infections of cattle are still not widely recognized is simply that insensitive diagnostic techniques give a misleading indication of the prevalence of these infections.

Seroprevalence

Perez-Martinez and colleagues (1986) demonstrated that the standard CFT for bovine antibodies against chlamydial agents was unable to detect even high antibody levels. Addition of bovine complement was necessary to obtain improved sensitivity because bovine antibodies in essence do not bind guinea pig complement. The lack of sensitivity of the standard CFT is obvious in Figure 1 from research on synthetic antigen ELISAs for chlamydial antigens which compared these assays to the CFT (Kaltenboeck *et al.*, 1997a). In a random survey of 40 sera from Alabama cattle herds with abortion problems, ELISAs against peptides of the *C. abortus* major outer membrane protein or against recombinant chlamydial LPS invariably detected very high antibody levels. In fact, immunoglobulin-rich sera from gnotobiotic calves challenged with bovine coronavirus had to be used as negative controls because it was impossible to find any other *Chlamydophila*-negative bovine sera. In comparison, the CFT titers of all but one serum sample were negative. The single positive serum had a low titer of 1:10 (Figure 1).

The authors have comparatively examined 317 additional bovine sera with high-sensitivity ELISA methods using chemiluminescent substrate and *C. abortus* and *C. pecorum* peptide and lysed elementary body antigens, and have failed to detect a single truly anti-*Chlamydophila* negative serum despite consistently negative reactivity of several control sera from gnotobiotic calves (data not shown). These ELISAs have a wide dynamic range and are highly informative because the resultant luminescence signal is linear over 5 orders of magnitude. The data indicate that signals from all antigens are highly correlated, but agreement between whole organism antigens is better than that between peptide antigens. The serological cross-reactivity between whole organism *C. abortus* and *C. pecorum* antigens is 83% (Jee *et al.*, submitted). In examinations of sera from *C. abortus* challenged heifers, IgM antibodies provided the best functional correlation between fertility outcome and pre- or post-challenge anti-*C. abortus* antibodies, or their ratios (DeGraves *et al.*, 2004; Jee *et al.*, submitted).

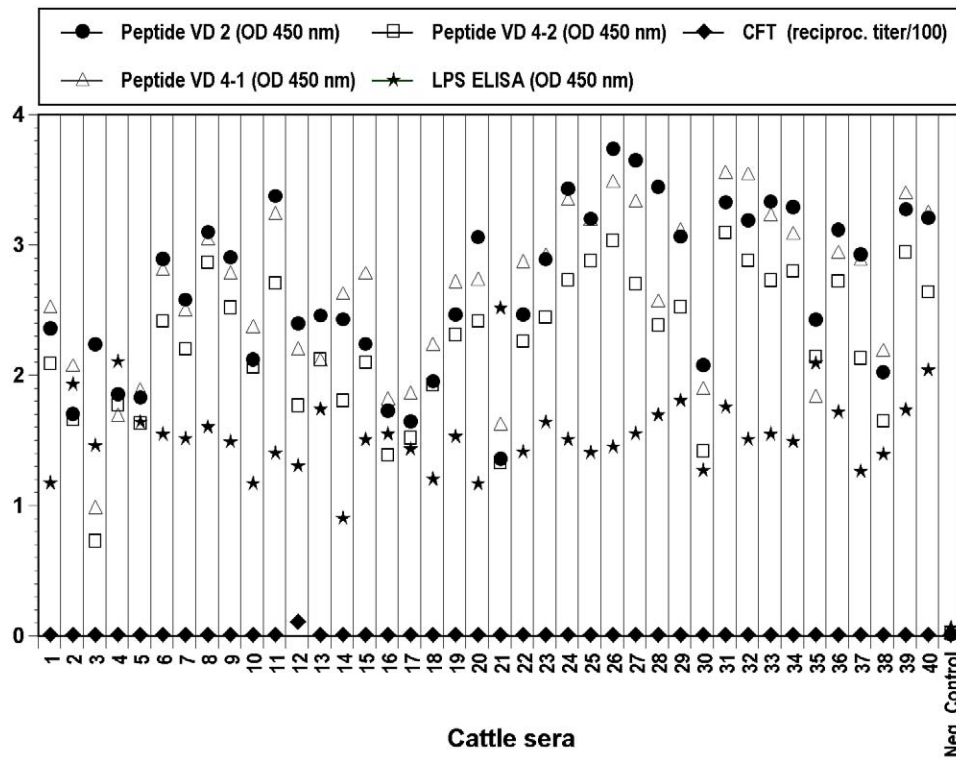


Figure 1. *Chlamydophila* spp. ELISA and CFT data obtained from 40 bovine sera from herds with abortion (Kaltenboeck *et al.*, 1997a). The CFT test used standard *Chlamydophila* CFT antigen, ELISAs used *C. abortus* major outer membrane protein (MOMP)-derived peptides from variable domains (VD) 2 or 4, or recombinant chlamydial lipopolysaccharide (LPS). An immunoglobulin-rich serum from a gnotobiotic calf after challenge with bovine coronavirus was used as negative control serum. Animal sera are indicated on the abscissa

A major determinant of sensitivity in assays of bovine antibodies against *Chlamy-dophila* spp. is the negative control serum. Given the overwhelming seroprevalence of *Chlamy-dophila* spp., many sera used as negative controls cannot be truly seronegative. Thus, the vast majority of seroprevalence studies will show variable proportions of false negative data, indicating as negative those chlamydia-positive animals that have serum anti-*Chlamy-dophila* antibody levels below the arbitrary cut-off imposed by the control sera.

Detection of chlamydial agents

The high seroprevalence of *Chlamydomphila* spp. in cattle poses the challenging question why we do not also routinely detect a high prevalence of the agents. The answer might be that standard chicken embryo and cell culture methods for detection are not sensitive enough to detect low amounts of chlamydiae (Huang *et al.*, 2001). Such low amounts would be expected if chlamydial infections were persistent at the individual host or herd level in an endemic disease situation.

Application of PCR to the direct detection of chlamydial agents is an obvious answer. Following initial reports that PCR detection of chlamydial DNA was possible, specific, and sensitive (Hewinson *et al.*, 1997; Kaltenboeck *et al.*, 1997b), the limitations of early PCR methods were recognized: (1) nested PCR methods were necessary to maximize sensitivity and specificity; and (2) the quantity and stability of the specimen imposes major constraints on the detection limits of the methods (DeGraves *et al.*, 2003b). Therefore, such PCR methods, while an improvement, are problematic because they lack the robustness needed for routine diagnostic application or large scale research.

The advent of single-tube real-time PCR platforms has removed most limitations to routine diagnostic use of PCR methodology, and has added the benefit of quantification and differentiation of the nucleic acid targets (DeGraves *et al.*, 2003a). This research group has adapted the LightCycler[®] real-time PCR as a high-throughput platform with extreme sensitivity for routine detection and quantification of even single DNA targets by modifying assay chemistry (Huang *et al.*, 2001) and nucleic acid extraction, and adopting specimen collection and preservation with guanidinium thiocyanate buffer before freezing (DeGraves *et al.*, 2003b).

Application of these technologies has already demonstrated the surprisingly high prevalence of 53% vaginal infection of virgin heifers with *C. abortus* and *C. pecorum* (Table I, DeGraves *et al.*, 2003a). These data also clearly demonstrate that these genital infections are transmitted by non-venereal means, and that typically very low amounts of *Chlamydomphila* genomes are present. They also show substantial differences between sensitivities of different PCR methods, as evident in the overall 22% prevalence found by *Chlamydomphila ompA* PCR and the 53% prevalence of *Chlamydomphila* spp. infection found by 23S rRNA PCR in the same extracted specimens. The lower sensitivity of the *ompA* PCR is related to the longer amplification fragment as compared the 23S rRNA PCR (287 bp vs. 168 bp) and the higher degree of degeneracy of the *ompA* primers (DeGraves *et al.*, 2003b).

Upon investigation of the acquisition of these infections by female calves in serial sampling beginning at birth, it became evident that the calves acquired natural infection with *C. abortus* and *C. pecorum* at overall 61% prevalence primarily in the first 2 months after birth, without showing signs of clinical disease (Table II, Jee *et al.*, in press). The percentage of *Chlamydomphila*-positive calves and the level of infection showed a highly significant quadratic, but not linear, increase as the number of calves per weekly group was increased (Jee *et al.*, 2004). Thus, crowding greatly increased the prevalence and intensity of *Chlamydomphila* spp. vaginal infections in calves.

TABLE I
Prevalence of *Chlamydomphila* spp. vaginal infection in heifers as determined by *Chlamydomphila* 23S rRNA and *Chlamydomphila omp1* FRET-qPCRs (DeGraves *et al.*, 2003a)

qPCR method ^a	<i>C. abortus</i> positive	<i>C. pecorum</i> positive	Total <i>Chlamydomphila</i> positive	<i>Chlamydomphila</i> genomes per PCR positive specimen (mean \pm SD)
23S rRNA	17.6%	37.3%	51.0%	0.51 \pm 1.9
<i>omp1</i>	7.8%	15.7%	21.6%	0.80 \pm 1.0
Total ^b	23.5%	39.2%	52.9%	0.59 \pm 1.3

^aVaginal cytobrush swabs were collected 4 times at weekly intervals from a herd of 51 clinically normal virgin Holstein heifers. DNA from swab specimens was extracted, and each sample was examined by a single *Chlamydomphila* 23S rRNA, *C. abortus omp1*, and *C. pecorum omp1* qPCR. Heifers were scored as positive if any of the 4 specimens was positive

TABLE II
Prevalence of *C. abortus*, *C. pecorum*, and total *Chlamydomphila* spp. DNA in calves (Jee *et al.*, in press)

	Specimen	Positive ^a	<i>Chlamydomphila</i> genomes per positive qPCR
<i>C. abortus</i>	Calf, vaginal swab	9.8%	2.7 \pm 1.7
<i>C. pecorum</i>	Calf, vaginal swab	51.2%	10.4 \pm 6.1
<i>Chlamydomphila</i> spp.	Calf, vaginal swab	61.0%	9.2 \pm 5.4

^aVaginal cytobrush swabs were collected for 12 weeks at weekly intervals from 41 female calves born over a period of 11 months in a herd of 140 dairy cows. DNA from swab specimens was extracted, and each sample was examined by a single *Chlamydomphila* 23S rRNA qPCR. Calves were scored positive if *Chlamydomphila* spp. DNA was detected in one or more qPCRs

The authors conclude that sample collection and preservation, nucleic acid extraction, as well as the type of PCR profoundly influence *Chlamydomphila* spp. detection in bovine specimens. Results of epidemiological studies may thus range from low single digit to above 50% prevalence for bovine vaginal *Chlamydomphila* spp. infection. For reliable and comparable data it will be imperative to standardize the overall methodology of PCR testing.

THE EFFECT OF LOW-LEVEL CHLAMYDIAL INFECTIONS OF CATTLE ON HEALTH AND PRODUCTION

The high seroprevalence of chlamydiae in cattle, confirmed by chlamydial genomic DNA prevalence data, poses the challenge of determining the health consequences, if any, of these endemic infections. Economic losses caused by late-term *C. abortus* infection and subsequent epizootic bovine abortion are readily apparent. However, infection may result in unrecognized economic losses as the consequence of subclinical infertility (Bowen *et al.*, 1978). Bovine infertility associated with *C. abortus* and *C. pecorum* infection has been addressed, but not extensively characterized (Jones *et al.*, 1998; Storz *et al.*, 1968, 1976; Wehner and Wehr, 1980; Wittenbrink *et al.*, 1988, 1993). It is recognized that bulls can shed *C. abortus* in semen; that breeding bulls which shed *C. abortus* in semen have decreased fertility; that semen spiked with *C. abortus* is associated with reduced fertility; and that uterine challenge with *C. abortus* can cause metritis. As described above, cattle typically experience a first infection with *Chlamydomphila* spp. at an early age without any clinical signs of disease, and respond by establishing immune responses as evidenced by sustained high antibody levels. However, it is unclear if infection is cleared from the tissues (Papp *et al.*, 1993, 1994), and what the consequences of persistent or repeated infections are.

To approach this question, the authors investigated the influence of subclinical uterine and systemic infection with *C. abortus* on the fertility of virgin heifers (DeGraves *et al.*, 2004). A herd of 30 Holstein heifers, all highly positive for serum antibodies against *C. abortus*, was estrus-induced by injection with dinoprost tromethamine. Twenty heifers responding with estrus in the first challenge round were inseminated with the semen of a bull with high fertility, and simultaneously received an intracervical challenge of 0, 10^4 , 10^5 , 10^6 , or 10^8 inclusion forming units (IFU) of *C. abortus* type strain B577 (Storz, 1966) via the cervix. Two weeks later, the remaining 10 heifers were injected again, and similarly bred and inoculated in the second challenge round. Pregnancy was determined after 42 days by rectal palpation. Second-round heifers were considered cohort-challenged at the time of insemination by contact with *C. abortus*-shedding first-round animals. Pregnancy results indicated that the highest challenge dose abolished fertility, demonstrating that previous infection and established immunity against *C. abortus* did not protect against chlamydial disease (DeGraves *et al.*, 2004). The data also indicated that pregnancy results at lower uterine challenge were strongly influenced by concomitant cohort challenge, as well as by the level of pre-challenge immunity against *C. abortus*, expressed as pre-challenge anti-*C. abortus* IgM serum antibodies. Logistic regression modeling predicted fertility of heifers challenged with *C. abortus* (Figure 2). This study demonstrated that subclinical chlamydial infections may potentially have a profound negative effect on bovine fertility, and that these losses may be reduced with appropriate herd management practices.

The risk factors for reduction of bovine fertility identified in the study may provide insight for the development of viable prophylactic and therapeutic measures. Transmission frequency of *Chlamydomphila* spp may be decreased by reducing group size and population density. Vaccination may also serve as a component of a prophylactic or

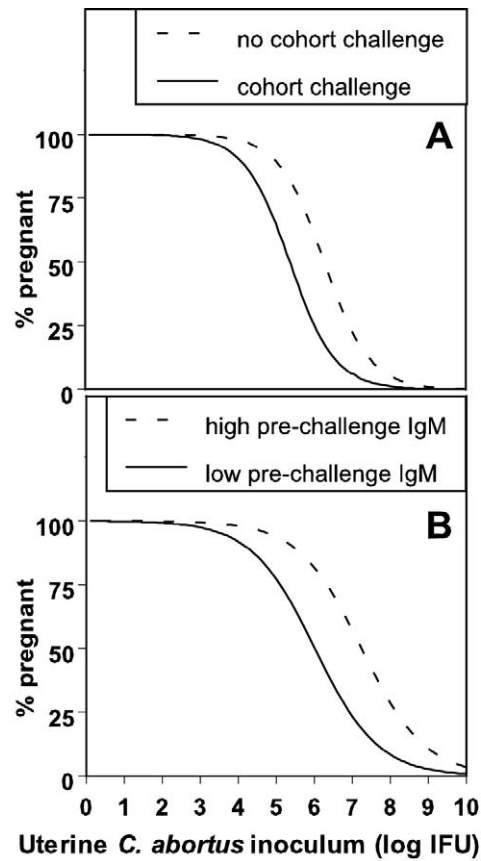


Figure 2. Cohort challenge and pre-challenge anti-*C. abortus* serum IgM levels strongly modulate fertility after uterine challenge with *C. abortus*. Fertility of challenged heifers (percent animals pregnant) is significantly predicted in logistic regression models by uterine *C. abortus* inoculum dose and cohort challenge by *C. abortus* (A), or by uterine inoculum dose and concentration of IgM against *C. abortus* (B). The solid line represents fertility in dependence on uterine inoculum under conditions of cohort challenge to *C. abortus* (A) or under condition of below-median (low) levels of anti-*C. abortus* IgM (B). The dashed line represents fertility without cohort challenge (A) or with above-median (high) levels of anti-*C. abortus* IgM (B). These logistic regression models of fertility of heifers with established immunity against *C. abortus* indicate that (A) with cohort challenge a uterine infection of $10^{5.38}$ IFU of *C. abortus* is necessary to reduce fertility of heifers from 100% to 50%, as compared to the 8.5-fold higher dose of $10^{6.31}$ IFU required for the same reduction without cohort challenge; and (B) at low pre-challenge anti-*C. abortus* IgM levels $10^{6.01}$ intrauterine IFU of *C. abortus* reduce fertility of heifers from 100% to 50%, as compared to the 17-fold higher dose of $10^{7.24}$ IFU required for the same reduction at high pre-challenge anti-*C. abortus* IgM levels.

therapeutic program, by protecting individual animals from disease and by reducing *Chlamydophila* transmission within the herd.

VACCINATION FOR PROTECTION AGAINST *C. ABORTUS*-INDUCED INFERTILITY

Several reasons make a vaccine against bovine chlamydial infections desirable: (1) it is likely that vaccination can create a differential in immunity to *C. abortus* that would aid in evaluating the impact of subclinical chlamydial infection on bovine fertility in controlled trials; (2) prophylaxis or therapy of chlamydial infections by vaccination is highly preferable in production animals as compared to regimens using antibiotics; (3) successful vaccination might substantially improve bovine herd fertility.

Conventional approaches to searching for vaccines against *Chlamydia*-induced disease have led to whole organism or subunit vaccines with less than optimal responses, including inconsistent protection in animal models (Rank, 1999) and exacerbation of disease in human trachoma field trials (Ward, 1999). A leading candidate for a vaccine, the major outer membrane protein (MOMP), has been tested as a genetic vaccine in several host species with variable and inconclusive results (Pal *et al.*, 1999; Tan *et al.*, 1990). The authors chose expression library immunization (ELI) for identification of protective vaccine candidate genes (Barry *et al.*, 1995). ELI is an unbiased whole-genome approach in which pools of expression plasmids containing inserts from the *C. abortus* genome were tested in a mouse pneumonia model for the ability to confer protection against challenge infection. Pools of protective plasmids were fractionated into smaller pools, until single protective gene candidates were tested (Stemke-Hale *et al.*, in press). Five gene fragments conferred protection at levels as good or better than the live-vaccine control of mice that had received a low-level respiratory *C. abortus* inoculation one month prior to the high-dose challenge inoculation. Four of these fragments (*dnaX₂*, *gatA*, *gatC*, *pbp3*) encoded peptides of cytosolic proteins; only one (*pmp5*) encoded a peptide of an outer membrane protein, traditionally considered the best vaccine candidates. The *dnaX₂* fragment, encoding a portion of the *C. abortus* DNA polymerase, completely protected mice from *C. abortus* disease, better than the best protection achieved with live vaccination by low-level intranasal *C. abortus* inoculation. (Stemke-Hale *et al.*, in press).

The protective genes and their corresponding proteins were used in a preliminary vaccine trial to evaluate protection against *C. abortus* (DeGraves *et al.*, 2002). The five most protective genes were selected for vaccination of virgin heifers in a *C. abortus* cohort challenge model. This vaccine was composed of a pool of plasmids that contained the full ORFs of the genes, recoded for mammalian codon usage. A corresponding recombinant protein vaccine against *C. abortus* was also developed. This vaccine was Alum-Quil A based and contained affinity-purified full-length proteins expressed in *E. coli*, using the same genes that were incorporated into the genetic vaccine.

A natural Holstein heifer infection model was used in which intracervically inoculated – but not bred – heifers were placed in contact with herd mates that were

TABLE III

Pregnancy rates in vaccinated heifers 42 days after breeding and cohort challenge with *C. abortus* (DeGraves *et al.*, 2002)

Vaccine Group	Pregnant	Not pregnant
Control	6 (50%)	6 (50%)
Genetic	4 (80%)	1 (20%)
Protein	5 (83%)	1 (17%)

bred 2 weeks later and were indirectly *C. abortus* challenged by contact exposure. The Holstein herd had a 53% vaginal *Chlamydophila* spp. PCR pre-challenge prevalence, without significant difference between vaccinated and non-vaccinated heifers. The experimental herd mates were used to evaluate the genetic and recombinant protein vaccines, compared to mock vaccinated controls. Twenty-seven heifers were synchronized for estrus and then inoculated with an intrauterine dose of *C. abortus*. When the challenged heifers were at maximum shedding of *C. abortus* as determined by real-time PCR of vaginal swabs, estrus was synchronized in experimental heifers ($n = 24$). Experimental heifers were then monitored for estrus with a computerized estrus detection system and bred by artificial insemination. Pregnancy was determined by rectal palpation at 42 days post breeding. The results shown in Table III suggest protection by both genetic and protein vaccines from *C. abortus*-induced infertility. The odds ratio for improvement of fertility with vaccination against *C. abortus* is 4.5 ($p = 0.12$).

The ~32% increase in fertility of vaccinated heifers as compared to the 50% first-service conception rate of control heifers would represent a substantial improvement in bovine herd health. Thus, immunization with a subunit *C. abortus* vaccine may hold promise to improve bovine herd fertility. It is important to note that the vaccines did not create a fresh adaptive immune response to *C. abortus* in naive heifers, but rather enhanced and/or modulated an existing response in animals with *Chlamydophila* spp. infection. Thus, the vaccines were used as therapeutic, not prophylactic vaccines, and may be more appropriately termed ‘antigen-specific immune modulators’.

CONCLUSIONS

The results presented of *Chlamydophila* prevalence studies, and of challenge experiments with and without vaccination indicate that (1) *C. abortus* and *C. pecorum* are endemic at low levels in cattle and infect virtually 100% of the animals; (2) *Chlamydophila* spp. infections are acquired early in life by non-sexual transmission; (3) such infections typically do not result in clinical disease; (4) *C. abortus* (and possibly

C. pecorum) infections at the time of breeding have the potential to substantially reduce fertility in cattle; and (5) enhanced immunity mediated by recent natural infection or by vaccination may protect cattle from suppression of fertility by *C. abortus* (and possibly *C. pecorum*).

These conclusions suggest that subclinical *Chlamydophila* spp. infections have the potential to cause substantial production losses in bovine herds due to reduced fertility. A renewed focus towards quantitative prospective studies of subtle disease manifestations is warranted to improve our understanding of the impact of *Chlamydophila* spp. infections on bovine herd health. These infections may also negatively affect frequencies and intensities of bovine herd health problems such as mastitis, and respiratory and neonatal diseases. Rational application of standardized, highly sensitive PCR and serological methodology will be required for conclusive results. Furthermore, combination *C. abortus*–*C. pecorum* vaccines may aid in prevention of losses by these infections, but also serve together with antibiotics as ‘perturbation’ tools in prospective intervention studies on the impact of *Chlamydophila* spp. infections on bovine herd health.

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