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Chlamydiosis in Goats (16 Jan 2001)

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Although most of the work on chlamydial infections of small ruminants concerns ewes, chlamydiosis has an economic and public health impact in numerous goat farms throughout the world. Chlamydial abortions were reported for the first time in Germany in 1959. After that the disease was diagnosed in Bulgaria, Spain, USA, France, India, Japan, United Kingdom, Chad, Greece, and Tunisia. In many areas, chlamydial abortion is the second cause of infectious abortions after brucellosis, and the main cause in most of the countries where brucellosis is controlled.

Aetiology

The disease is due to a small Gram-negative bacterium, *Chlamydia psittaci*, which grows in the cytoplasm of eucaryotic cells in a unique cycle of development in which a resistant infectious form, the elementary bodies (EB) alternate with a metabolically active non-infectious form the reticulate bodies (RB). The EB attaches to the membrane of the host cell and promotes its own endocytose in a membrane limited vacuole called the inclusion, which does not fuse with lysosomes. Then the EB transforms in RB, which replicates by binary fission. After several divisions, the RBs, which fill all the inclusion, transform back into infectious EBs. These EBs are released through host cell lysis or extrusion of the inclusion out of the host cell [1].

Taxonomy

Chlamydia psittaci is one of the four species of the genus *Chlamydia*, which also includes *Chlamydia trachomatis*, *Chlamydia pneumoniae* and *Chlamydia pecorum*.

Chlamydia trachomatis and *Chlamydia pneumoniae* are both human pathogens. *C. psittaci* infects a wide variety of birds, mammals and occasionally humans and *C. pecorum* contaminates ruminants, swine and koalas. In ewes and goats *C. pecorum* causes pneumonia, conjunctivitis and arthritis but is very often isolated from asymptomatic intestinal infections [2].

Except in very rare instances, strains inducing abortion in goats belong to *C. psittaci* serotype-1, which is also responsible for pneumonia, conjunctivitis, arthritis and sometimes, intestinal infections without any clinical signs. The *C. psittaci* species is very heterogeneous and before the fourth species *C. pecorum* appeared [3].

Phylogenetic analyses of 16S and 23S rRNA genes suggest the existence of nine differentiated species in the *Chlamydiaceae*, and lead Everett et al., [4] to propose the creation of two new genera *Chlamydia* and *Chlamydophila*. The genus *Chlamydia*, which corresponds to the old *Chlamydia trachomatis* denomination includes 3 species: *C. trachomatis* (human strains), *C. suis* (porcine strains related to *C. trachomatis* isolated from spontaneous abortions, vaginal infections, and pneumoniae) and *C. muridarum* (mouse-hamster strains).

The genus *Chlamydophila* regroups 6 species: *C. pneumoniae*, *C. pecorum*, *C. psittaci* (previous *Chlamydia psittaci* avian strains), *C. caviae*, the agent of guinea pig inclusion conjunctivitis (GPIC), *C. felis* (*C. psittaci* strains that infect cats) and *C. abortus* (classical serotype-1 *Chlamydia psittaci* strains). The results are in agreement with those obtained through phylogenetic analyses of five other coding genes (GroEL, KDO-transferase, MOMP, 60-kDa cysteine-rich protein, and cysteine-rich lipoprotein) [5] and the biological properties of the strains [6]. The restriction length polymorphism analysis (RFLP) of the ribosomal intergenic spacer domain of 16S-23S rRNA genes provides a rapid and reproducible method for identifying and classifying the chlamydial strains in the new species [7]. However, some members of the

scientific community do not agree with the proposal for a new Chlamydial taxonomy, as it does not take into account the whole genome of the bacteria.

Clinical Signs

Chlamydiosis is clinically characterized by abortion during the last months of pregnancy, stillbirths or premature births of weak kids with low birthweight. Abortions occur without previous clinical specific signs even if some goats may develop persistent cough without breathlessness, or arthritis and keratoconjunctivitis. In experimental infections, slight vaginal discharge was observed the day before abortion on some goats [8]. Retained placentas and metritis are not usual, even if they are more frequent than in ewes [9]. After the abortion, goats may recover rapidly [10] or may present brown discharge from the vagina. In experimental infections [8] or in some natural infections with a high rate of abortions [11] only 50% or less of goats that aborted, recovered quickly whereas post abortive sickness in ewes is unusual. This could be due to virulence differences between strains since very little is known on virulence mechanisms of strains. No difference in virulence could be shown in mouse models between ovine and caprine strains [12], but amplified fragment length polymorphism (AFLP) revealed genomic differences between caprine strain AC1 and ovine serotype-1 *C. psittaci* strains [13].

Although we have demonstrated that servicing infected goats could result in infected sires [14], until now no epididymitis due to *C. psittaci* has been described in sires. This is probably due to the very small number of studies on caprine chlamydiosis rather than a greater susceptibility of rams and bulls to chlamydial infections.

In a newly infected flock the rate of abortion is severe. Frequently 30% or more, sometimes 90% of pregnant does may abort and milk production may decrease. The high rate of abortion is observed for 2 or 3 years after which the disease takes on a cyclic nature: 10% of pregnant females will abort every year for several years until a new outbreak occurs and then all the yearlings will abort. The high level of immunity produced after abortion is responsible of the cyclic evolution of the disease in the herd: it is exceptional for a goat to abort twice. Papp and Shewen [15] have shown that some of the ewes that aborted can become chronically infected. Chlamydial antigens and DNA can be detected in the vagina, uterus and uterine tubes during the peri-ovulatory period of ewes that aborted. No research has been done to determine the incidence of chronic infections in goat herds.

The fetus does not display specific macroscopic lesion. Kids delivered close to term may be covered by brown material. Clear or blood-stained diffuse edema [16], blood-stained fluids in abdominal and pleural cavities and petechiae on the tongue, in the buccal cavity and on the hooves are often observed.

Transmission of the Disease

Infected does excreted large numbers of *Chlamydiae* in placenta and fetal fluids at the time of kidding and at the time of abortion. Some goats may shed *Chlamydia* in vaginal fluids from more than two weeks before abortion to more than two weeks after abortion. This may explain the higher incidence of abortion in newly infected herds of goats, since the susceptibility to infection varies in relation to the physiological status of the animal. Goats that are less than 100 days pregnant are more susceptible than those at the end of gestation or those that are barren. Smaller amounts of *Chlamydiae* can also be shed in urine, milk and feces during several days after abortion.

Young goats born from infected mothers may retain the infection in the herd or transmit it to other herds. The survey of a group of 27 yearlings in an infected herd during their first year of life demonstrated how they could spread the disease by not being detected by their serological response. These young goats could be divided into 3 groups according to gestation/parturition. The first group kidded normally a live kid, the second group was barren or had aborted too early in pregnancy to be detected and in the third group goats had aborted. The complement fixing (CF) antibodies of the two first groups increased to reach a maximum (1/80 - 1/160) at the time of breeding, then antibody levels decreased until the time of kidding. The third group had a CF antibody titer $\leq 1/40$ which is not considered as significant until the onset of abortions. The role the venereal transmission of chlamydiosis by males still needs to be investigated. However, genital infections in rams and bulls result in male infertility and sterility rather than abortion in females. The role that the disease plays in inapparent intestinal infection and its influence in the epidemiology of chlamydial abortion needs to be explored. The recent identification of molecular markers for caprine intestinal strains [13] would allow such studies.

Diagnosis

The diagnosis is usually performed by the detection of bacteria in smears or impression of the placenta combined with serological analysis of at least ten sera samples.

Staining of *Chlamydia* by the Stamp, Gimenez or Machiavello methods is quick and can be undertaken easily in most laboratories but its interpretation is often tricky as it requires an experienced person to differentiate *Chlamydia* from *Brucella* and *Coxiella*. Immunofluorescence using immunoglobulin conjugates marked with fluorescent labeled isothiocyanate, increases the sensitivity and specificity of the detection of chlamydia in smears or placenta impressions.

The presence of chlamydial antigens in ground placenta or vaginal swabs sampled just after abortion may be detected by ELISA with diagnostic kits developed for human *C. trachomatis* infections [17,18].

In human medicine, polymerase chain reaction (PCR) or its variation, ligase chain reaction (LCR) are considered to be the most sensitive diagnostic methods available for diagnosis of *Chlamydia*. Several primers common to all type of *Chlamydia*, as *Omp1*, the gene coding for the major outer membrane protein [19], or specific of *C. psittaci* [20] or *C. pecorum* [21] or of the serotype-1 *C. psittaci* strains [22] have been developed for veterinary application. But this technic remains expensive.

The complement fixation test (CFT) is the most widely used and considered being the gold standard for serological diagnosis. However, CFT is not very sensitive and not specific because the test uses an antigen i common with *C. pecorum*, which most goats harbor in their intestine. Therefore, positive reactions with titers between 1:10 and 1:40 are not specific for abortion but may relate to an intestinal infection with *C. pecorum*. The CFT test should preferably be done 3 to 6 weeks after abortion or lambing, when the antibody response is at its maximum level. The CFT test cannot be used for individual diagnosis or to detect infection in young or in males [23].

Several attempts were undertaken [24-29] to develop more specific techniques, which would distinguish between *C. psittaci* and *C. pecorum* infections. However, none of these tests was sufficiently sensitive and specific. Recently, a new indirect enzyme-linked immunosorbent assay (ELISA) based on a recombinant antigen that express a part of a protein of 80 - 90 kDa has been developed [30,31]. The test reacts with serum antibodies [32] elicited early against these highly immunogenic [33-35] multigenic family proteins [36]. This test has high sensitivity and high specificity [37].

Treatment

Tetracyclines affect the replication of chlamydia and can be effective in preventing abortions. The injection of 20 mg/kg of **oxytetracycline** given by intramuscular route at 105 and 120 days of pregnancy can prevent abortion but cannot prevent the chlamydial shedding at kidding.

Vaccination

Killed vaccines could reduce the incidence of abortion but not the excretion of *Chlamydiae* at kidding. Abortion induces a strong enough immunity to withstand later challenges [38], a live vaccine constituted with a temperature sensitive mutant of *C. psittaci* strains was developed [39]. Susceptible goats were vaccinated before mating [14] and no interference with the subsequent gestation was noted. Goats were protected against chlamydial abortion and chlamydial shedding at kidding was prevented. Nevertheless, when all goats in an infected herd are vaccinated the first year and all replacement animals are vaccinated on subsequent years, it could take about 3 years before abortions stop. This is due to latent infection in goats, goats that were infected before vaccination but had not aborted. Vaccination will not change the course of a latent infection. These goats may abort or may kid healthy or infected kid at term and may or may not shed *Chlamydiae*. As long as goats with a latent infection are present in a herd, it is not advisable to stop vaccinating (or else abortions would start anew), nor is it possible to sell vaccinated animals, excepted to breeders who vaccinate their flock regularly.

Future Prospects

The development of a vaccine, as efficient as the live vaccine mentioned, that would protect and allow the serological detection of infected goats in vaccinated flocks, would be very useful for the control of the disease. Depletion of the 'diagnosis specific antigen' from the live vaccine is not currently an option since suitable genetic and molecular methodologies are not yet available and its seems difficult to delete this protein family. For these reasons, an acellular vaccine, which protects against abortion and excretion would be of interest. Previous studies have shown that both T and B lymphocytes are involved in protective immunity [40]. In murine models, CD8⁺ T cells (Lyt 2⁺ in mouse) play a major role in protection following transfer of primed spleen cells [41]. To date, *C. psittaci* T-cell antigens and epitopes are unknown. Although caution must be exercised in extrapolating *C. trachomatis* results to *C. psittaci*, several T-cell epitopes have been identified on *C. trachomatis* major outer membrane protein (MOMP) [42]

The role of antibodies in preventing placental and fetal infection by *C. psittaci* has been demonstrated in mice. Passive transfer of specific polyclonal sera induces significant immunity albeit lower than T

cell-mediated protection [40]. Monoclonal antibodies (Mabs) which neutralize *C. psittaci* infectivity *in vitro* confer a remarkable immunity to pregnant mice after intravenous challenge since abortion and fetal colonization are eliminated [40]. Humoral immunity is involved in protection. Its effectiveness depends on the concentration of specific antibodies against the appropriate epitope. All protective Mabs isolated to date react with thermosensitive conformational epitopes located on a MOMP oligomer [43].

Hence, the MOMP oligomer could be a potential vaccine component [44]. It is not feasible, however, to produce this antigen by extraction from *chlamydiae* as cost of production would vastly exceed that a farmer would consider affordable for a caprine or ovine vaccine. Therefore, alternative methods of vaccine production must be investigated.

The generation of recombinant MOMP oligomer may be difficult. The gene encoding MOMP, *Omp1*, is well characterized, but high level expression of the full length gene from an unregulated promoter is toxic to *E. coli*. For this reason, different experimental strategies for the generation of the protective epitopes were undertaken. We tried to mimic the protective epitopes with monoclonal anti-idiotypic antibodies, or conformationally constrained peptide mimotope, but none of them were efficient. We decided to assess the vaccination of mice with DNA vaccine as DNA vaccination with MOMP gene protected mice against *C. trachomatis* [45] and turkeys against *C. psittaci* [46]. Until now only partial protection was obtained but further research (on target gene, concentration of DNA, route of vaccination, etc.) is needed to know whether mice could be protected with a DNA vaccine as efficiently as with live vaccine.

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