

Recent advances in the understanding of *Chlamydophila pecorum* infections, sixteen years after it was named as the fourth species of the Chlamydiaceae family

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Abstract – *Chlamydophila pecorum* found in the intestine and vaginal mucus of asymptomatic ruminants has also been associated with different pathological conditions in ruminants, swine and koalas. Some endangered species such as water buffalos and bandicoots have also been found to be infected by *C. pecorum*. The persistence of *C. pecorum* strains in the intestine and vaginal mucus of ruminants could cause long-term sub-clinical infection affecting the animal's health. *C. pecorum* strains present many genetic and antigenic variations, but coding tandem repeats have recently been found in some *C. pecorum* genes, allowing *C. pecorum* strains isolated from sick animals to be differentiated from those isolated from asymptomatic animals. This review provides an update on *C. pecorum* infections in different animal hosts and the implications for animal health. The taxonomy, typing and genetic aspects of *C. pecorum* are also reviewed.

***Chlamydophila pecorum* / pathogenesis / typing / epidemiology**

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1. INTRODUCTION

Chlamydiae are obligate intracellular bacteria that develop inside eukaryotic cells within membrane-bound vacuoles termed inclusions, with a unique biphasic developmental cycle alternating between an extracellular metabolically inert form (elementary body, EB) and an intracellular metabolically active replicating form (reticulate body, RB) [52]. *Chlamydia trachomatis* and *Chlamydia psittaci* were the first two species to be assigned to the genus *Chlamydia* on the basis of differences in inclusion morphology, glycogen content, susceptibility to sulfadiazine and natural host [25, 54]. *C. trachomatis* encompasses all *Chlamydiae* found in man. *C. psittaci* comprises all other *Chlamydiae* found in birds and animals except the mouse pneumonitis agent, classified as *C. trachomatis* on the basis of its biological properties. *C. pneumoniae* is separated from the other two species on the basis of ultrastructural differences in the elementary bodies, DNA homology values and serological reactions [26].

Until 1993, *C. pecorum* strains were considered as members of the *C. psittaci* species, sharing many similar phenotypic characteristics, including inclusion morphology, the absence of glycogen in inclusions and sulfadiazine resistance. The word “pecorum” comes from the Latin word for flocks of sheep or herds of cattle [20]. The first isolation of *C. pecorum* was from a sporadic encephalomyelitis of cattle reported by McNutt and Waller [51], and at that time it was considered as a *C. psittaci* strain.

C. psittaci strains isolated from ruminants were subdivided into two serotypes using a plaque-reduction test [69, 70]: serotype 1 comprises the strains causing abortion and enteritis (*C. abortus*), and serotype 2 consists in the strains inducing polyarthritis and conjunctivitis (*C. pecorum*). Further work divided *C. psittaci* strains isolated from ruminants into three main biotypes based on their culture characteristics, although only a small number of strains was used in this study [73]. Biotype 1 comprises strains causing abortion (*C. abortus* strains in the classification [16]) with compact inclusion morphology and inclusions appearing within 48 h. Biotype 2 comprises strains causing polyarthritis,

conjunctivitis, and encephalomyelitis. Strains isolated from faecal samples were included in biotype 3. The two latter biotypes, which correspond to *C. pecorum* strains, have diffuse inclusion morphology and inclusions appearing within 24 h. The abortion type grows well in a sheep fibroblast culture, unlike the enteric type [57]. A micro-immunofluorescence (MIF) test confirmed the two serotypes of *C. psittaci* identified by a plaque reduction test (serotype 2 corresponding to *C. pecorum*) [14]. Following subcutaneous inoculation into the footpad of mice, the *C. psittaci* strains were subdivided into three groups: (1) highly invasive strains causing splenomegaly and massive infection of the spleen; (2) invasive strains inducing a moderate enlargement of the spleen with a low level of splenic infection; and (3) non-invasive strains (*C. pecorum*) inducing neither increased spleen weight nor splenic infection¹. The invasive and highly invasive strains corresponded to serotype 1, i.e. *C. abortus*, in the new classification [16].

Genetic differences between the *C. pecorum* and *C. psittaci* strains were identified through restriction endonuclease analysis [18, 67], DNA-DNA hybridization, and Southern blot analysis with genomic DNA probes [18]. In 1992, Fukushi and Hirai [19] suggested separating the *C. pecorum* species from *C. psittaci* based on the results of DNA-DNA hybridization and immunological analysis. *C. pecorum* strains exhibit 10% or less DNA relatedness to strains of the other three Chlamydial species (*C. trachomatis*, *C. psittaci* and *C. pneumoniae*), while the homology levels are 88% or greater within the *C. pecorum* species. Restriction enzyme and gene probe analysis indicated that the Chlamydial strains infecting koalas belong to two distinct genotypes of *C. psittaci*, one corresponding to the strains causing urogenital diseases and similar to the SBE *C. pecorum* strain and the other grouping the ocular isolates [22]. This observation was

¹ Rodolakis A., A mouse model for studying the invasive and abortive properties of ruminant isolates, Chlamydial diseases of ruminants, Commission of the European Communities, Luxembourg, 1986, pp. 133–137.

confirmed by *ompA* (major outer membrane protein-MOMP gene) sequence analysis, indicating that the two Chlamydial species infecting koala are *C. pecorum* and *C. pneumoniae* [23].

New taxonomic methods were developed with the discovery of the polymerase chain reaction (PCR) and sequence analysis. This made it possible to analyse a greater number of strains using a small DNA sample, unlike former methods (biological, morphological, and DNA-DNA hybridization techniques, etc.) which required isolation and culture of the bacteria, a difficult procedure for an obligate intracellular bacterium such as *Chlamydia*.

The isolates of *Chlamydia* were grouped into two lineages using the 16S/23S intergenic spacer and domain I of the 23S gene [15]: *C. trachomatis* comprising human, swine, and mouse-hamster groups, and non-*C. trachomatis* comprising *Chlamydia pecorum*, *Chlamydia pneumoniae*, and *Chlamydia psittaci* abortion, avian, feline, and guinea pig groups. Then in 1999, Everett et al. [16] proposed a new classification dividing the Chlamydiaceae family into two genera, *Chlamydia* and *Chlamydomphila*, the former comprising 3 species, *Chlamydia trachomatis*, *Chlamydia muridarum* and *Chlamydia suis*, and the latter 6 species, *Chlamydomphila psittaci*, *Chlamydomphila abortus*, *Chlamydomphila caviae*, *Chlamydomphila felis*, *Chlamydomphila pneumoniae* and *Chlamydomphila pecorum*. Some *Chlamydiae* specialists have rejected this classification, particularly the division of the Chlamydiaceae family into two genera. According to these specialists [71], the new classification scheme is based on minor sequence differences in the 16S and 23S rRNA genes, and some of the new species include too few isolates. However, this new classification was confirmed by sequence analysis of five genes: MOMP, GroEL chaperonin, KDO-transferase, small cysteine-rich lipoprotein and 60 kDa cysteine-rich protein [5]. The majority of published studies on *Chlamydiae* isolated from animals cite this new classification.

Currently, sequencing or PCR-RFLP analysis of the *ompA* gene or the 16s/23s rRNA intergenic spacer appears to have facilitated the characterization of the different isolates of *Chlamydia*. Many studies have classified

different Chlamydial strains isolated from ruminants, pigs, koalas, wild animals and birds under the *C. pecorum* species.

The aim of this review was to further our understanding of the role of *C. pecorum* infections in animal health, focusing on previous and recent advances into the epidemiological and molecular aspects of this bacteria.

2. *C. PECORUM* INFECTIONS

2.1. In cattle

C. pecorum is probably the predominant Chlamydial species infecting the intestine [46] and genital tract [34] of healthy cattle. In a study using quantitative PCR, 67% of vaginal cytobrush specimens taken from heifers with high anti-Chlamydial antibody titres were found to be *C. pecorum* positive [11]. An investigative study of Chlamydial infections of female calves, using serial sampling from birth, showed that 51% of calves become infected with *C. pecorum* within the first 2 months of life [34]. These data demonstrate clearly that these genital infections are not necessarily transmitted by the venereal route. On the contrary, a low level of *C. pecorum* infection was found in the semen and preputial washing samples (1–2/120) taken from healthy bulls [41].

C. pecorum strains in cattle cause numerous diseases, such as sporadic bovine encephalomyelitis, polyarthritis, pneumonia, enteritis, vaginitis and endometritis [13, 39, 51, 76, 82, 83].

Chlamydial infections, including *C. pecorum*, can substantially reduce cattle fertility during the breeding season [38]. In a study in Germany, Sting et al. [75] found that direct detection of *C. burnetii* and *Chlamydiae* singly or combined, revealed significantly higher values of infection for cattle from positive herds than from control herds. Cows with insemination rates ≥ 2 excreted more *Chlamydia* via the genitals and presented more antibodies against *C. burnetii* than those with insemination rates < 2 . However, the ELISA capture and the complement fixation tests (CFT) used to detect the bacteria and the antibodies did not differentiate between *C. abortus* and *C. pecorum*.

In a study of Swedish cattle, however, the same seroprevalence (28%) of Chlamydial infection (using the Chekit ELISA) was observed in cows with reproductive problems and healthy controls [24], from which the authors concluded that *C. pecorum* is probably more widespread than *C. abortus*, and that *Chlamydophila* spp. is not related to reproduction disorders in Swedish cattle. However, in this study infected cases and controls were selected from the same herds. In addition, serial sampling to evaluate the infection status of the animals was not carried out, and the prevalence of the bacteria was very low.

Nevertheless, intestinal infections with *Chlamydophila* spp. (including *C. pecorum*) in cattle may contribute to long-term effects on animal health resulting in economic losses (e.g. reduced bodyweight), despite the absence of marked clinical signs [62]. In addition, respiratory Chlamydial infection due to *C. pecorum* and/or *C. abortus* could be associated with chronic inflammation of the lungs and airways affecting long-term lung function [33].

2.2. In small ruminants

As in cattle, *C. pecorum* strains are commonly found in the intestine of healthy animals such as sheep, with colonization beginning at 3 months of age when the young start to graze [8]. However, some isolates of *C. pecorum* have been associated with conjunctivitis, arthritis, and orchitis [65, 77].

Chlamydial abortion in small ruminants is generally due to *C. abortus*, but some *C. pecorum* strains have been isolated from ovine and caprine abortion cases, particularly in Morocco and Tunisia [63]. However, the intradermal or intravenous inoculation of pregnant ewes with *C. pecorum* strains isolated from ovine abortion cases did not induce the same abortion rate as *C. abortus* (1 out of 5) [66]. However, intraperitoneal inoculation of pregnant mice with these *C. pecorum* strains did not cause abortion, although the placentas were infected [63]. This study therefore suggests that the outbred mouse is not a suitable model for *C. pecorum* infection as previously shown [65]. None of the *C. pecorum* strains tested in the mouse model

was invasive. Other co-factors such as nutrition, parasites or the animal breed could play a role in abortion in small ruminants infected with *C. pecorum*.

Several studies have tested the pathogenicity of ovine intestinal *C. pecorum* strains, particularly in relation to abortion, using mouse or sheep models. For example, the oral infection of pregnant ewes with *C. pecorum*, alone or together with *Fasciola hepatica*, did not result in tissue invasion [58]. In another study, the subcutaneous injection of pregnant ewes or mice with intestinal *C. pecorum* strains did not lead to placental infection [1, 66, 81]. Only intravenous inoculation resulted in placental infection, but the infected pregnant ewes did not abort and the lambs' weight was not affected [58]. Intestinal *C. pecorum* infection in the mouse model is mainly controlled by the non-specific immune response [4]. All these studies suggest that intestinal *C. pecorum* strains are unable to pass through the intestinal epithelium but that they may persist in the intestine for a long time.

2.3. In swine

In swine, *C. pecorum* infection has been associated with pneumonia, polyarthritis, pleuritis, pericarditis and abortion [35–37]. Mixed infections of *C. trachomatis* and *C. pecorum* are probably the main cause of Chlamydial abortion in swine [72]. Unlike bovine Chlamydial infection, *Chlamydia*-like organisms have frequently been detected in vaginal and cervical swabs taken from healthy sows [6].

C. pecorum has been found only sporadically (1–2%) in semen and faeces samples from boars. *C. psittaci* has frequently been found in semen samples, while *C. suis* is the predominant Chlamydial species found in faeces samples [40].

2.4. In koalas

In koalas, *C. pecorum* strains cause conjunctivitis, urinary and reproductive tract disease and infertility [9, 50]. The level of *C. pecorum* infection in free-range koala populations could reach up to 85%, with 17% associated with clinical disease [32]. Young koalas have been

found to have a high level of *C. pecorum* infection, 58% involving both ocular and urogenital infections, suggesting that mother-offspring transmission is a major transmission route among the koala population. In addition, *C. pecorum* could be more pathogenic for koalas than *C. pneumoniae* species [32].

2.5. In other animals

A strain of *C. pecorum* that causes conjunctivitis has been isolated from the western barred bandicoot in Australia [80]. Three faecal samples from healthy pigeons in Japan were found to be *C. pecorum* positive using PCR [79]. Recently, a mixed infection with *C. pecorum* and *C. abortus* caused abortion in water buffalo in Italy [27].

3. THE TYPING OF *C. PECORUM* STRAINS

Simple typing methods and molecular markers are indispensable for epidemiological studies of *C. pecorum* infections and can also help improve the understanding of the pathogenicity of this *Chlamydophila* species.

Ovine and bovine *C. pecorum* strains present many genetic and antigenic variations [18]. Eight distinct *ompA* gene restriction patterns have been identified among 12 *C. pecorum* strains [12]. A MIF test identified 16 serotypes among 18 strains using a panel of monoclonal antibodies [68]. In contrast, the *C. pecorum* strains isolated from swine and koalas appear more homogenous. Eight swine strains were classified into 2 genotypes using the *ompA* sequence analysis [35]. Fifteen koala *C. pecorum* strains were grouped into 5 genotypes using the *ompA* sequence analysis [31]. Variant coding tandem repeats (CTR) in the *incA* gene enabled 19 *C. pecorum* strains to be classified into 3 groups based on the presence of various motifs. Seven out of eight strains isolated from animals with different clinical signs were found in a single group [84]. In that study the multi- virulence-locus sequence typing (MVLST) technique was used to classify 19 *C. pecorum* strains in 4 groups, one containing only 6 strains of different clinical and geographical origins.

A comparison of the number of repetitions of the CTR in the ORF663 gene showed that the strains isolated from sick animals had fewer repetitions than those isolated from the faeces of asymptomatic animals [85]. These studies suggest that differences exist between the *C. pecorum* strains isolated from asymptomatic animals and those isolated from sick animals.

4. GENETICS

4.1. Tandem repeats

Little is currently known about the genetics of *C. pecorum* because its genome sequence is not yet available. Recently, the sequence analysis of 12 genes demonstrated that *ompA* of *C. pecorum* (like that of *C. trachomatis* and *C. psittaci*) is a highly polymorphic gene with more than 300 polymorphic nucleotides. House-keeping genes in the *C. pecorum* strains were found to be very homogenous (less than 5 polymorphic nucleotides). Interestingly, 2 CTR variants have been identified: the CTR of *incA* (9–18 nucleotides) which contains different motifs and is rich in alanine and proline, and the CTR of ORF663 (15 nucleotides) which is rich in serine, proline and lysine. Such CTR may be involved in the virulence of *C. pecorum* [84, 85]. Another tandem repeat variant was previously identified in the *C. pecorum* genome (8 nucleotides) in the intergenic space *rrn-nqrF* (the 5S rRNA gene and the gene for subunit F of the Na⁺-translocating NADH-quinone reductase) [45]. In general, the tandem repeats are not very numerous in *C. trachomatis* and *C. pneumoniae* genomes [3]. The two tandem repeats found in the *Chlamydiae* genome are conserved in all Chlamydial species, one at the N-terminal of Pmp (polymorphic membrane protein) [28], and the other in the intergenic space of the ORF8 and ORF1 of the Chlamydial plasmid [78].

Bioinformatics technology has developed software for detecting the variable number tandem repeats (VNTR) on the bacterial genome. VNTR analysis for *C. trachomatis* strains has identified some tandem repeats, most having a single nucleotide [56]. In contrast, VNTR analysis for *C. psittaci* and *C. abortus*

shows a high discrimination power, although the number of repeats does not vary greatly between strains (2–6 copies) [42, 43].

Thus, the sequence of the *C. pecorum* genome could reveal some surprises regarding the existence and variability of the tandem repeats between the different *C. pecorum* strains.

4.2. Plasmids

The presence of plasmids in *C. trachomatis* and *C. psittaci* was first described in 1980 [47]. A plasmid has also been detected in *C. caviae*, *C. felis* and *C. pecorum* using the DNA-DNA hybridization method [22, 49], but not in *C. abortus* strains [48]. Restriction enzyme analysis has demonstrated that these 3 plasmids are closely related.

An average copy number of 4.0 ± 0.8 plasmids per chromosome has been determined in the EB of *C. trachomatis* and *C. pneumoniae* [59]. In addition, sequence analysis of a 7.5 kb DNA plasmid isolated from *C. trachomatis*, trachoma biovar, has identified 8 open reading frames and a region composed of four 22-bp repeats [10, 74]. Although the hypothetical origin of replication for the *C. trachomatis* plasmid appears to be near a region which contains four 22-bp tandem repeats [78], Chlamydial plasmids are unable to replicate in *Escherichia coli* [55].

Two 7.4-kb plasmids from two *C. pecorum* strains (bovine and koala) have been characterized by restriction endonuclease analysis. These plasmids, showing considerable cross-hybridization, are quite relatively distinct from the *C. psittaci* and *C. trachomatis* plasmids [30]. Some *C. pecorum* strains do not harbour plasmids (3 out of 11 isolates studied) since they cannot be found by Southern blot analysis [2]. A great number of *Chlamydia* strains do not have plasmids, suggesting that they are not required for Chlamydial growth.

The *C. pecorum* plasmid sequence is not yet available. It would be interesting to sequence it and compare its polymorphism to different *C. pecorum* strains. Its sequence may differ from the other Chlamydial plasmids. The presence of the plasmid in some *C. pecorum* strains could play a role in pathogenesis, as

shown recently in *C. trachomatis* infection. The *C. trachomatis* L2 plasmid could regulate the expression of some chromosomal genes such as *glgA*, encoding glycogen synthase [7]. Furthermore, the plasmid-deficient *C. muridarum* mutant strain can infect the murine genital tract, but fails to cause disease in the oviduct. Mice infected with this strain have been shown to be protected against oviduct disease when challenged with the virulent *C. muridarum* strain [53].

4.3. Chlamydiaphage

In addition to plasmids, some *Chlamydiae* strains harbor phages. In 1982, the first phage (Chp1) infection was described in an avian *C. psittaci* isolate [64]. Later, several phages were isolated from different Chlamydial species: *C. pneumoniae* (CPAR39), *C. caviae* (CPG1), and *C. abortus* (Chp2) [29, 44, 61]. A Chlamydiaphage (Chp3) belonging to the Microviridae was detected in the *C. pecorum* T52 strain isolated from sheep. Its genome is 4 554 bp long and encodes 8 open reading frames organized in the same genome structure as other Chlamydiaphages [17, 21]. Chlamydiaphages are unable to infect all Chlamydial species [17, 21].

Although no evidence has been found for the integration of phage DNA into the Chlamydial chromosome [48], Chlamydiaphages could play a role in the pathogenesis of *Chlamydia*, since the presence of CPG1 phage in *C. caviae* infection delays the appearance of the peak level of *Chlamydiae* in animals and decreases the pathologic response [60].

5. CONCLUSION

The pathogenesis of *C. pecorum* species is still unclear. This species should receive more attention from *Chlamydia* specialists for three reasons. Firstly, the persistence of *C. pecorum* strains in the intestine and vaginal mucus of ruminants could cause a long-lasting sub-clinical infection affecting the animal's health. Secondly, some endangered species such as koalas and bandicoots can be infected by *C. pecorum*

strains, which could further reduce the number of these animals due to abortion and infertility. Thirdly, the CTR found in the *inCA* and ORF663 genes could help differentiate between *C. pecorum* strains isolated from sick animals and those isolated from asymptomatic animals. This type of CTR appears to be involved in bacterial virulence and identification of its role in the biology of the *C. pecorum* species could be the key to understanding the pathogenesis of some *C. pecorum* strains.

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