Ovine toxoplasmosis: a review

D Buxton MRCPath MRCVS *Department of Pathology and Immunology, Moredun Research Institute, 408 Gilmerton Road, Edinburgh EH17 7JH*

Keywords: Toxoplasma gondii; toxoplasmosis; cats; sheep; abortion

Paper read to Section of Comparative Medicine, 21 February 1990

Introduction

Toxoplasma gondii is a protozoan parasite with a worldwide distribution, capable of infecting all warm-blooded animals including man. In the 1950s it was recognized as a significant cause of abortion in sheep[1,2] and subsequently also in goats[3,4], but to date seems not to cause disease in cattle or deer.

Life cycle

Two recent reviews give accounts of the life cycle of *T. gondii*[5,6] which comprises an asexual cycle with little host specificity and a sexual cycle, confined to the enteroepithelial cells of cats and resulting in the production of oocysts (Figure 1). In the asexual cycle there are two developmental stages, the tachyzoite (fast multiplying form 5 μm×1.5-2 μm) and the bradyzoite (slow multiplying form of similar size and shape). The tachyzoite actively penetrates the host cell, becomes surrounded by a parasitophorous vacuole and multiplies by endodyogeny. Multiplication continues until the cell ruptures, when the organisms are released locally and into the bloodstream to parasitize further cells. This process continues until the host dies or, more usually, develops immunity to the infection, the onset of which coincides with the establishment of chronic infection. Extracellular parasites are eliminated, intracellular multiplication slows and tissue cysts, containing bradyzoites, develop (the second stage of the asexual cycle). Cysts are found most frequently in brain and skeletal muscle and represent the quiescent stage of the parasite within the host. When a cyst ruptures the bradyzoites are released to enter other cells and so the asexual cycle is complete. In some species, such as sheep, goats, pigs and man, cysts may remain for the remainder of the life of the animal, while in cattle and deer the host may eventually become clear of infection.

The sexual cycle is initiated when a non-immune cat ingests tissue cysts, tachyzoites or oocysts. In the case of meat containing tissue cysts, the cyst wall is dissolved by proteolytic enzymes in the small intestine and the released bradyzoites can then penetrate the adjacent epithelial cells. In these cells the toxoplasms pass through a schizogonic cycle followed by gametogony which gives rise to oocysts (10×12 μm in diameter) which are then excreted in the faeces. Following sporulation over the next one to 5 days they become infective and can remain so for over a year[7].

By comparison, ingestion of tachyzoites or sporulated oocysts by susceptible cats readily causes infection but with a very much smaller output of oocysts, with shedding occurring only after 20 days and in a minority of the challenged animals[8].

Infection in the environment

The ingestion of tissue cysts by cats is of great significance, in terms of spread of infection to other animals (Figure 2). Oocysts may be shed continuously in the faeces from 4 until 14 days after infection, with an expected peak output of tens of millions at 6-8 days[9]. Recrudescence of infection may occur if the cat is experimentally stressed[10] and perhaps also through unrelated illness. This can result in the re-excretion of oocysts in smaller numbers for a shorter time than in a primary infection.

Cats acquire infection as a result of hunting so that many will have seroconverted by adulthood. Although less than 1% may be shedding oocysts at any one time[5], infection may be more prevalent in young cats taking up hunting for the first time.

Female feral cats can produce two to three litters a year, each of up to eight kittens, and may rear their young communally[11]. Numbers of young cats are also dependent upon the density of breeding adults. In rural areas male cats may have territories of 60-80 hectares (250-200 acres) while females usually only occupy a 10th of this area[11]. In an urban environment these territories are considerably smaller[12]. The area occupied by feral cats is influenced by the supply of food, which includes mice, voles, shrews, rats, rabbits and small birds[11].

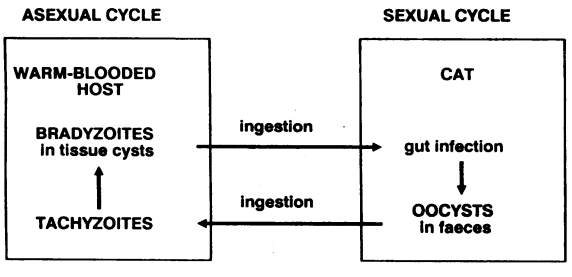

Figure 1. The life cycle of the intracellular protozoan parasite Toxoplasma gondii

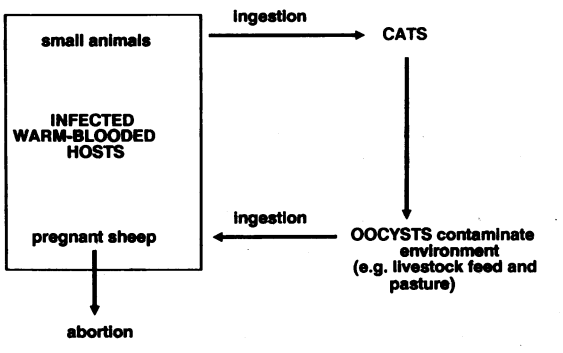

Figure 2. The spread of Toxoplasma *infection to susceptible pregnant sheep from infected cat faeces deposited in the environment*

0141-0768/90/080509-03/$02.00/0

The most important sources of feline infection are chronically-infected birds and rodents[6,13], particularly the latter because they can pass *T. gondii* infection from generation to generation without causing overt clinical disease[14-16]. In this way a reservoir of *T. gondii* tissue cyst infection can exist in a particular location for a long time, with the potential for infecting cats and triggering massive oocyst excretion.

The available epidemiological and experimental evidence suggests that, in the UK, sheep are frequently maintained in an environment significantly contaminated with oocysts and that infection follows ingestion of infected food[17,18]. Perhaps the most common source of infection is contaminated pasture. Certainly, fields treated with manure and bedding from farm buildings where cats live can cause infection[19]. Careless storage of farm feeds may also pose a risk[20]. Fifty grams of infected cat faeces may contain as many as 10 million oocysts[9]. If in a hypothetical situation this was evenly dispersed throughout 10 tonnes of concentrated animal feed then each kilogram could contain between five and 25 sheep-infective doses[21]. The extent of environmental contamination with *T. gondii* oocysts is thus related to the distribution and behaviour of cats.

Measures to reduce environmental contamination by oocysts should be aimed at reducing the number of cats capable of shedding oocysts. This would include attempts to limit their breeding. If male cats are caught, neutered and returned to their colonies the stability of the colony is maintained; fertile male cats do not challenge the neutered males[12] and breeding is controlled. Thus the maintenance of a small healthy population of mature cats will reduce oocyst excretion as well as help to control rodents. Sheep feed should be kept covered at all times to prevent its contamination by cat faeces.

Infection in the pregnant ewe

Abortions and neonatal mortality occur when sheep, (and goats) suffer a primary infection during pregnancy[5]. In the UK, toxoplasmosis is a primary cause of loss in 10-20% of flocks with an abortion problem, giving an annual incidence in the breeding ewe population of 1-2%[22,23]. Sporulated *T. gondii* oocysts, ingested by susceptible pregnant sheep, excyst in the digestive tract and release sporozoites to penetrate the intestinal epithelium. By 4 days, organisms can be found in the mesenteric lymph nodes, where they multiply causing marked lymph node enlargement, sometimes with focal necrosis[24]. Around the 5th day toxoplasms are released to cause a parasitaemia, which may last until the 12th day[25,26]. Coinciding with the parasitaemia the ewe displays a febrile response which can exceed 41°C around day 6 or 7[27].

Many tissues become infected in this way. The cessation of the parasitaemia coincides with the onset of an effective maternal immune response and infection then persists as bradyzoites within tissue cysts.

In pregnant animals the gravid uterus is an 'immunologically privileged' site[28]. On the uterine side maternal immunological responses are suppressed while the ability of the fetus, with its placenta, to recognize and respond to a pathogen commences during the first half of gestation and develops for the remainder of pregnancy, so that lambs at birth are immunocompetent. During a *T. gondii* parasitaemia in the dam, tachyzoites are able to parasitise the caruncular septa, the maternal tissues of the placentome. They then invade adjacent trophoblast cells of the fetal villi, and from there, the rest of the fetus, between 5 and 10 days after the onset of parasitaemia[29]. However, the outcome of infection is influenced by the stage of gestation at which it commences.

Infection in early gestation is rapidly fatal[18,30] due to the absence of a fetal immune response to inhibit parasite multiplication[29]. Subsequent resorption of the fetus can be mistaken for infertility[30]. Infection in mid gestation may also be fatal and give rise to a mummified fetus often alongside a sibling which is born alive but weakly or which dies late in gestation. Infection in late pregnancy will normally cause fetal infection but because, at this stage, the competence of the fetal immune system is well advanced, the parasite will be resisted and the lamb born live, infected and immune[18]. When infection in the placentome is initiated, parasite multiplication causes multiple foci of necrosis[29]. These foci of tissue damage expand throughout the remainder of gestation until abortion or birth when they may be macroscopically visible as white spots in the cotyledons of the shed placenta, a feature used to aid diagnosis[1,31].

Diagnosis is also helped by histological examination of the brain where there may be both primary and secondary lesions[32,33]. Glial foci, surrounding a necrotic and sometimes mineralized centre, often associated with a mild lymphoid meningitis, represent a fetal immune response following direct damage by parasite multiplication. Focal leukomalacia is also common and is thought to be due to fetal anoxia in late gestation caused by advanced focal necrosis in the placentome preventing sufficient oxygen transfer from mother to fetus[33]. Focal inflammatory lesions and associated diffuse lymphoid infiltrates may also be found in the liver, lung and heart and less frequently in kidneys and skeletal muscle[33].

The ovine fetal immune system starts to respond to *T. gondii* at or soon after 60 days gestation when both humoral and cellular reactions can be detected[29]. Specific circulating anti-*T. gondii* IgM and IgG, detectable after 30 days of maternal infection, can be used in the diagnosis of *T. gondii* abortion[29]. Infection of pregnant and non-pregnant ewes provokes substantial immunity, so that a uterine *T. gondii* infection will not develop in a future gestation[21].

Vaccination

The natural development of protective immunity to subsequent challenge offers the prospect of an effective vaccine. However, neither a killed whole *T. gondii* tachyzoite vaccine[34] nor one in Freund's incomplete adjuvant[35] protected sheep against experimental challenge with *T. gondii*. Recently a *T. gondii* membrane 'iscom' vaccine was tested first in mice[36] then in sheep. While it induced a substantial antibody response in sheep it did not appear to induce significant protection[37,38].

Research with a live attenuated strain of *T. gondii* in New Zealand[39] has been sufficiently encouraging for it to be marketed commercially in that country. Live *T. gondii* tachyzoites are injected into sheep where they induce a limited infection but do not form bradyzoites in tissue cysts. The advantages of the vaccine are that it protects the sheep and does not leave the animal latently infected, which could pose a public health hazard if its meat was eaten. The disadvantages

of the vaccine are that it has a very short shelf-life and could be a hazard to those using it or handling meat from animals killed soon after vaccination.

Whether this vaccine is eventually used in other countries remains to be seen. In the meantime, in the absence of anything else, the ionophore monensin which has significant anti-*T. gondii* activity in sheep may be used both to prevent and suppress ovine toxoplasmosis[27,40], but continued research for an effective killed vaccine is essential if this zoonotic infection[41] of sheep is to be mastered.

References

1 Hartley WJ, Jebson JL, McFarlane D. New Zealand II type abortion in ewes. *Aust Vet J* 1954;**30**:216-18

2 Hartley WJ, Marshall SC. Toxoplasmosis as a cause of ovine perinatal mortality. *N Z Vet J* 1957;**5**:119-24

3 Munday BL, Mason RW. Toxoplasmosis as a cause of perinatal death in goats. *Aust Vet J* 1979;**55**:485-7

4 Dubey JP. Epizootic toxoplasmosis associated with abortion in dairy goats in Montana. *J Am Vet Med Assoc* 1981;**178**:661-70

5 Dubey JP, Beattie CP. *Toxoplasmosis of animals*. Boca Raton, Florida: CRC Press, 1988

6 Jackson MH, Hutchison WM. The prevalence and source of *Toxoplasma* infection in the environment. In: Baker JR, Muller R, ed. *Advances in parasitology*, vol. 28, London: Academic Press, 1989:55-105

7 Dubey JP. *Toxoplasma, Hammondia, Besnoitia, Sarcocystis*, and other tissue cyst-forming coccidia of man and animals. In: Kreier JP, ed. *Parasitic protozoa*, vol. III. London: Academic Press, 1977

8 Dubey JP, Frenkel JK. Feline toxoplasmosis from acutely infected mice and the development of *Toxoplasma* cysts. *J Protozool* 1976;**23**:537-46

9 Dubey JP, Frenkel JK. Cyst-induced toxoplasmosis in cats. *J Protozool* 1972;**19**:155-77

10 Dubey JP, Frenkel JK. Immunity to feline toxoplasmosis: modification by administration of corticosteroids. *Vet Pathol* 1974;**11**:350

11 Macdonald D. The behaviour and ecology of farm cats. In: *The ecology and control of feral cats*. Potters Bar, Herts: University Federation for Animal Welfare, 1980

12 Tabor R. The behaviour and ecology of farm cats. In: *The ecology and control of feral cats*. Potters Bar, Herts: University Federation for Animal Welfare, 1980

13 Peach W, Fowler J, Hay J. Incidence of *Toxoplasma* infection in a population of European starlings *Sturnus vulgaris* from central England. *Ann Trop Med Parasitol* 1989;**83**:173-7

14 Eichenwald H. Experimental toxoplasmosis. *Am J Dis Child* 1948;**76**:307-15

15 Beverley JKA. Congenital transmission of toxoplasmosis through successive generations of mice. *Nature* 1959; **183**:1348-9

16 De Roever-Bonnet H. Congenital *Toxoplasma* infections in mice and hamsters infected with avirulent and virulent strains. *Trop Geogr Med* 1969;**21**:443-50

17 Blewett DA. The epidemiology of ovine toxoplasmosis. I. The interpretation of data for the prevalence of antibody in sheep and other host species. *Br Vet J* 1983;**139**:537-45

18 Blewett DA, Watson WA. The epidemiology of ovine toxoplasmosis. II. Possible sources of infection in outbreaks of clinical disease. *Br Vet J* 1983;**139**:546-55

19 Faull WB, Clarkson MJ, Winter AC. Toxoplasmosis in a flock of sheep: some investigations into its source contrand ol. *Vet Rec* 1986;**119**:491-3

20 Plant JW, Richardson N, Moyle GG. *Toxoplasma* infection and abortion in sheep associated with feeding of grain contaminated with cat faeces. *Aust Vet J* 1974;**50**:19-21

21 McColgan C, Buxton D, Blewett DA. Titration of *Toxoplasma gondii* oocysts in non-pregnant sheep and the effects of subsequent challenge during pregnancy. *Vet Rec* 1988;**123**:467-70

22 Blewett DA, Watson WA. The epidemiology of ovine toxoplasmosis. III. Observations on outbreaks of clinical toxoplasmosis in relation to possible mechanisms of transmission. *Br Vet J* 1984;**140**:54-63

23 Blewett DA, Trees AJ. The epidemiology of ovine toxoplasmosis with especial respect to control. *Br Vet J* 1987;**143**:128-35

24 Dubey JP. Experimental toxoplasmosis in sheep fed *Toxoplasma gondii* oocysts. *Int Goat Sheep Res* 1984; **2**:93-104

25 Dubey JP, Sharma SP. Parasitemia and tissue infection in sheep fed *Toxoplasma gondii* oocysts. *J Parasitol* 1980;**66**:111-14

26 Reid HW, Buxton D, Gardiner AC, Pow I, Finlayson J, MacLean MJ. Immunosuppression in toxoplasmosis: studies in lambs and sheep infected with louping-ill virus. *J Comp Pathol* 1982;**92**:181-90

27 Buxton D, Blewett DA, Trees AJ, McColgan C, Finlayson J. Further studies in the use of monensin in the control of experimental ovine toxoplasmosis. *J Comp Pathol* 1988;**98**:225-36

28 Tomasi TB. Mechanisms of immunosuppression in neonatal and pregnant mice. In: Wegmann TG, Gill TT with Cumming CD, Nisbet-Brown, eds. *Immunology and reproduction*, chapter 14, New York: Oxford University Press, 1983

29 Buxton D, Finlayson J. Experimental infection of pregnant sheep with *Toxoplasma gondii*: pathological and immunological observations on the placenta and foetus. *J Comp Pathol* 1986;**96**:319-33

30 Johnston WS. An investigation into toxoplasmosis as a cause of barrenness in ewes. *Vet Rec* 1988;**122**:283-4

31 Beverley JKA, Watson WA, Payne JM. The pathology of the placenta in ovine abortion due to toxoplasmosis. *Vet Rec* 1971;**88**:124-8

32 Hartley WJ, Kater JC. The pathology of *Toxoplasma* infection in the pregnant ewe. *Res Vet Sci* 1963;**4**:326-32

33 Buxton D, Gilmour JS, Angus KW, Blewett DA, Miller JK. Perinatal changes in lambs infected with *Toxoplasma gondii*. *Res Vet Sci* 1982;**32**:170-6

34 Beverley JKA, Archer JF, Watson WA, Fawcett AR. Trial of a killed vaccine in the prevention of ovine abortion due to toxoplasmosis. *Br Vet J* 1983;**127**:529-35

35 Wilkins MF, O'Connell E, Te Punga WA. Toxoplasmosis in sheep. I. Effect of a killed vaccine on lamb losses caused by experimental challenge with *Toxoplasma gondii*. *N Z Vet J* 1987;**35**:31-4

36 Uggla A, Araujo FG, Lunden A, Lovgren K, Remington JS, Morein B. Immunizing effects in mice of two *Toxoplasma gondii* iscom preparations. *J Vet Med B* 1988;**35**:311-14

37 Buxton D, Uggla A, Lovgren K, *et al.* Trial of a novel experimental *Toxoplasma* iscom vaccine in pregnant sheep. *Br Vet J* 1989;**145**:451-7

38 Fredriksson G, Buxton D, Uggla A, Kindahl H, Edqvist L-E. The effect of *Toxoplasma gondii* infection in unvaccinated and iscom-vaccinated pregnant ewes, as monitored by levels of 15-ketodihydroprostoglandind-F_2 alpha, progesterone and oestrone sulphate. *J Vet Med B* (in press)

39 Wilkins MF, O'Connell E, Te Punga WA. Toxoplasmosis in sheep. III. Further evaluation of the ability of a live *Toxoplasma gondii* vaccine to prevent lamb losses and reduce congenital infection, following experimental oral challenge. *N Z Vet J* 1988;**36**:86-9

40 Buxton D, Donald KM, Finlayson J. Monensin and the control of experimental ovine toxoplasmosis: a systemic effect. *Vet Rec* 1987;**120**:618-19

41 Buxton D. Toxoplasmosis. *Practitioner* 1990;**234**:42-4

(Accepted 30 March 1990)