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Review Article
Chlamydophila abortus Infection in Small Ruminants: A Review

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Abstract
Ovine enzootic abortion caused by *Chlamydophila abortus* (*C. abortus*). The disease is common in Europe, North America and some parts of Africa. It has great importance to decrease the economic losses as a result of abortion in small ruminants. Some of the abortions are due to pathogens that can cause zoonosis, threatening the public health other than the negative financial effects relating to animal production, animal products and animal health and public health importance in human. Serological detection of chlamydial infection in animal is of a little value due to cross reaction with other chlamydial species. Complement fixation test is recommended by OIE for detection of chlamydial antibodies in affected animals despite ELISA test is more sensitive and specific. The PCR technique is more sensitive and specific can overcome on the disadvantage of serological test and differentiate between different *Chlamydia* species.

Key words: Control, diagnosis, ovine chlamydiosis, pathogenesis, taxonomy, PCR, typing

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INTRODUCTION

Chlamydophila abortus, formally called *C. psittaci* serotype 1 is a non-motile, coccoid, obligate intracellular parasite. It belongs to the family Chlamydiaceae, which was recently reclassified and now comprises 11 separate species[1,2]. It is one of the most important causes of reproductive failure in sheep and goats[3,4]. In sheep the disease is usually manifested as abortion in the last 2-3 weeks of gestation, while the goats can abort at any stage of pregnancy, but most abortions are during the last 2-3 weeks of gestation[5,6]. The *C. abortus* has an affinity for placental tissues. In rams and bucks, *C. abortus* can cause orchitis and seminal vesiculitis, resulting in the shedding of the organism in semen[7].

The ELISA kits are commercially available for the detection of *C. abortus* antibodies in animals and this assay are more sensitive and specific as compared to Complement Fixation Test (CFT)[8].

Molecular techniques such as conventional and real-time PCR have been used to detect and differentiate between different chlamydial strains of *C. abortus* in abortion cases. Amplification of the outer membrane protein 2 gene (OMP2) was found to be a reliable procedure to diagnose *C. abortus* infection[9,10]. Furthermore, the multiple loci variable tandem repeats analysis (MLVA) allowed the direct typing of clinical samples[11].

CHLAMYDIACEAE FAMILY AND TAXONOMY

Members of the Chlamydiales are a unique and closely related group of Gram-negative obligate intracellular bacteria. Despite the evolutionary maintenance of a complex developmental cycle and a relatively small genome size (about 1000 kb). This bacterium has a unique life cycle inside the host cells in two different forms: Non infective Reticular Body (RB) and infective Elementary Body (EB)[12,13].

The systematic taxonomy established for Chlamydiae in 1999 uses up-to-date, prevailing criteria for bacterial classification, including DNA-DNA reassociation, 16S and 23S ribosomal RNA gene similarity, sequence similarity clustering of protein coding genes and genome size[1]. Until the family Chlamydiaceae was redefined by splitting it into two genera which included 11 species[14,11].

The genus *Chlamydia* comprises currently *C. trachomatis* occurs in humans, which cause trachoma, pneumonia and arthritis, *C. suis* (formerly porcine *Chlamydia trachomatis*) causes enteritis, conjunctivitis and pneumonia in swine and *C. muridarum* (formerly *Chlamydia trachomatis* of mice) causes respiratory infections in mouse and guinea pig.

Whereas, *Chlamydophila* comprises *C. psittaci* causes human psittacosis, avian chlamydiosis and abortions in bovine, *C. abortus* (formerly *C. psittaci* serotype 1) causes ovine enzootic abortion in sheep, *C. caviae* (formerly *C. psittaci* guinea pig strains) causes inclusion conjunctivitis in guinea pigs, *C. felis* (formerly *C. psittaci*, feline strains) causes feline pneumonitis in cats, *C. pneumonia* (formerly *C. pneumonia*) causes respiratory infections in humans and horses and *C. pecorum* (formerly *C. pecorum*) occurs in sheep, goats and cattle causes pneumonia, enteritis, infectious polyarthritis and abortions[15,16].

EPIDEMIOLOGY OF OVINE CHLAMYDIOSIS AND TRANSMISSION OF *C. abortus*

The *C. abortus* is considered the main cause of reproductive losses in sheep and goat at the global level with the exception of Australia and New Zealand that are free of disease. In Northern Europe OEA is the major cause of infectious abortions[17]. In the UK, for example, OEA accounted for 44% of diagnosed abortions due to infectious agents between the years 1995-2008. Moreover, more than 56% of small ruminant abortions in Spain were caused by this disease[16]. The *C. abortus* plays a substantial role in abortion in sheep and goat in Jordan1 and cause abortion in sheep with incidence rate 9.07% in Saudi Arabia[16]. There is few epidemiological data about the chlamydia in animal in Egypt but Osman *et al.*[5] detected 68% serologically positive aborted ewes with CFT.

The source of infection with disease may be the contaminated environment of the farm with infected vaginal or uterine discharge of infected animals. Moreover, the infected animal can be shedding the microorganism after abortion for several weeks[18].

Ingestion of infectious agents through feed or water that has been contaminated with abortion materials, uterine discharges and feces is a known route of infection[19]. Animals usually are protected after abortion but they can continue to shed.

Inhalation of contaminated dust, aerosols or droplets[18]. Mechanical transmission or venereal transmission of infectious agent in both directions during copulation between males and females[7]. If rams develop orchitis, their semen becomes infectious during mating[20]. Vertical transmission can have occurred from mother to lamb or kid. Newborns acquire the infection from their carrier mothers in two ways: Either during passage through the birth canal, in which case abortion is likely after maturity in the next year (second gestation) or by infection congenitally in-utero, in which case it will abort at its first gestation[21].

DISEASE (OVINE CHLAMYDOPHILOSIS)

Ovine enzootic abortion is caused by *C. abortus* which is a non-motile, Gram-negative, pleomorphic, obligate intracellular bacterium of the family Chlamydiaceae[12].

The *C. abortus* can be found in feces, urine, uterine discharges and less commonly in the milk of mothers that have suffered from abortion. Sheep and goats can also be asymptomatic chronic carriers[5].

This disease causes various reproductive failures in sheep and goats such as: Abortion (expulsion of the fetus prior to the normal end of pregnancy) mainly late in the last 2-3 weeks of pregnancy, dead lambs, premature births, stillbirths (live for not more than 48 h) and the birth of weak lambs with low birth-weight. Furthermore, *C. abortus* can cause mummification and maceration in sheep and goats, in addition to resorption of fetuses in sheep flocks[22,23].

This enzootic disease is very resilient and difficult to control with its periodic recurrence in the flock and the longevity of the disease-causing organism in farms and host animal bodies and is endemic to large parts of the world[24]. This disease is commonly characterized with 'Storms of abortions' that are cyclical every 3 or 4 years within an infected flock[21,25].

VIRULENCE FACTORS AND PATHOGENESIS

Incubation periods can be variable, but abortion usually occurs 5-6 weeks after the gain of the infection. Sheep infected with *C. abortus* out with pregnancy or during the early-to mid-stages of pregnancy showed no clinical signs, but the abortion is most commonly in the last 2-3 weeks of gestation[4]. In addition to abortion, ewes secreted a vaginal discharge around the time of abortion, but otherwise appear healthy. Consequently, *C. abortus* infection often processed unnoticed in sheep flocks until it is too late to implement preventative measures during the lambing period[15]. Following abortion, aborted fetuses are usually well-developed and do not have gross anatomical abnormalities, thickened, necrotic placental membranes and placental exudate, suggesting that placentitis is a key element in the pathogenesis of OEA[26].

The pathogenesis of *C. abortus* retrieved to three main virulence factors described by Carter and Wise[25]. The lipopolysaccharide genus-specific antigen or complement fixation antigen is thought to be a virulence factor and to encourage inflammatory reactions in the host. Another virulence attributed to *Chlamydia* is *Chlamydia* protease/or proteasome-like activity factor (CPAF), which enables *Chlamydia* to escape recognition by T-cells through diminishing the presence of host transcription factors that are associated with the production of Major Histocompatability Complex (MHC), consequently the interactions between immune cells (leukocytes and WBCs) mediated by (MHC) are disconnected and so to give the *Chlamydia* another chance to live and replicate. Another putative virulence factor is the type III secretion apparatus, which opens up a hole in the vacuole membrane to facilitate conveyance of pathogenic products into the cytosol of the host cell[26,18].

DIAGNOSIS

Accurate diagnosis of OEA caused by *C. abortus* is considered difficult. This difficulty is due to the intracellular viability, unique cycle of development and infection inside and outside the host, shortage of bacterium number in the uterine shedding over time, short and late antibody responses after abortion, contamination of fetus and fetal membranes with environmental agents, improper or inadequate selection and collection of samples, cross reactions with other related chlamydial strains or Gram-negative bacteria and the absence of predictive signs of abortion before occurring in most abortion cases. That's because most of the obvious normal-birth signs (enlargement, redness and discharges of vulva, restlessness and other behavioral changes) that usually happen at the last 48 h of normal birth are less obvious or even non-existent at the time of abortion[27].

Cytological staining: *Chlamydia* can be detected macroscopically in stained smears of exudates and feces or in impression smears of liver and spleen by a variety of techniques, using Giemsa, Stamp and Giménez stains. The modified Giménez technique[28], using carbol fuchsin and malachite green is routinely used by several laboratories for detection of chlamydial inclusions in smears. The bacteria appear as red to purple stained. The cytological examination can be helpful but are less sensitive and specific than immunochemical stainings or other diagnostic methods as PCR detection, since other Gram-negative bacteria like for instance *E. coli* may also stain red due to their affinity for carbol fuchsin.

Isolation of the organism: Tissue specimens, fecal samples and swabs are routinely used as samples for the isolation of Chlamydiae. The processing of the samples is similar for inoculation of cell cultures, embryonated eggs or laboratory animals[29].

Diluents such as phosphate-buffered saline (PBS, pH 7.2) and cell culture media often are used to prepare a 20-40% suspension of the homogenized sample. These diluents with antibiotics are satisfactory when the samples are to be inoculated within 24 h and will not be frozen[30].

Cell cultures are the most common and convenient method for the isolation of *C. abortus*. The most commonly used cell lines are BGM, vero and L-929, although a number of other cell cultures can be used. Following by identification with direct Fluorescent Antibody (FA) or another appropriate technique of staining of the infected monolayer[31,25].

In another hand, numerous laboratories isolate the chlamydiae on embryonated chicken egg. The standard procedure is to inject up to 0.3 mL of inoculum into the yolk sacs of 6-day-old embryos[32]. Replication of the chlamydiae may be cause death of the embryo within 5-12 days after inoculation. Chlamydial infection typically causes vascular congestion of the yolk-sac membranes, which are harvested and homogenized as a 20% yolk-sac suspension. The identification of *C. abortus* usually performed by staining of yolk-sac impression smears by either cytological or immunohostochemical staining[30,5].

Serological examination: There is some limitation for serological diagnosis of *Chlamydia* abortion as false positive result due to cross reaction with other related chlamydial strains as *C. pecorum*[33,34]. Serological diagnosis enables detecting Chlamydial antibodies in case of infection after 90 days of pregnancy due to the underlying nature of the pathogen that takes a long time in multiplying in the placenta[35]. Serology depends on detection of rising levels of *Chlamydophila abortus*-specific immunoglobulin G (IgG) antibodies in the animal sera between the acute and convalescent phases of infection[36].

Serological tests for *Chlamydia* are available in different natures and variable specificities, two tests are used mainly.

Complement Fixation Test (CFT): The first described serological test that was based on LPS and was previously the most used test in veterinary laboratories[37]. The CFT is the most widely used test and is still recommended by the office international des epizootic (OIE).

It can show rise in serum antibodies level at the time of abortion and stay as well for 6 weeks at least[38]. The test lacks specificity because it detects genus-specific LPS antigen which cross reacts with other members of the genus *Chlamydophila* such as *C. pecorum*[36,39].

Enzyme linked immunosorbent assay (ELISA): Depends on the binding of specific antibodies against the targeted antigens of the targeted organism, targeted antigens are bound by serum which is detected and categorized by color change to describe the status of the tested animal[8]. The ELISA using purified LPS have been developed in an attempt to produce more specific test that able to differentiate between *C. pecorum* and *C. abortus* infections, but most of them were not sufficiently sensitive and specific[40,10].

More specific indirect ELISAs based on recombinant antigen preparations such as Major Outer Membrane Protein (MOMP) and Polymorphic Outer Membrane Protein (POMP) that express a part of a protein at 80-90 kDa have been developed and shown to be more sensitive and specific than CFT in differentiating animals infected with *C. abortus* from those infected with *C. pecorum*[36,41].

MOLECULAR DIAGNOSIS

Polymerase chain reaction: Polymerase chain reaction is one of the most modern advanced techniques used for accurate diagnosis of the causative agent[42]. The PCR assay can differentiate between different bacterial causes incriminating in abortion in sheep using one multiplex PCR as described by Reisberg *et al.*[43]. The previous published PCR assay for *C. abortus* based on two different genomic target regions for amplification, namely the ribosomal RNA gene region[44,45] and the gene encoding the MOMP antigen designated omp1 or ompA[9,10].

If the goal is to define *Chlamydophila's* prevalence in a large group of animals, PCR should be the first choice, although more reliable tests imply more expensive tests. If the investment had limitations, an ELISA test can be the most adequate choice for the diagnosis. If the group of animals is just of some units, then cultured cells can be a possibility if the proper laboratory conditions are available[39,27].

Microarray: Rapid detection of bacterial pathogens remains a challenge to diagnosticians in veterinary medicine, particularly when slow-growing or non-culturable microorganisms are involved, as is the case with *Chlamydia* spp.[29,46].

Micro-arrays can be coupled with PCR where they serve as a set of parallel dot-blots to enhance product detection and identification of bacterial isolates. Recently, Schnee and Sachse[2] developed a DNA microarray-based detection and identification method for *Chlamydia* and *Chlamydophila* spp. The test was developed using the ArrayTube platform

(CLONDIAG® chip technologies). Unique species-specific hybridization patterns were obtained for all 9 species of the family Chlamydiaceae on both microarray types.

The DNA microarray-based approach, which is capable of verifying the exact nucleotide sequence of a target region through hybridization, appears a promising alternative. Moreover, a DNA microarray assay can be designed to target a large number of genomic loci (limited only by the size of the array) to ensure discrimination between microbial species, strains, geno-types, serotypes, resistance types, etc.[46]. They described DNA-microarray procedure for detection of 11 *Chlamydia* spp.

Typing: Recently, various literatures studied molecular typing of *C. abortus* strains using multiple-locus variable-number tandem repeat (VNTR) analysis (MLVA), as well as a different approach using a multilocus sequence typing (MLST) system was used to differentiate of *C. abortus* strains into distinct genotypes[47,48].

The MLVA typing method, based on the analysis of five VNTR loci, that able to the clustering of 145 *C. abortus* strains into six genotypes[47,11]. In contrast, MLST analysis targeting seven house-keeping genes[43], recognized four Sequence Types (STs) among the 16 *C. abortus* strains examined[48]. Taken together, MLST and MLVA analyses demonstrated that *C. abortus*, similarly to other genetically monomorphic bacteria has a predominantly clonal population structure consisting of subpopulations, many of which seem to be associated with particular geographic regions. Both techniques effectively distinguished the "LLG/POS variant" lineage from the "Clonal complex" one, sup-porting the notion that possibly two separate host niche adaptations of *C. abortus* have occurred through time. In combination, MLST and MLVA may provide additional information into the origins and evolutionary relationships of circulating *C. abortus* populations[11].

Treatment and control: The aborted animal, their live births and contact pregnant animals should be treated with injectable, long acting oxytetracycline (20 mg kg^{-1} body weight by IM route) to suppress the organism's multiplication[3] to reduce the infection severity and/or the abortion losses. This treatment is started 6 and 3 weeks before lambing and repeated every ten days or every two weeks until delivery time by subcutaneous route[17].

However, Rekiki *et al.*[49] warned against repeated tetracycline treatments, which could result in the development of antibiotic resistance.

The control of the disease should be performed through removing the abortion materials, fetuses and contaminated bedding from the barn, burning or disinfection followed by burying them deeply (not less than 1.5 m), besides cleaning and disinfecting the abortion site and all premises carefully[17].

Vaccination protocols on infected farms will considerably reduce abortion occurrence, it will not necessarily stop all abortion cases nor eradicate the disease because of re-shedding of organisms especially at the next birth[28]. Killed vaccines can reduce the incidence of abortion as well as the shedding of *C. abortus* at kidding, but unfortunately they do not completely stop *Chlamydophila* shedding, which leads to endemic cycles of infection that have serious consequences regarding the epidemiology of chlamydophilosis[18].

CONCLUSION

The *C. abortus* is one of most important infectious cause of abortion in small ruminants worldwide. In sheep the disease manifested as abortion in last 2-3 weeks of gestation period. The serological examination is most commonly used technique for diagnosis and epidemiological studies. Molecular diagnosis such as conventional and real-time PCR used to identify *C. abortus* in abortion cases based on OMP2 gene.

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