Recent advances on ovine chlamydial abortion

Annie Rodolakis, Jesus Salinas, John Papp

Vet. Res. 29 (1998) 275–288

Review article

Recent advances on ovine chlamydial abortion

Annie Rodolakis[a]*, Jesus Salinas[b], John Papp[c]

[a] Pathologie infectieuse et immunologie, Inra, Centre de Tours-Nouzilly, 37380 Nouzilly, France
[b] Department of Patologia Animal, Facultad Veterinaria, Universidad de Murcia Campus de Espinardo, Murcia, Spain
[c] Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON NIG2W1, Canada

(Received 15 September 1997; accepted 12 March 1998)

Abstract – This paper reviews new findings on ovine chlamydial abortion. Concerning chlamydial taxonomy, with the recent advances due to the analysis of the ribosomal genes, nine genotypic groups were identified separated into two lineages. It also describes the transmission of the disease, the site of entry of the organism and chlamydial shedding by underlying the role of latent infections. Recent results also concern the pathogenesis, with the kinetics of placental colonization and placental pathology leading to abortion in ruminants. Studies using experimental infection in a pregnant mouse model have allowed the identification of target placental cells and have shown the differential evolution of the infection according to the stage of pregnancy. Different diagnosis techniques are compared (ELISA, PCR and immunofluorescence for the direct diagnosis) but also the possibilities of distinction between antibodies against *Chlamydia pecorum* and *Chlamydia psittaci* (for serodiagnosis). The recent advances on prophylaxis are presented, as well as the research efforts needs for the next century. © Inra/Elsevier, Paris

chlamydiosis / taxonomy / transmission / pathogenesis / diagnosis / vaccine

Résumé – Avancées récentes sur la chlamydiose ovine abortive. Cet article est une revue des résultats récents sur divers aspects de la chlamydiose ovine. Concernant la taxonomie des chlamydia et plus spécialement les modifications apportées par l'analyse de la séquence des gènes ribosomaux, neuf groupes génétiques, séparés en deux lignées, ont été identifiés d'après la séquence de l'espace intergénique 16S-23S. La transmission de la maladie est ensuite décrite, de même que les sites de pénétration de la bactérie et l'excrétion des chlamydia, en soulignant tout particulièrement l'importance des infections latentes. Les nouvelles données concernent également la pathogénie par la cinétique de la colonisation du placenta et de l'induction des lésions aboutissant à l'avortement chez les ruminants. Des études menées lors d'infections expérimentales de la

* Correspondence and reprints
Tel.: (33) 2 47 42 78 88; fax: (33) 2 47 42 77 79; e-mail: Annie.Rodolakis@tours.inra.fr

souris gestante ont permis d'identifier les cellules placentaires cibles et de suivre la progression de cette infection suivant le stade de gestation où elle se produit. Les avantages et les inconvénients des techniques Elisa, PCR ou d'immunofluorescence pour la mise en évidence des chlamydia, ainsi que les possibilités de distinction des anticorps dirigés contre *Chlamydia psittaci* ou *Chlamydia pecorum* pour le diagnostic sérologique, sont abordés, ainsi que les méthodes actuelles de prophylaxie. La revue fait également le point sur les efforts de recherche à faire dans les années à venir.

chlamydiose / taxonomie / transmisssion / pathogénie / diagnostic / vaccin

Plan

1. INTRODUCTION

Chlamydia spp. are obligatory intracellular bacteria that replicate in the phagosome. They are widely distributed in the animal kingdom. In ruminants *Chlamydia* is responsible for several syndromes or infections which can be classified into three groups:

- clinically inapparent infections such as those of the intestine;
- local infections limited to the epithelial cells of the mucous surfaces, as they are the entry portal for chlamydiae, and as such may cause enteritis, conjunctivitis, mastitis or genital infections such as epididymitis and orchitis;
- systemic infections spread by the blood after the microorganism has entered the body through infected mucous membranes and leading to abortion, pneumonitis, polyarthritis and encephalomyelitis.

Abortion, known as 'enzootic abortion of ewes' (EAE) has economic and public health impacts. It is probably the most dramatic chlamydial infection of ruminants. This disorder induces abortions during the last weeks of pregnancy or premature births of weak lambs of low weight.

Losses due to this disease are difficult to evaluate accurately, but they are probably the most severe encountered by producers. The birth of lambs or kids is required regardless of whether production is oriented towards meat, milk, wool or replacement stock. During the acute phase of the disease 30 % of the pregnant ewes and sometimes more than 60 % of the pregnant goats may abort. However, the infected animal is then afforded a very efficient protective immunity and after 2–3 years a prevalence of abortion of 5–10 % is usual, but 5 years later an explosive outbreak affecting all the yearlings can occur.

Several cases of human abortions followed by severe complications have been associated with exposure to infected placentas from ewes or does that aborted due to *C. psittaci* (Buxton, 1986). The prevalence of such infections remains unknown, as infectious causes are not systematically searched for in human abortions.

2. RECENT ADVANCES IN CHLAMYDIAL TAXONOMY

The genus *Chlamydia* includes four species, *C. trachomatis*, *C. pneumoniae*, *C. psittaci*, *C. pecorum*, and more than 30 serovars.

2.1. *Chlamydia pecorum*

C. pecorum strains exhibit 20 % or less DNA homology with strains of the three other species (Fukushi and Hirai, 1992). Ruminant and porcine strains which were previously classified as the serotypes 2, 3, 4, 6 and 9 of *C. psittaci* by microimmunofluorescence (MIF) test were included in the *C. pecorum* species on the basis of *Omp* 1 [gene coding for the major outer membrane protein (MOMP)] sequence and restriction fragment length polymorphism pattern (RFLP) analysis (Storz et al., 1994). The *C. pecorum* strains constitute a heterogeneous group with different *Alu* 1 restriction patterns of the *Omp* 1 gene. However, all the patterns shared a 180-bp fragment or a corresponding restricted fragment of 110 and 70 bp (Denamur et al, 1991). They also present numerous antigenic variations (Salinas et al., 1995) but share at least two different epitopes recognized by species specific monoclonal antibodies (Mab) (Salinas et al., 1996). Most of these strains present a low degree of virulence and are considered to have placentopathogenic potential but not the inherent ability to cross-breach the intestinal barrier required for hematologic dissemination and placental localization. They mostly induce clinically inapparent intestinal infections or local infections limited to the epithelial cells of the mucous surface. The inability of *C. pecorum* isolates to invade deeper tissues was confirmed by inoculating the footpads of mice and examining the animals for splenic infection (Rodolakis et al., 1989a). In comparison, chlamydiae isolated from cases of EAE were invasive and were detected in the spleens of mice following footpad inoculation.

2.2. Identification of two lineages in the Chlamydiaceae family

The need to distinguish several separate species from *C. psittaci* was underlined during the 7th International Symposium on Human Chlamydial Infection at Harrison Hot Springs in 1990 (Storz et al., 1994). Indeed the *C. psittaci* species is a very heterogeneous taxon which contained almost all the animal strains before the reclassification of *C. pecorum* strains. The analysis of the ribosomal intergenic spacer domain 1 of the 23S rRNA gene, after PCR amplification provides a rapid and reproducible method for identifying and classifying chlamydial strains (Everett and

Andersern, 1997), easier and more precise than the current methods used to classify chlamydial strains (DNA–DNA hybridization, RFLP of *Omp* 1 gene, serological or biological properties). This technique, which is in agreement with those used previously, has allowed the identification of two lineages: *C. trachomatis* and non-*C. trachomatis*. Three genotypic groups constitute the *C. trachomatis* lineage (human, swine and mouse-hamster) while the non-*C. trachomatis* lineage regroups six distinct genotypic groups (*C. pecorum*, *C. pneumoniae* and *C. psittaci* avian, abortion, feline and guinea pig).

The *C. psittaci* abortion group corresponds to the serotype 1 of *C. psittaci* which is invasive in the mouse model. The homogeneity of this group at the genomic level was demonstrated by RFLP analysis of total DNA (Rodolakis and Souriau, 1992) or of the *Omp* 1 gene (Denamur et al., 1991). Mab raised against invasive-serotype 1 strain AB7 underlined its antigenic homogeneity (Salinas et al., 1995). Strains of the *C. psittaci* abortion group were characterized at the genomic level by RAPD-PCR (Sidi Boumedine et al., 1996) and amplified length polymorphism (AFLP), in order to obtain genetic markers essential for epidemiological investigations. These two techniques allow the identification of specific fragments that can be used to construct PCR primers for detection and rapid identification of chlamydial strains from clinical samples. In particular a fragment specific for French strains was identified by AFLP and may indicate a similar origin for all French ruminant isolates.

3. TRANSMISSION OF CHLAMYDIAL ABORTION AND CHLAMYDIAL SHEDDING

Investigations on transmission of *C. psittaci* among sheep initially focused on the peri-parturient period owing to the potential for exposure to infected placentas from ewes that aborted or gave birth to weak lambs. Environmental contamination as a source of infection was confirmed when pregnant ewes acquired *C. psittaci* after being housed with ewes that aborted (Blewett et al., 1982). Ingestion of *C. psittaci* from environmental sources may be the precursor to placental infection in pregnant ewes and/or inapparent intestinal colonization. Experimental oral inoculation of pregnant ewes with *C. psittaci* has induced abortion which was similar to that occurring with the natural disease (McEwen et al., 1951a; Parker et al., 1966; Wilsmore et al., 1984; Dawson et al., 1986). However, the site of entry of the organism may be anterior to the rumen since inoculation of the tonsillar crypts and not intraruminal inoculation induced disease (Jones and Anderson, 1988). Chlamydiae may gain access to the nasopharyngeal mucosae when ewes nuzzle, lick or ingest infected placentas or fetal tissues. In an attempt to mimic peri-parturient transmission, infected placenta was rubbed over the muzzle of ewes that recently lambed (Wilsmore et al., 1984). When these ewes were bred the following year, two out of seven aborted in late pregnancy owing to *C. psittaci*. Therefore, ewes can become infected at the conclusion of one pregnancy and develop clinical signs of infection during the next pregnancy.

Isolation of chlamydiae from the feces of healthy sheep suggested a possible source for the continual presence of the organism within flocks (Storz, 1964). Pregnant ewes intramuscularly inoculated with chlamydiae recovered from homologous or heterologous feces developed placental infection and pathology characteristic of enzootic abortion. However, fecal chlamydial isolates typically belong to *C. pecorum* and although abortogenic when systemically injected into pregnant ewes,

they are not considered to be able to invade beyond the intestinal tract (Rodolakis and Souriau, 1989). In addition, *C. pecorum* strains, as soon as they reach the placenta of pregnant mice, seem able to cross it more easily than strains of *C. psittaci* abortion group (Rodolakis et al., 1989a).

C. psittaci, being a reproductive tract pathogen in sheep, may persist in the uterus and uterine tubes of ewes that abort or give birth to weak lambs (Papp and Shewen, 1996a). Although fertility and fetal development were not compromised in chronically infected ewes, the potential for transmission to other ewes was apparent during the peri-ovulatory period when there was enhanced excretion of *C. psittaci* from the reproductive tract (Papp et al., 1994). Suggestive of venereal transmission, a preliminary study demonstrated that the vaginal mucosae of non-pregnant ewes was susceptible to *C. psittaci* infection such that the subsequent pregnancy resulted in the birth of weak chlamydial infected lambs (Papp and Shewen, 1996b). The potential for venereal transmission of *C. psittaci* among sheep has been investigated, without much success, by artificially inseminating ewes with contaminated ram semen (Wilsmore et al., 1984; Appleyard et al., 1985). However, a more definitive study would mimic natural reproduction by breeding rams to chronically infected ewes and then to naïve ewes.

4. CLINICAL DISEASE AND PATHOGENESIS

4.1. In ruminants

Whatever the route of transmission of the disease, nasopharyngeal or venereal, *C. psittaci* can cause infection via epithelial cells and macrophages (Kuo, 1988) with hematologic or lymphatic dissemination to different tissues, principally those of the lung, liver and spleen. Amin and Wilsmore (1995) detected chlamydial antigen in cells from different organs from ewes 2 d post-infection (pi.) but did not isolate *C. psittaci*, which may indicate a non-infectious form of the organism or a low level of infection. It has been suggested, on the basis of antigen and specific DNA detection, that the bacteria may reside in lymphoid tissues which drain the inoculation site (Huang et al., 1990; Buxton et al., 1996). These authors further suggest that *C. psittaci* can exist in a non-infectious or immature state as a consequence of the immune response of the ewe. The marked expansion of the medullar cords and of the paracortical zone, accompanied by substantial follicular development in the local lymph node indicates T- and B-cell stimulation as well as local production of IFN-γ, which may act in a chlamydiostatic fashion (Graham et al., 1995). However, the cells in which *Chlamydia* may be found have yet to be identified.

Placental pathology, which occurs as a result of the massive multiplication of chlamydia, becomes evident after 90 d of pregnancy (Buxton et al., 1990). Towards 60 d of pregnancy, normal physiological invasion of the caruncular stroma by chorionic villi coincides with haemorrhage from maternal vessels, accompanied by the formation of haematomas. These haematomas could be the means by which the chlamydiae pass from maternal blood supply and come into contact with the chorionic epithelium (Buxton et al., 1990). However, the placenta continues to be relatively resistant and no pathological change appears until 90 d of pregnancy, when infection can be detected by culture (Papp et al., 1993). Different endocrine mechanisms and/or changes related with the immune response may play an important part, permitting the rapid multiplication of chlamydia in the trophoblast at the

limbus of the placentomal hilus, accompanied by the appearance of local necrosis and contiguous spread of infection to involve the cotyledonary and intercotyledonary placenta and apposing endometrium (Buxton et al., 1990). At the same time, the fetus shows focal necrosis in the liver and other organs, suggesting the microorganism has cross-breached the maternal–fetal junction (Buxton et al., 1990).

During the final stages of pregnancy (days 125–140) the placenta is seriously infected by *C. psittaci* and alterations in the foetoplacental binding may lead to death and/or infection of the fetus, culminating in abortion. A lower level of infection may give rise to the premature birth of weak lambs of low weight that frequently die during the perinatal period. However, ewes are rarely affected clinically and remain fertile following abortion. In contrast, there is an increased frequency of placenta retention, endometritis and vaginitis in goats (Rodolakis et al., 1984) and cows (Wittenbrink et al., 1993).

In the fetus, there is an early immune response even before 90 d of pregnancy (Buxton et al., 1990) with the stimulation of B- and T-cells in the lymphoid tissues, and anti-chlamydiae IgM and IgG can be detected. This demonstrates that *C. psittaci* is capable of reaching the fetus even before the first lesions can be observed in the placenta. However, the level of infection in fetuses and the pathological changes are always lower than in placenta (Buxton et al., 1990).

One of the most important characteristics of chlamydiosis is the long-term immunity it confers to ewes, which do not suffer any more from chlamydial abortion. Such immunity may prevent future chlamydaemias and stop placental colonization even though cases have been described of ewes carrying and excreting the microorganism from the reproductive tract during the periovulation period (Papp et al., 1994). However, the following gestation remains normal with no evidence of chlamydiae in the vaginal, placental or neonatal samples. In these ewes with chronic infection, chlamydial antigen or DNA can be detected in the vagina, uterus and oviduct, with the endometrial cells in the basal stroma being the predominant site of infection (Papp and Shewen, 1996b). However, no sign of any pathology related to the persistent infection is presented except for an increase in the number of plasma cells and intraepithelial lymphocytes in the uterus. The *C. psittaci*-specific IgG antibody responses in these ewes is apparently sufficient to eliminate chronic infection (Papp and Shewen, 1996b).

Jones (1995) raised several unresolved questions concerning the pathogenesis of the disease. For example, in which tissues and cells is *C. psittaci* found in a latent state? What factor or factors inhibit its growth? Why do *C. psittaci* prefer trophoblastic cells in ruminant, human or murine placenta? What are the factor(s) responsible for *C. psittaci* changing from its latent to its active state at about 90 d of pregnancy in the ewe?

4.2. In the mouse

Some of these questions may be addressed in a mouse model, in which inoculation at mid-pregnancy (day 11) with strains of *C. psittaci* serotype 1, induce colonization of the placenta and subsequent abortion (Rodolakis, 1976). These abortions appear similar in certain respects to those observed in cases of natural or experimentally induced abortion in small ruminants. This model was used to demonstrate that Lyt-2$^+$T-cells (CD8 T-cells) played a major role in the protection (Buzoni-Gatel et al., 1992). There was a dramatic decrease in protection following in vivo depletion of Lyt-2$^+$T-cells.

Although Lyt-2$^+$T-cells were the main cells responsible for immunity, the lack of complete reduction in protection after Lyt-2$^+$T-cells depletion may indicate that other cellular phenotypes were involved in protective mechanisms.

Recent studies (Buendia et al., 1998) have shown that abortion in this animal model occurred in the final stages of pregnancy (about day 20) whether inoculation with *C. psittaci* is carried out near the early pregnancy (day 7) or mid-pregnancy (day 11). In both cases the primary target organs of colonization were the liver and spleen after intraperitoneal inoculation with the serotype 1 of *C. psittaci*, and chlamydial antigen could be detected 2 d pi. inside macrophages and neutrophils (unpublished data).

Regardless of the time of infection, chlamydial antigens are not detected in the placenta before day 5 pi. In mice inoculated at mid-pregnancy, immunoreaction is detected in the metrial gland and decidua basalis mainly in trophoblastic cells, neutrophils, and granulate metrial gland (GMG) cells (Sánchez et al., 1996). GMG cells are the most numerous lymphoid cells in the utero-placental unit in rodent pregnancy, being morphologically, antigenically and functionally related to NK cells (Croy, 1994). The metrial gland becomes infected by chlamydiae from day 6–7 pi. (day 17–18 of pregnancy), reaching the maximum level at day 9 pi., just before abortion.

In mice inoculated during early pregnancy, chlamydial antigens are also detected from day 5 pi. in trophoblastic cells and neutrophils in the decidua basalis albeit in small amounts (Buendia et al., 1998), but the GMG cells are not affected until day 16 of pregnancy (day 9 pi.). At days 10–11 pi. (days 17–18 of pregnancy) the metrial gland begins to be invaded, with a maximum degree of infection occurring at about day 13 pi. (day 20 of pregnancy), resulting in abortion.

The pattern of placental colonization is similar in both cases, early and mid-pregnancy inoculations, although it occurs a few days later if inoculation is carried out at the beginning of pregnancy. Chlamydial antigen can be detected 5 d pi. although massive colonization of the placenta does not occur until day 17–18 of pregnancy. The factor(s) which prevent the massive multiplication of chlamydiae and the ensuing grave pathological changes until this time has passed, are still unknown.

GMG cells begin to lose their functional character in natural conditions from the 16th day of pregnancy (Delgado et al., 1996) which may allow them to become susceptible to pathogenic microorganism attack. However, only their susceptibility to infection by *C. psittaci* has been described (Sánchez et al., 1996). One of the functions of GMG cells is, probably, to protect the placenta against such infectious agents (Croy, 1994). The infection of these cells by *C. psittaci* at about day 16 of pregnancy would speed up the process of natural degeneration. The consequent liberation of cytotoxic proteins which, in normal physiological conditions play a part in facilitating natural birth (Croy, 1994), together with the important infiltration of neutrophils and direct damage by *C. psittaci* might be sufficient to initiate abortion in the final stages of pregnancy.

Despite the similarities, we can not extrapolate the results of the mouse model directly to sheep, since there are substantial differences in the type of placenta and local immune response. However, it is well known that cells exist in endometrial tissues which are morphologically and functionally analogous to the GMG cells of pregnant mouse uterus although they are $\gamma\delta$ TCR$^+$ CD8$^+$ (Hansen and Liu, 1996), but it is not known whether these cells develop NK activity in sheep. During pregnancy, these lymphoid cells secrete

inhibiting cytokines such as transforming growth factor-β and others which stimulate placental growth and the secretion of hormones, such as prostaglandin (PG) E_2. Furthermore, trophoblastic cells produce interferon-tau (IFN-τ) which inhibits lymphocyte proliferation and increases the killing activity of NK cells (Hansen and Liu, 1996). These cytokines, together with hormones such as progesterone, progressively reduce the number and activity of the endometrium lymphocytes, except the γδ TCR^+ $CD8^+$ large granulated lymphocyte subpopulation, which increases in the luminal epitelium in the interplacentomal endometrium. At the end of pregnancy, these cells may represent up to 10 % of the cells of this epithelium (Lee et al., 1992). It would be very interesting to study the role of these lymphoid cells in the ovine chlamydial abortion.

Graham et al. (1995) showed that ovine IFN-γ has a strong chlamydiostatic effect, blocking the use of the L-tryptophan by chlamydiae. However, no study has demonstrated the presence of IFN-γ in ovine placenta during any stage of pregnancy. Furthermore, the equilibrium between the local production of IFN-γ in any organ and the concentration of tryptophan in plasma might be a mechanism by which infections remain latent in any sheep organ.

Hormonal changes during pregnancy have also been investigated and progesterone, oestradiol 17-β and PGE_2 levels have been shown to be altered when the placenta becomes infected by *C. psittaci* (Leaver et al., 1989).

5. DIAGNOSIS

Diagnosis of the disease is usually made by examination of fixed and stained impressions or smears prepared from the placenta associated with serological analysis of sera samples from about ten aborted or lambing ewes by complement fixation (CF) test or ELISA. These techniques, which are often the only methods available for a large number of laboratories, lack sensitivity and specificity. Indeed, chlamydiae stained by Gimenez, Machiavello or Giemsa methods and microscopically examined appear as small bright coccoid structures either individually or in groups. This method of detection may be time consuming and subject to error since *Chlamydia* may be confused with *Coxiella* or *Brucella*. In all cases bacteriological analysis should be undertaken together with serological analysis to avoid interpretative errors. Isolation of chlamydiae in embryonated hen eggs or cell culture is not routinely performed by veterinary laboratories because it is labor intensive and prone to contamination.

5.1. New specific and sensitive methods for direct diagnosis

New more specific and sensitive methods than bacteriological methods, dependent on evidence of the presence of chlamydial antigens, are being developed. The presence of chlamydial antigen in samples is detected by immunoglobulin conjugates marked with fluorescent labelled isothiocyanate (immunofluorescence) or by alkaline phosphatase (ELISA). Diagnostic kits are available for detection of *C. trachomatis* infections in humans which can be used for detecting the presence of chlamydiae in vaginal swabs of aborted ewes, using antibodies raised against specific antigens of *C. psittaci* (Amin and Wilsmore, 1994; Wilsmore and Davidson, 1991). In spite of their cost, they can be of interest if the laboratory is not involved in regular chlamydial diagnosis or for confirmation of doubtful bacteriological results. For veterinary laboratories, the detection of chlamydial antigen by immunofluorescence or ELISA is more sensitive than iso-

lation, whereas in human medicine it is the opposite (Wilsmore and Davidson, 1991; Black, 1997). This is probably due to the fragility of the chlamydiae and differences in the sampling conditions. In human medicine, the patients go directly to the laboratory for sampling or samples are sent very quickly and under the best conditions to the analytical laboratory treatment which results in a good chance of survival of chlamydiae.

However, polymerase chain reaction (PCR) and ligase chain reaction (LCR) are considered to be the best diagnostic methods in human medicine, as they allow the detection of the greatest number of infected individuals. Many PCR primers which allow chlamydial detection have been described. Some of the diagnostic kits available for human medicine can be used in veterinary medicine, because they work by priming the oligonucleotides of the *Omp* 1 gene common to all types of chlamydiae. The species discrimination is possible after RFLP (restriction fragments length polymorphism) analysis of the amplified fragment (Denamur et al., 1991). Likewise, this distinction can be performed by amplification and RFLP analysis of the 16S-23S rRNA spacer region (Anderson et al., 1996). Several PCR primers for veterinary use which allow chlamydial detection and which distinguish between chlamydial species by the length of the *Omp* 1 (Kaltenboek et al., 1992) or *Omp* 2 (Sheehy et al., 1996) amplified fragments have been proposed. With the primers identified by RAPD-PCR (random amplified polymorphic DNA) it is possible to perform a multiplex PCR allowing a rapid and direct identification of *C. psittaci* or *C. pecorum*, as well as the characterization of some particular abortion strains (Sidi Boumedine et al., 1996).

Nevertheless these techniques require expensive apparatus and reagents, as well as experienced personnel working with very strict precautions to avoid false positive results. However false negative results due to the presence of inhibitors in the samples are a larger problem than false positive since they are difficult to detect.

5.2. Improvement of serological diagnosis

The complement fixation (CF) test is widely used for the detection of chlamydial antibodies in the blood of the animals. It is an easy method but it is hampered by *C. pecorum* that most ruminants harbor in their intestine, as the antigen used is common to all the genus *Chlamydiae*. This has resulted in standardization of the method and fixing of a threshold value to separate animals considered as positive. This implies:

- CF test cannot be used for individual diagnosis;
- it must be carried out 3–6 weeks after abortion or lambing;
- it does not allow the detection of the infection in lambs or in rams.

Other techniques such as ELISA or immunofluorescence, can be used. They present the same drawbacks as they use the same type of antigen but they suppress the problem of anticomplementary sera.

Several attempts to develop more specific techniques which distinguish between *C. psittaci* and *C. pecorum* infections were reported:

- ELISA with soluble antigen obtained by treatment of purified *C. psittaci* strain, isolated from abortion, with sodium deoxycholate (Sting and Hafez, 1992), or antigen preparations derived from *C. psittaci* strain isolated from abortion or *C. pecorum* strain, treated by SDS (Markey et al., 1993), or with LPS extracted from chlamydiae (Sting and Hafez, 1992; Jones et al., 1997) or recombinant LPS (Griffiths et al., 1996) as antigen;

- an indirect ELISA with a soluble antigen obtained by treatment of purified chlamydiae from *C. psittaci* abortion group with sarkosyl then sodium periodate to reduce the reaction due to residual LPS (Anderson et al., 1995);
- an indirect immuno-fluorescence test (IFAT) (Markey et al., 1993).

None of these tests, however, was sufficiently sensitive and specific. Use of more specific antigens will improve diagnosis of EAE. For this purpose Mab raised against *C. psittaci* strain isolated from ovine abortions was used to characterize chlamydial antigens. A highly immunogenic antigen, serotype 1-specific, located on the 80- to 90-kDa protein region of *C. psittaci* was identified (Souriau et al., 1994). The antibodies against this antigen appear very early in the infection and as soon as 8 d after experimental inoculation. This antigen was recombinantly expressed in *E. coli* (Rodolakis et al., 1995) and partially purified. The first results in ELISA tests have been very promising and the validation of its use is in progress.

6. PROPHYLAXIS OF THE DISEASE

Contrary to *C. trachomatis*, which can give rise to multiple episodes of infection in human with an apparent lack of natural immunity, a first exposure of ruminants to *C. psittaci* induces an immunity strong enough to withstand later challenges (Rodolakis and Souriau, 1980). This implied that vaccination could control the chlamydial abortion in goats and ewes, and had prompted researches for commercial inactivated vaccines. An adjuvant vaccine whose value has been subject to discussion was developed a long time ago (McEwen et al., 1951b). Killed vaccines could reduce the incidence of abortion but not the shedding of chlamydiae at lambing (Rodolakis and Souriau, 1979) leading to endemic cycle of infection, which has serious consequences on the epidemiology of the disease. In the UK, systematic utilization of this type of vaccine may have exerted a selective pressure towards higher virulence of field strains and/or antigenic shift from the initial type used in vaccine production (Aitken et al., 1986).

The inactivated vaccines hitherto employed have been developed and used empirically without identification of the immunoprotective antigens of *C. psittaci* or assessment of the host protective mechanisms. The level of complement fixing (CF) antibodies induced in rabbits was used to control vaccine variation but these antibodies are not considered protective; so variation of the efficiency of the different batches of commercial vaccines could be frequent through lack of an accurate control method. This was confirmed by the mouse model where the vaccine potency was estimated by recording the average number of living infant mice per litter at birth and their survival during their first week (Rodolakis et al., 1981). Indeed, due to their rapid reproductive capacity, mice could provide an excellent model to investigate the efficiency of vaccines. This test demonstrated the possibility of inducing a good immune response with inactivated vaccines but requires large amounts of chlamydiae and thus may not be cost effective for sheep producers. An avirulent live vaccine would not require as much chlamydiae to obtain protection and may reproduce the immune mechanisms of the natural disease better than a killed vaccine.

For this purpose a temperature-sensitive mutant of *C. psittaci* AB7 designed 1B was obtained (Rodolakis, 1983) the virulence of which was reduced for ewes, goats and mice. When it was given to susceptible ewes (Rodolakis and Souriau, 1983) or goats (Rodolakis and Souriau, 1986) before mating it did not interfere with their subsequent gestation, protected

them against abortion and prevented chlamydial shedding at delivery.

The stability of the strains was evaluated in cell culture by serial passages at the restrictive temperature or in persistent infection (for 6 months), by one passage in pregnant mice and ten passages in non-pregnant mice, and three passages in lambs (Rodolakis et al., 1989b). All passaged strains obtained did not differ from the original 1B strain in virulence tests in mice. In addition their thermolability at 51 °C was the same as 1B strain. Even if the virulence factors and genetic localization of the mutation are still unknown, strain 1B thus appears as very stable, which is an important characteristic for a live vaccine. The efficiency of vaccine against challenge with four different isolates from killed vaccine failures was demonstrated (Rodolakis and Bernard, 1984; Chalmers et al., 1997). It can be used simultaneously with live *Brucella* Rev 1 vaccine, and live *Salmonella* Rv6 vaccine (Souriau et al., 1988). The compatibility of the chlamydial vaccine 1B with live *Toxoplasma* vaccine is acceptable if the vaccines are injected at different sites (Chalmers et al., 1997). The vaccine 1B is now available in the UK, France and Spain (Enzovax, Intervet, Tecvax Chlamydia Vetoquinol). One vaccination is sufficient to protect more than three successive gestations. So in case of chlamydial outbreak, the first year, all the flock must be vaccinated before mating, then the following years, only replacement animals need to be vaccinated.

7. FUTURE PROSPECTS

To control the disease, future research on EAE would concern the following.

The *improvement of diagnosis*, with better detection of chlamydiae in the samples and a more sensitive and specific serodiagnosis. This could be achieved by PCR or derived techniques and the recombinant antigen expressing the highly immunogenic diagnostic antigen serotype 1-specific located on the 80- to 90-kDa protein region of *C. psittaci* (Rodolakis et al., 1995).

The *development of an acellular Chlamydia vaccine* which protects against abortion and chlamydial shedding at parturition and does not hamper serodiagnosis. In the mouse model, both cellular and humoral immunity induced protection (Buzoni-Gatel et al., 1987). By passive transfer of Mab, pregnant mice can be protected against abortions and fetal and placental colonizations lowered by 5 log PFU (Buzoni-Gatel et al., 1990). All the protective Mab obtained were serotype 1-specific and recognized heat-sensitive epitopes located on an oligomer of the MOMP of *C. psittaci* (De Sa et al., 1995; McCafferty et al., 1995). Mice vaccinated with the purified MOMP oligomer alone or in combination with cross-reactive LPS from *Salmonella minnesota* Re were protected against abortion, and placental infection was considerably reduced. Excretion of chlamydiae could certainly be prevented with such vaccines. However, vaccine trials with a subcellular outer membrane fraction (Tan et al., 1990) or recombinant MOMP (Herring et al., 1994) previously performed in ewes were disappointing. The presence of protective epitopes in these vaccines was not assessed. This underlines the importance of having an accurate method to control vaccines. As cell culture yields of *C. psittaci* are poor, the purification of MOMP oligomer from chlamydiae is prohibitively expensive for an ovine vaccine. However the development of an acellular vaccine composed by a protective antigen different from the diagnosis antigen will allow the detection of infected animals even in vaccinated flocks. This could be very helpful if it is as efficient as the live vaccine.

Epidemiological studies needing to be performed in the future include the role of inter species contamination in EAE, and the eventuality of reservoirs (birds, cats, dogs or wild rodents). These studies would become possible with a better knowledge of chlamydial taxonomy and the identification of strain markers by RAPD-PCR and AFLP.

The *pathogenesis of the disease*. Future research in this field should attempt to determine the factor or factors that allow *C. psittaci* to persist but also those which determine the complete invasion of ovine placenta during the last third of pregnancy.

REFERENCES

Aitken I.D., Anderson I.E. Robinson G.W., Ovine chlamydial abortion: limitation of inactived vaccine, in: Aitken I.D. (Ed.), Chlamydial Disease of Ruminants, Commission of the European Communities, Luxembourg, 1986, pp. 55–65.

Amin J.D., Wilsmore A.J., Detection of *Chlamydia psittaci* (*ovis*) antigen in tissue section and McCoy cells using streptavidin-biotin and IMAGEN^{TM} staining method, Br. Vet. J. 150 (1994) 555–560.

Amin J.D., Wilsmore A.J., Studies on the early phase of the pathogenesis of ovine enzootic abortion in the non-pregnant ewe, Br. Vet. J. 151 (1995) 141–155.

Anderson I.E., Baxter S.I.F., Dunbar S., Rae A.G., Philips H.L., Clarkson M.J., Herring A.J., Analysis of the genomes of chlamydial isolates from ruminants and pigs support the adoption of the new species *Chlamydia pecorum*, Int. J. Syst. Bacteriol. 46 (1996) 245–251.

Anderson I.E., Herring A.J., Jones G.E., Low J.C., Greig A., Development and evaluation of an indirect ELISA to detect antibodies to abortion strains of *Chlamydia psittaci* in sheep sera, Vet. Microbiol. 43 (1995) 1–12.

Appleyard W.T., Aitken I.D., Anderson I.E., Attempted venereal transmission of *Chlamydia psittaci* in sheep, Vet. Rec. 116 (1985) 535–538.

Black C.M., Current methods of Laboratory diagnosis of *Chlamydia trachomatis* infections, Clin. Microbiol. Rev. 10 (1997) 160–184.

Blewett D.A., Gisemba F., Miller J.K., Johnson F.W.A., Clarkson M.J., Ovine enzootic abortion: The acquisition of infection and consequent abortion within a single lambing season, Vet. Rec. 111 (1982) 499–501.

Buendía A.J., Sánchez J., Martínez M.C., Cámara P., Navarro J.A., Rodolakis A., Salinas J., The pathogenic mechanism of chlamydial abortion in a mouse model is not dependent on the time of inoculation, Infect. Immun. (1998) in press.

Buxton D., Potential danger to pregnant women of *Chlamydia psittaci* from sheep, Vet. Rec. 118 (1986) 150–151.

Buxton D., Barlow R.M., Finlayson J., Anderson I.E., Mackellar A., Observations on the pathogenesis of *Chlamydia psittaci* infection of pregnant sheep, J. Comp. Pathol. 102 (1990) 221–237.

Buxton D., Rae A.E., Maley S.W., Thomson K.M., Livingstone M., Jones G.E., Herring A.J., Pathogenesis of *Chlamydia psittaci* infection in sheep: detection of the organism in a serial study of the lymph node, J. Comp. Pathol. 114 (1996) 221–230.

Buzoni-Gatel D., Bernard F., Andersen A.A., Rodolakis A., Protective effect of polyclonal and monoclonal antibodies against abortion in mice infected by *Chlamydia psittaci*, Vaccine 8 (1990) 342–346.

Buzoni-Gatel D., Guilloteau L., Bernard F., Bernard S., Chardes T., Rocca A., Protection against *Chlamydia psittaci* in mice confered by Lyt-2^{+} T cells, Immunology 77 (1992) 284–288.

Buzoni-Gatel D., Rodolakis A., Plommet M., T cell mediated and humoral immunity in a mouse model *Chlamydia psittaci* systemic infection, Res. Vet. Sci. 43 (1987) 59–63.

Chalmers W.S.K., Simpson J., Lee S.J., Baxendale W., Use of a live Chlamydial vaccine to prevent ovine enzootic abortion, Vet. Rec. 141 (1997) 63–67.

Croy B.A., Granulated metrial gland cells: hypotheses concerning possible functions during murine gestation, J. Reprod. Immunol. 27 (1994) 85–94.

Dawson M., Zaghloul A., Wilsmore A.J., Ovine enzootic abortion: experimental studies of immune responses, Res. Vet. Sci. 40 (1986) 59–64.

De Sa C., Souriau A., Bernard F., Salinas J., Rodolakis A., An oligomer of the major outer membrane protein of *Chlamydia psittaci* is recognized by monoclonal antibodies which protect mice from abortion, Infect. Immun. 63 (1995) 4912–4916.

Delgado S.R., Mc Bey B.A., Yamashiro S., Fujita J., Kiso Y., Croy B.A., Accounting for the peripartum loss of granulated metrial gland cells, a natural killer cell population from the pregnant mouse uterus, J. Leukoc. Biol. 59 (1996) 262–269.

Denamur E., Sayada C., Souriau A., Orfila J., Rodolakis A., Elion J., Restriction pattern of outer menbrane gene provides evidence for

homogeneous invasive group among ruminant isolates of *Chlamydia psittaci*, J. Gen. Microbiol. 137 (1991) 2525–2530.

Everett K.D.E., Andersern A.A., The ribosomal intergenic spacer and domain I of the 23S rRNA gene are phylogenetic markers for *Chlamydia* spp., Int. J. Syst. Bacteriol. 47 (1997) 461–473.

Fukushi H., Hirai K., Proposal of *Chlamydia pecorum* sp.nov for *Chlamydia* strains derived from ruminants, Int. J. Syst. Bacteriol. 42 (1992) 306–308.

Graham S.P., Jones G.E., MacLean M., Livingstone M., Entrican G., Recombinant ovine interferon gamma inhibits the multiplication of *Chlamydia psittaci* in ovine cells, J. Comp. Pathol. 112 (1995) 185–195.

Griffiths P.C., Plater J.M., Horigan M.W., Rose M.P.M., Venable C., Dawson M., Serological diagnosis of ovine enzootic abortion by comparative inclusion immunofluorescence assay, recombinant lipopolysaccharide enzyme-linked Immunosorbent assay and complement fixation test, J. Clin. Microbiol. 34 (1996) 1512–1518.

Hansen P.J., Liu W.-J., Immunological aspects of pregnancy: concepts and speculations using the sheep as a model, Anim. Reprod. Sci. 42 (1996) 483–493.

Herring A.J., McCafferty M.C., Jones G.E., Dunbar S., Andersen A.A., Vaccination against chlamydial abortion in sheep: problems and progress with a recombinant vaccine, in: Orfila J., Byrne G.I., Chernesky M.A., Grayston J.T., Jones R.B., Ridway G.L., Saikku P., Schachter J., Stamm W.E., Stephens R.S. (Eds.), Chlamydial Infections, Esculapio Bologne, 1994, pp. 118–121.

Huang H.-S., Buxton D., Anderson I.E., The ovine immune response to *Chlamydia psittaci;* histopathology of the lymph node, J. Comp. Pathol. 102 (1990) 89–97.

Jones G.E., *Chlamydia psittaci*: prevailing problems in pathogenesis, Br. Vet. J. 151 (1995) 115–118.

Jones G.E., Anderson I.E., *Chlamydia psittaci*: is tonsillar tissue the portal of entry in ovine enzootic abortion? Res. Vet. Sci. 44 (1988) 260–261.

Jones G.E., Low J.C., Machell J., Armstrong K., Comparison of five tests for the detection of antibodies against chlamydial (enzootic) abortion of ewes, Vet. Rec. 141 (1997) 164–168.

Kaltenboek B., Kousoulas K.G., Storz J., Two step polymerase chain reactions and restriction endonuclease analysis detect and differentiate *Omp*A DNA of *Chlamydia* spp, J. Clin. Microbiol. 30 (1992) 1098–1104.

Kuo C.-C., Host response, in: Barron A.L. (Ed.), Microbiology of the Chlamydiae, CRC press, Boca Raton, FL, 1988, pp. 193–208.

Leaver H.A., Howie A., Aitken I.D., Appleyard B.W., Anderson I.E., Jones G., Hay L.A., Williams G.E., Buxton D., Changes in progesterone, oestradiol 17β, and intrauterine prostaglandin E_2 during late gestation in sheep experimentally infected with an ovine abortion strain of *Chlamydia psittaci*, J. Gen. Microbiol. 135 (1989) 565–573.

Lee C.S., Meeusen E., Gogolin-Ewens K., Brandon M.R., Quantitative and qualitative changes in the intraephitelial lymphocyte population in the uterus of non-pregnant and pregnant sheep, Am. J. Reprod. Immunol. 28 (1992) 90–96.

Markey B.K., Mc Nulty M.S., Todd D., Comparaison of serological tests for diagnosis of *Chlamydia psittaci* infection of sheep, Vet. Microbiol. 36 (1993) 233–252.

McCafferty M.C., Herring A.J., Andersen A.A., Jones G.E., Electrophoretic analysis of the major outer membrane protein of *Chlamydia psittaci* reveals multimers which are recognized by protective monoclonal antibodies, Infect. Immun. 63 (1995) 2387–2389.

McEwen A.D., Stamp J.T., Littlejohn A.I., Enzootic abortion in ewes. II Immunization and infection experiments, Vet. Rec. 63 (1951a) 197–201.

McEwen A.D., Littlejohn A.I., Foggie A., Enzootic abortion in ewes. Some aspects of infection and resistance, Vet. Rec. 63 (1951b) 489–492.

Papp J.R., Shewen P.E., Localization of chronic *Chlamydia psittaci* infection in the reproductive tract of sheep, J. Infect. Dis. 174 (1996a) 1296–1302.

Papp J.R., Shewen P.E., Pregnancy failure following vaginal infection of sheep with *Chlamydia psittaci* prior to breeding, Infect. Immun. 64 (1996b) 1116–1125.

Papp J.R., Shewen P.E., Gartley C.J., *Chlamydia psittaci* infection and associated infertility in sheep, Can. J. Vet. Res. 57 (1993) 185–189.

Papp J.R., Shewen P.E., Gartley C.J., Abortion and subsequent excretion of Chlamydiae from the reproductive tract of sheep during estrus, Infect. Immun. 62 (1994) 3786–3792.

Parker H.D., Hawkins Jr W.W., Brenner E., Epizootiologic studies of ovine virus abortion, Am. J. Vet. Res. 27 (1966) 869–877.

Rodolakis A., Infection abortive de la souris gestante inoculée par voie intrapéritonéale avec *Chlamydia ovis*, Ann. Rech. Vet. 7 (1976) 195–205.

Rodolakis A., *In vitro* and *in vivo* properties of chemically induced temperature-sensitive mutants of *Chlamydia psittaci* var *ovis*: Screening in a murine model, Infect. Immun. 42 (1983) 525–530.

Rodolakis A., Bernard F., Vaccination with temperature-sensitive mutant of *Chlamydia psittaci* against enzootic abortion of ewes, Vet. Rec. 114 (1984) 193–194.

Rodolakis A., Bernard F., Lantier F., Mouse models for evaluation of virulence of *Chlamydia psittaci* isolated from ruminants, Res. Vet. Sci. 46 (1989a) 34–39.

Rodolakis A., Bernard F., Souriau A., Stability and safety of a chlamydial live vaccine against enzootic abortion of ewes, in: Meheus A., Spier R.E. (Eds.), Vaccines for Sexually Transmitted Diseases, Butterworths, London, 1989b, pp. 92–97.

Rodolakis A., Boullet C., Souriau A., *Chlamydia psittaci* experimental abortion in goats, Am. J. Vet. Res. 45 (1984) 2086–2089.

Rodolakis A., Gestin L., Bertin A., Méthode de contrôle des vaccins contre la chlamydiose abortive ovine utilisant la souris gestante, Ann. Rech. Vet. 12 (1981) 371–377.

Rodolakis A., Souriau A., Clinical evaluation of a commercial vaccine against chlamydial abortion of ewes, Ann. Rech. Vet. 10 (1979) 41–48

Rodolakis A., Souriau A., Clinical evaluation of immunity following experimental or natural infection of ewes with *Chlamydia psittaci* (var *ovis*), Ann. Rech. Vet. 11 (1980) 215–223.

Rodolakis A., Souriau A., Response of ewes to temperature-sensitive mutants of *Chlamydia psittaci* (var ovis) obtained by NTG mutagenis, Ann. Rech. Vet. 14 (1983) 155–161.

Rodolakis A., Souriau A., Response of goats to vaccination with temperature-sensitive mutants of *Chlamydia psittaci* obtained by nitrosoguanidine mutagenesis, Am. J. Vet. Res. 47 (1986) 2627–2631

Rodolakis A., Souriau A., Variations in the virulence of strains of *Chlamydia psittaci* for pregnant ewes, Vet. Rec. 125 (1989) 87–90.

Rodolakis A., Souriau A., Restriction endonuclease analysis of DNA from ruminant *Chlamydia pittaci* and its relation with virulence, Vet. Microbiol. 31 (1992) 263–271

Rodolakis A., Souriau A., Salinas J., De Sa C., Layachi K., A specific antigen for diagnosis of chlamydial abortion located on 80 to 90 kilodalton protein region of *Chlamydia psittaci*, Biol. Cell 81(2 encart):B7, 1995.

Salinas J., Souriau A., Cuello F., Rodolakis A., Antigenic diversity of ruminant *Chlamydia psittaci* strains demonstrated by the indirect microimmunofluorescence test with monoclonal antibodies, Vet. Microbiol. 43 (1995) 219–226.

Salinas J., Souriau A., De Sa C., Andersen A.A., Rodolakis A., Serotype 2-specific antigens from ruminant strains of *Chlamydia pecorum* detected by monoclonal antibodies, Comp. Microbiol. Immunol. Infect. Dis. 19 (1996) 155–161.

Sánchez J., Buendía A.J., Salinas J., Bernabé A., Rodolakis A., Stewart I.J., Murine granulated material gland cells are susceptible to *Chlamydia psittaci* infection in vivo, Infect. Immun. 64 (1996) 3897–3900.

Sheehy N., Markey B., Gleson M., Quinn P.J., Differentiation of *Chlamydia psittaci* and *C pecorum* strains by species-specific PCR, J. Clin. Micobiol. 34 (1996) 3175–3179.

Sidi Boumedine K., Souriau A., Rodolakis A., Association of RAPD-PCR and specific DNA probes a method for detection and typing of ruminants chlamydial strains, in: Strary A. (Ed.), Proceedings of the Third European Society for Chlamydia Research, Study Group for STD and Dermatological Microbiology of the Austrian Society for Dermatology and Venerology, Vienna, 1996, p. 314.

Souriau A., Bosseray N., Rodolakis A., Lantier F., Plommet M., Anti-chlamydial immunity in ewes conferred by vaccination with a combination of three live chlamydia, brucella and salmonella vaccines, Vet. Rec. 123 (1988) 29–32.

Souriau A., Salinas J., De Sa C., Layachi K., Rodolakis A., Identification of subspecies- and serotype 1-specific epitopes on the 80- to 90-kilodalton protein region of *Chlamydia psittaci* that may be useful for diagnosis of chlamydial induced abortion, Am. J. Vet. Res. 4 (1994) 510–514.

Sting R., Hafez H.M., Purification of *Chlamydia psittaci* Antigen by affinity chromatography on polymixin B agarose for use in the Enzyme-linked Immunosorbent Assay (ELISA), Zbt. Bakt. 277 (1992) 436–445.

Storz J (1964) Superinfection of pregnant ewes latently infected with a psittacosis-lymphogranuloma agent, Cornell Vet. 53, 469–480.

Storz J., Baghian A., Kousoulas K.G., Advances in detection and differentiation of Chlamydiae from animals, in: Orfila J., Byrne G.I., Chernesky M.A., Grayston J.T., Jones R.B., Ridway G.L., Saikku P., Schachter J., Stamm W.E., Stephens R.S. (Eds.), Chlamydial Infections, Esculapio, Bologne, 1994, pp. 563–573.

Tan T.W., Herring A.J., Anderson I.E., Jones G.E., Protection of sheep against *Chlamydia psittaci* infection with a subcellular vaccine containing the major outer membrane protein, Infect. Immun. 58 (1990) 3101–3108.

Wilsmore A.J., Parsons V., Dawson M., Experiments to demonstrate routes of transmission of ovine enzootic abortion, Br. Vet. J. 140 (1984) 380–391.

Wilsmore A.J., Davidson I., Clearview rapid test compared with other methods to diagnose chlamydial infection, Vet. Rec. 128 (1991) 503–504.

Wittenbrink M.M., Schoon H.A., Bisping W., Binder A., Infection of the bovine female genital tract with *Chlamydia psittaci* as a possible cause of infertility, Reprod. Dom. Anim. 28 (1993) 129–136.