



RESEARCH COMMUNICATION

Isolation of *Toxoplasma gondii* from goats with a history of reproductive disorders and the prevalence of *Toxoplasma* and chlamydial antibodies

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ABSTRACT

SHARMA, S.P., BAIPOLEDI, E.K., NYANGE, J.F.C. & TLAGAE, L. 2003. Isolation of *Toxoplasma gondii* from goats with a history of reproductive disorders and the prevalence of *Toxoplasma* and chlamydial antibodies. *Onderstepoort Journal of Veterinary Research*, 65–68

The prevalence of antibodies to *Toxoplasma gondii* and *Chlamydia psittaci* was assessed in goats with a history of abortion, stillbirth and neonatal mortality. Antibodies were detected in 540 (30%) and 57 (3.2%) goats out of 1799 tested by indirect haemagglutination and complement fixation tests, respectively. *Toxoplasma gondii* was isolated for the first time in Botswana from 22 out of 81 sets (27.2%) of foetal tissues, maternal and foetal cotyledons and uterine tissues of goats which had previously aborted or given birth to stillborn or weak kids that died within two days of birth. These results implicate *T. gondii* and *C. psittaci*, but especially the former, to be associated with caprine reproductive problems and require appropriate control measures.

Keywords: Abortion, *Chlamydia psittaci*, foetal tissues, neonatal mortality, prevalence, *Toxoplasma gondii*

INTRODUCTION

Toxoplasmosis and chlamydiosis have long been recognized as causes of abortion, stillbirth and neonatal mortality in sheep and goats (Dubey, Sharma, Lopes, Williams, Williams & Weisbrode 1980; Gunson, Acland, Gillette & Pearson 1983; Blewett & Watson 1984; Dubey & Beattie 1988; Freyre, Bonino, Falcon, Castells, Correa & Casaretto 1997; Bisson, Maley, Rubaire-Akiki & Wastling 2000). Abortion and stillbirth may also occur in small ruminants infected with *Campylobacter foetus*, *Listeria*

monocytogenes, *Salmonella* spp., *Leptospira* spp. and *Brucella* spp., *Coxiella burnetti*, Rift valley fever and Wesselbron viruses and Caprine herpesvirus (Saito, Gribble, Berrios, Knight & McKercher 1974; Radostits, Blood & Gay 1994). Binta, Monyame, Ndebele, Mushi & Raborokgwe (1998a) and Binta, Mushi, Raborokgwe, Monyame & Ndebele (1998b) reported antibody to *C. psittaci* and *T. gondii* in 23.2% and 10.1% of goats, respectively, in Botswana. Detection of high antibody titres to any of these infective organisms may be of some diagnostic significance but isolation of the causative organism is required to arrive at a conclusive diagnosis. The aim of this investigation was to determine the seroprevalence of toxoplasma and chlamydial infections as well as to isolate *T. gondii* from foetal

and maternal tissues of goats which had previously aborted or given birth to stillborn or weak kids that had died within two days of birth.

A total of 1 799 goats which had recently experienced abortion, stillbirth and neonatal deaths were tested serologically over a four year period from 1998–2001 to determine the titres of antibody to *T. gondii* and *C. psittaci*. Prior to testing, the sera were inactivated at 60°C for an hour to destroy anti-toxoplasma factor (Te Punga & Penrose 1964). Indirect haemagglutination and complement fixation tests using commercial kits (Cellognost® Toxoplasmosis H, Behring Diagnostics and Ornithosis-Complement fixation test, Dade Behring Marburg, Gm bH, Germany) were performed in order to detect antibody to *Toxoplasma* and *Chlamydia*, respectively. Where deemed necessary, sera were screened at the Onderstepoort Veterinary Institute, Onderstepoort, South Africa for antibody to *Brucella* spp. (complement fixation test), *Leptospira interrogans* (microscopic agglutination test), Rift Valley fever virus (haemagglutination inhibition test) and blue-tongue and Wesselsbron viruses (ELISA).

In an attempt to isolate *T. gondii* by inoculation into mice, tissues from 81 aborted, stillborn foetuses and weak kids that had died within a day or two of birth were collected at autopsy at the National Veterinary Laboratory, Gaborone during the years 1998–2001. The tissues included liver, lung, kidney and spleen from 74 kids, placental and maternal cotyledons as well as amniotic fluid from four and uterine tissues from three nanny goats. The tissues of each set were pooled and homogenized in five volumes of physiologic saline (0.85 % NaCl) in a Waring blender (M/S Dynamic Corporation of America, New Hartford, Connecticut, USA). The homogenate was filtered through a sieve, and 0.25 ml of antibiotic solution containing 1 000 units of penicillin and 100 µg of streptomycin was added per millilitre of the homogenate. It was kept at room

temperature for about 30 min. One millilitre of the tissue homogenate was injected intraperitoneally into each of four albino mice. Impression smears from mesenteric lymph nodes, spleen and liver of mice that died within a week of their inoculation were stained with Giemsa and examined for tachyzoites of *T. gondii*. Squash smears were made from the brain of mice killed after eight weeks of their inoculation and examined for the presence of *Toxoplasma* cysts.

Foetal tissues were also cultured on blood and McConkey agars. If tissues were fresh and gross pathological changes were observed, the tissues were also fixed in 10 % formol saline, processed, and stained with haematoxylin and eosin for histopathological examination. Impression smears made from the cotyledons were examined for the presence of *C. psittaci* after staining with Gimenez stain.

Antibody titres positive to *Toxoplasma* and *Chlamydia* (1:64 or above and 1:8 or above, respectively) were recorded in sera of 540 and 57 of 1 799 goats tested, representing infection rates of 30 and 3.2%, respectively (Table 1). Dual infections with *T. gondii* and *C. psittaci* were detected in 16 goats. Serum samples of 29 animals found positive for chlamydial antibodies, however, proved negative for *Toxoplasma* antibodies. *Toxoplasma* and *Chlamydia* infection rates recorded in this study varied greatly from those of the single investigation conducted by Binta *et al.* (1998a, b) which may probably be due to a smaller number of animals they examined. Moreover, depending on the management practices, the prevalence rates may vary widely between farms and localities. It was observed that in the majority of cases which occurred during winter and early spring, *T. gondii* seemed to have played a predominant role. Gunson *et al.* (1983) reported that a mixed infection of *C. psittaci* and *T. gondii* was associated with abortions and stillbirths in a dairy goat herd in southeastern Pennsylvania, USA. Dubey,

TABLE 1 Prevalence of antibodies to *Toxoplasma gondii* and *Chlamydia psittaci* in goats with a history of abortion, stillbirth and neonatal mortality

Year	Number of animals tested	Number (%) positive for toxoplasma antibody	Number (%) positive for chlamydial antibody
1998	424	50 (11.8)	10 (2.4)
1999	917	419 (45.7)	41 (4.5)
2000	265	45 (17.0)	3 (1.1)
2001	193	26 (13.5)	3 (1.6)
Total	1 799	540 (30.0)	57 (3.2)

Desmonts, Antunes & McDonald (1985) expressed the opinion that the presence of high antibody titres in does' serum are not necessarily diagnostic of recent infection as the concentration of antibody may remain high, even into the next breeding season. On account of the transport problems in different veterinary districts, a common constraint in our studies was the failure to collect and test paired serum samples from the affected goats in order to monitor fluctuations in the antibody titres.

Positive antibody titres to bluetongue, Wesselbron and Rift Valley fever viral infections were recorded in 13, 11 and two serum samples, respectively, indicating their minor role in inducing the reproductive problems in the goats. It is possible that these viral infections might have occurred when the does were not pregnant. None of the serum samples contained antibody to *Brucella* and *Leptospira* spp.

All the mice inoculated with material from four sets died within few hours to two days of inoculation because the tissues were probably from carcasses with extensive autolytic changes. Cysts of *T. gondii* were demonstrated in squash preparations of the brain of 22 out of the 81 inoculated mice (27.2%). There were variations in size and shape of the *Toxoplasma* cysts. These were ovoid to spherical, with thin smooth walls fully packed with bradyzoites, and had a mean diameter of $41.2 \pm 12.5 \mu\text{m}$.

Elementary bodies of *Chlamydia* spp. could not be found in the Gimenez stained impression smears. No other abortifacient bacterium was isolated on bacteriological culture of foetal and maternal tissues. Isolation of toxoplasma cysts from the caprine tissues soon after the episodes of abortions, stillbirths and neonatal deaths in the absence of any other potent abortifacient microorganisms confirmed the possible role of *T. gondii* in the induction of these disorders in goats. This is the first confirmation of the association of *T. gondii* with abortions in goats and stillbirths and neonatal mortalities in goat kids from Botswana.

The reasons for the failure to isolate *T. gondii* from other sets of aborted tissues could be:

- Extensive autolytic changes in tissues because of delayed submission of carcasses from the field, as well as foetal death and autolysis *in utero*.
- Abortion in the acute phase of *Toxoplasma* infection, when placentas and fetuses were not yet invaded by the organisms (Owen, Clarkson & Trees 1998).
- Inoculation of tissues without their prior digestion

in acid pepsin or trypsin (Sharma & Dubey 1981; Dubey 1998).

How goats acquired *Toxoplasma* and chlamydial infections in nature could not be established. The animals were possibly infected by intake of *Toxoplasma* oocysts excreted in the faeces of cats which are often found roaming and possibly contaminating the communal grazing lands and water pools in Botswana since they and other felids are the only known definitive hosts for *T. gondii*. It was also evident from the history that the majority of the abortions occurred in those animals which were housed in close contact with each other in kraals at kidding time where they eat the foetal membranes, in this way assisting the spread of the infection. The majority of goats in Botswana are free ranging and usually carry heavy burdens of gastro-intestinal parasites (Carmichael 1972), a factor which may contribute to their lowered resistance and greater susceptibility to these infections. For prevention, the restrictions on the entry of cats in kraals and communal grazing areas, regular deworming of goats, supplementation of fodder with concentrates and mineral mixtures, improved hygiene, and close monitoring of animals at kidding time would be useful in combating lowered productivity and minimizing the public health hazards.

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