Veterinary Microbiology 135 (2009) 134–141

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Molecular detection of *Chlamydophila abortus* in post-abortion sheep at oestrus and subsequent lambing

Morag Livingstone, Nicholas Wheelhouse, Stephen W. Maley, David Longbottom *

Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, Midlothian EH26 OPZ, UK

ARTICLE INFO

Keywords:
Chlamydophila abortus
Enzootic abortion of ewes
Oestrus
Real time PCR
Serological detection

ABSTRACT

Enzootic abortion of ewes (EAE), resulting from infection with the bacterium *Chlamydophila abortus* (*C. abortus*), is a major cause of lamb loss in Europe. The purpose of this study was to assess the potential impact of the shedding of organisms in post-abortion ewes at oestrus and subsequent lambing on the epidemiology of EAE. Using a newly developed *C. abortus* specific real-time PCR assay, few chlamydial genomes could be detected in vaginal swabs taken from post-abortion ewes at oestrus. At subsequent parturition, all ewes lambed normally with no macroscopic or microbiological evidence of infection. Real-time PCR analysis of placental samples identified very few or no chlamydial genomes, which contrasted significantly with samples taken at the time of abortion, where an average of 2.7×10^7 chlamydial genomes per microgram of total tissue DNA was detected. Few genomes could also be detected from vaginal and cervical tissue samples and lymph nodes taken post-mortem. The results, although not discounting the possibility of a chronic low level persistent infection in post-abortion ewes, suggest that the low levels of chlamydial DNA detected during the periovulation period and at lambing do not significantly impact on the epidemiology of EAE. In terms of flock management, the products of abortion should be considered the major and principal source of infection for transmission to naïve ewes.

1. Introduction

Chlamydophila abortus, the aetiological agent of enzootic abortion of ewes (EAE) (also known as ovine enzootic abortion or OEA), is a major cause of lamb loss in many sheep-rearing countries throughout the world. The disease also affects goats and, to a lesser degree, cattle, horses, pigs and deer, although little is known about the incidence of these infections due to a lack of epidemiological data (Longbottom and Coulter, 2003).

Animals infected prior to pregnancy exhibit no clinical signs of infection, with the organism entering into a latent phase. It is not until around day 90–95 of pregnancy that *C. abortus* can first be detected in the placenta (Buxton et al., 1990). The organism establishes itself in the trophoblast cells of the fetal chorionic epithelium, spreading to the surrounding intercotyledonary membranes, where it gives rise to the typical thickened and necrotic placental lesions that are associated with the disease (Buxton et al., 2002). Usually the first clinical manifestation of disease is abortion in the last 2–3 weeks of gestation or when the ewe gives birth to stillborn or weak lambs, although ewes may exhibit a vaginal discharge 1–2 days prior to abortion occurring (Aitken and Longbottom, 2007). These discharges, and those occurring following abortion or lambing, as well as the placentas, foetuses, and coats of lambs contain large numbers of infectious organisms that are the major source of infection for other susceptible animals (Longbottom and Coulter, 2003). During an extended lambing period it is possible for other naïve ewes to pick up infection and abort in the same pregnancy. Where ewes become infected after around 110 days of

* Corresponding author. Tel.: +44 131 445 5111; fax: +44 131 445 6235.
E-mail address: david.longbottom@moredun.ac.uk (D. Longbottom).

0378-1135/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2008.09.033

gestation (i.e., within the last 5 weeks of pregnancy) they would not be expected to abort in that pregnancy, although such animals may go on to abort in the subsequent pregnancy (Aitken and Longbottom, 2007).

Infection can also be spread via vertical transmission from ewe to offspring, however there is little evidence to suggest that this has a significant role to play in the spread of disease (Rodolakis and Bernard, 1977). Although ewes develop immunity and do not experience further EAE abortive episodes, this immunity is not thought to be sterile. Two studies in the 1990s have suggested that ewes may become persistently infected carriers, shedding chlamydiae during subsequent oestrus cycles, thereby providing an opportunity for venereal transmission during breeding (Papp et al., 1994; Papp and Shewen, 1996b). They may also shed infectious organisms at subsequent lambing, thus contributing to the spread of infection to naïve animals (Wilsmore et al., 1990).

Management strategies are principally concerned with the containment and control of disease at abortion or lambing time to prevent exposure of infection to naïve animals. Therefore, the suggestion by Papp et al. (1994) and Papp and Shewen (1996a) that post-abortion ewes continue to excrete organisms at oestrus raised new questions regarding the possibility of venereal or mechanical transmission of EAE by rams. Yet despite the potential importance of these findings, there have to date been no further published studies confirming these findings. Furthermore, in the decade since these initial studies were performed, there have been major advances in the development and availability of molecular diagnostic techniques that allow the rapid and accurate quantification of organisms, and, importantly, enable the discrimination of different chlamydial species. Therefore, the purpose of this study was to reinvestigate the level of chlamydial excretion that occurs at oestrus and subsequent lambings in post-abortion ewes, using a highly sensitive and newly developed *C. abortus*-specific real time PCR assay. The interpretation and potential impact of the results on the epidemiology of EAE are discussed.

2. Materials and methods

2.1. Animals and experimental design

Twenty adult Scottish Blackface ewes, from a flock known to be free of EAE and serologically negative for *C. abortus* antibodies by rOMP90-3 and rOMP90-4 indirect ELISA (Longbottom et al., 2002) were randomly assigned to two groups and mated, following synchronisation with progesterone sponges (Veramix, Upjohn Ltd., Crawley, UK). At day 75 of gestation ten ewes received a subcutaneous injection, over the left prefemoral lymph node, of 2×10^6 inclusion forming units (IFU) egg-grown *C. abortus* strain S26/3 (McClenaghan et al., 1984) in a volume of 1 ml. The remaining group of 10 ewes were housed separately from the infected animals and acted as negative control animals; receiving 1 ml of control inoculum, which was prepared from uninfected yolk sac material. All animals were monitored throughout pregnancy and blood samples were taken at regular intervals for serological analysis. At lambing or abortion, placentas and blood samples were collected for analysis.

Approximately 7 months post-parturition, all previously infected ewes and control ewes were synchronized, as described above, and successfully re-bred. Vaginal swabs were taken during the oestrus cycle, between 2 weeks pre- and post-ovulation. Blood was taken throughout pregnancy and 3 months post-lambing for serological analysis. At lambing, placentas were collected for analysis by real-time PCR. Three months following parturition, ewes were euthanized by intravenous injection of pentobarbitone sodium (Euthatal; Merial Animal Health Ltd., Harlow, UK). Reproductive tract tissue and lymph nodes were removed at necropsy. The care and use of experimental animals were approved by the Institute's Experiments and Ethical Review Committee and complied with both Home Office Regulations and all local animal health and welfare policies.

2.2. Sampling methods

Placentas were collected, macroscopically assessed for EAE lesions, and representative cotyledons removed for bacteriological and pathological analysis. Cotyledons and surrounding intercotyledonary membrane were placed in 4 ml sucrose–phosphate–glutamate buffer (SPG) (Spencer and Johnson, 1983) and immediately frozen at −20 °C for subsequent recovery of organisms in cell culture and for real-time PCR analysis. Similar samples were also stored at +4 °C for the subsequent preparation of smears. For the detection of organisms at oestrus, the external opening of the vagina was firstly cleaned with chlorhexidine gluconate solution prior to insertion of swabs. Two vaginal swabs per animal were then taken by rotating cotton-tipped swabs (Barloworld Scientific, Stone, UK) around the wall of the vagina, approximately 2 cm distal to the cervix, and placed in 2 ml SPG. All swabs were stored at −20 °C prior to DNA extraction. Sheep were bled at approximately monthly intervals by jugular venipuncture. At necropsy, samples of vagina (2 cm distal to the cervix) and cervix (2 cm distal to the uterus), as well as uterine, mesenteric and prefemoral lymph nodes, were placed in cryovials, immediately snap frozen in liquid nitrogen and stored at −70 °C until required for DNA extraction. Rigorous procedures were observed to ensure tissues were sampled from the same anatomical location in each animal. Strict aseptic precautions, including the use of new sets of instruments and blades, were applied between each sample to avoid cross-contamination.

2.3. Bacteriological analysis

Following parturition, smears of placental membranes or vaginal swabs were prepared and stained by the modified Ziehl–Neelsen (mZN) method (Stamp et al., 1950). Smears were then examined under high-power microscopy for the presence of chlamydial elementary bodies (EBs) and any other contaminating bacteria. Isolation of chlamydial organisms was attempted in cell culture. Placental cotyledons were aseptically ground in SPG and dilutions (1/60) prepared in complete RPMI medium containing 1 μg/ml