WIADOMOŚCI PARAZYTOLOGICZNE 2005 • tom 51 • suplement • s. 15-18

Toxoplasmosis in sheep; the possibility of endogenous transplacental transmission

Susan M. Rodger, Stephen W. Maley, Steve E. Wright, Annie Mackellar, Jill Sales*, and David Buxton

Moredun Research Institute, Pentlands Science Park, Bush Loan, by Edinburgh EH26 0PZ, Scotland, UK, E-mail: david.buxton@moredun.ac.uk. *BioSS, Kings Buildings, Edinburgh EH9 3JZ

Introduction

Toxoplasma abortion in sheep occurs when seronegative ewes suffer a primary toxoplasma infection in pregnancy. However if this occurs in the latter part of pregnancy the ewe may give birth to infected but clinically normal offspring (Watson and Beverley 1971, Blewett et al. 1982, Miller et al. 1982). Following infection sheep are considered to remain persistently infected for life, to be immune to the parasite and therefore unlikely to abort again with toxoplasmosis (Beverley and Watson 1971). This is the basis of the live vaccine (Toxovax(r), Intervet B.V.) marketed for the control of ovine toxoplasmosis (Buxton and Innes 1995). However in some cases a small proportion of persistently infected ewes may, in a subsequent pregnancy, transmit *Toxoplasma* to the foetus and abort (Hartley 1966).

Since the life cycle of *T. gondii* was fully described (Dubey and Frenkel 1972) evidence has indicated that environmental contamination with toxoplasma oocysts from cat faeces is the principal source of infection for sheep. There is a correlation between the presence of cats on farms and evidence of exposure of sheep to *Toxoplasma* (Skjerve et al. 1998), and serological surveys show a steadily increasing prevalence of infection with age (Waldeland 1977, Blewett 1983, Figliuolo et al. 2004). More specifically sheep have been shown to seroconvert to *T. gondii* while grazing certain pastures (Faull et al. 1986, Owen 1996) and outbreaks of toxoplasma abortion have also been associated with the contamination of feed with cat faeces (Plant et al. 1974).

However, recently it has been suggested that environmental contamination with toxoplasma oocysts is relatively less important as a source of infection and cause of ovine toxoplasmosis, and that vertical transmission from the persistently infected ewe to the foetus is more frequent than previously thought (Duncanson et al. 2001, Williams et al. 2005). This presumably follows recrudescence of persistent infection during pregnancy with the consequent placental transfer of the parasite to the developing foetus.

The following study investigated the incidence of endogenous transplacental transmission in clinically normal seropositive sheep, considered to be naturally and persistently infected with *T. gondii*.

Materials and Methods

Thirty one Scottish Blackface sheep with antibodies to *Toxoplasma* (Group 1) and a second group of 15, seronegative for *T. gondii* and presumed to be uninfected (Group 2), were monitored clinically and serologically throughout pregnancy. Maternal blood samples were examined for IgG antibodies to *T. gondii* by ELISA, with values ≥ 25% considered to be indicative of exposure to infection (Buxton et al. 1988). At lambing, pre-colostral blood samples were collected from the lambs for serology, by IFAT (OIE 2004), along with samples of placenta for testing for *T. gondii* DNA by PCR (Wastling et al. 1993) and for histopathology. In the case of stillbirth and perinatal mortality (deaths within 48 hours of birth) pleural fluid for serology and samples of lung, heart, liver, kidney, spleen and brain, together with

placenta were collected for histopathology. The latter two tissues were also examined by PCR. The lamb and foetal sera were tested for IgM and IgG antibodies to *T. gondii*, with titres ≥ 1/32 considered to be positive. Western blotting was carried out on two sera (Stanley et al. 2004) and antibodies were detected with a monoclonal antibody against ovine Ig-light chain.

Results

Group 1 produced 43 healthy viable lambs, three lambs stillborn at term and a fourth died soon after birth, all as a result of dystocia. A further two stillborn lambs were produced by a ewe at 137 days gestation that had developed an idiopathic transitory ataxia (group mean gestation 145 days – range 137-152 days). The 15 ewes in Group 2 produced 22 healthy viable lambs and no stillbirths or abortions (group mean gestation 146 days – range 142-153days). All the lamb and placental samples were negative by PCR for *T. gondii* DNA and no histopathological evidence was found to indicate exposure to *T. gondii* or any other infectious agent. All foetal fluid samples were negative for IgM and IgG by IFAT, with the exception of, in Group 1, live twin lambs which had IgM titres of 1/64 and IgG titres of 1/16 and 1/64. In Group 1 all but two ewes remained seropositive throughout. Group 1 showed a significant ($P \leq 0.0001$) dip in mean titre at the time of lambing, when compared with pre-mating titres but the mean returned to the pre-mating level in the four weeks after lambing. Group 2 remained seronegative throughout the study. Western blotting of the two lamb sera, when compared with a toxoplasma-positive ewe serum, showed three bands (at approximately 40, 45 and 60kD which correlated with three similar bands in the ewe serum.

Discussion

The findings indicate that vertical transmission was not prevalent in this flock, with no histopathological evidence of toxoplasma infection in any of the tissues examined. The PCR results were all negative as were the pre-colostral sera and foetal fluid samples for toxoplasma antibodies in all but two lambs. The antibody to *T. gondii* detected in the latter both by IFAT and western blotting means, however, that vertical transmission cannot be ruled out, despite the absence of histopathological evidence of infection and given the failure to detect *T. gondii*-specific DNA in placental samples. The scant evidence of vertical transmission is in accord with the findings of Munday (1972) where analysis of 178 precolostral lamb sera from 135 ewes persistently infected with *T. gondii*, collected over a four year period, failed to demonstrate any evidence of congenital transmission, and Hartley (1966), who demonstrated *Toxoplasma* in 3 non viable lambs in a group of 25 lambs born to 22 persistently infected ewes. More recently, in a study that relied solely on PCR data, a greater frequency was reported (41% to 69%) along with a marked incidence of abortion (mean of 8%, range of 4.5-20.6%). From dead lambs 90% of samples had detectable *T. gondii* DNA, compared with 46.4% of live lambs (Duncanson et al. 2001).

Hitherto, vertical transmission in sheep has not been considered to be common but it does occur with greater frequency in bovine neosporosis, caused by the closely related protozoan *Neospora caninum*. It would appear that the hormonal and immunological consequences of pregnancy permit the recrudescence of a persistent neospora infection and a transient parasitaemia (Buxton et al. 2002, Innes et al. 2002). At the placenta pro-inflammatory cytokines, that normally limit multiplication of the parasite, are down-regulated to allow the dam to accommodate the foetus. However this also favours the establishment of a placental neospora infection and its consequent spread to the developing calf (Buxton et al. 2002, Innes et al. 2002). The parasite itself, however, once in the placenta may invoke pro-inflammatory cytokines which may then terminate pregnancy (Innes et al. 2004). Thus there is a fine balance as to whether the parasite precipitates abortion or causes a persistent infection of a live viable foetus. It seems reasonable to suggest that a balance between host immune response and parasite virulence may also determine the prevalence of vertical transmission of *T. gondii* in sheep. *Toxoplasma* exists in several strains, over 95% of which may be broadly subdivided into types I, II and III. Type I is acutely virulent in mice while type II (the most common in persistently infected animals) and type III are defined as non-virulent. Their prevalence in sheep is not known but it is of interest that clinical human infections are more often associated with type I strains (Sibley et al. 2003). Could the type of *Toxoplasma* influence vertical transmission in sheep?

It is clear from our study and previous reports in the literature that ovine toxoplasmosis is predomi-

nantly the result of primary infection during pregnancy and that the risk to susceptible sheep is from toxoplasma oocysts contaminating food and water. However there is a case to be made for suggesting that in some instances recrudescence of persistent infection in pregnant sheep may occur with greater frequency, leading to endogenous transplacental transmission and repeat abortions (Duncanson et al. 2001, Williams et al. 2005). The frequency of the latter and whether it is the result of a particular genetic trait of the parasite, rather than the genetics of the sheep or differences in flock management, warrants further investigation.

Acknowledgements

We thank John Huntley for expert guidance with the western blotting. Moredun Research Institute and BioSS receive funding from the Scottish Executive Environment and Rural Affairs Department.

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