New Zealand Veterinary Journal

ISSN: 0048-0169 (Print) 1176-0710 (Online) Journal homepage: www.tandfonline.com/journals/tnzv20

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To cite this article: L.D. Hale & D.D. Heath (1996) The serological response of dogs to *Taenia ovis* , New Zealand Veterinary Journal, 44:5, 165-169, DOI: 10.1080/00480169.1996.35965

To link to this article: https://doi.org/10.1080/00480169.1996.35965

General Articles

The serological response of dogs to *Taenia ovis*

L.D. Hale* and D.D. Heath*†

Abstract

The effect of 4-weekly anthelmintic dosing and other treatment regimes on the serological response of dogs to *Taenia ovis* was examined. Most dogs which were frequently fed infected meat and dosed with a cestocidal anthelmintic at 4-week intervals eventually showed a positive ELISA absorbance. The absence of dosing, or intermittent dosing, of repeatedly infected dogs raised ELISA absorbances to very high levels in most dogs and these absorbances took an increasingly longer time to fall after each new infection. The feeding of large numbers of frozen, dead cysts to sensitised dogs raised absorbance levels. The serological test for *T. ovis* infections in dogs does not detect false postives. Positive tests result from the dog being exposed to *T. ovis* scolex secretory antigen. (New Zealand Veterinary Journal 44, 165–169, 1996.)

Introduction

Existing control of *Taenia ovis* using the serological response of dogs to *T. ovis* is based on the assumption of a 6-week infection[1][2][3]. It is now known that *T. ovis* in New Zealand has a prepatent period of less than 6 weeks[4] and some veterinarians are recommending that dogs be dosed at 4-weekly intervals to prevent the cycle continuing. This paper describes how the serological test performs under different regimes of infection and dosing.

Materials and Methods

Cysts

Sheep infected with 10 000–20 000 *T. ovis* eggs were immunosuppressed weekly by a subcutaneous injection of 0.5 mg/kg dexamethasone trimethylacetate (Opticortenol, Ciba Geigy) and necropsied after 8 weeks of infection. The following day, cysts were removed intact in small pieces of meat and fed to the dogs.

Dogs

The dogs in this study were bred at Wallaceville Animal Research Centre from stock dogs of mainly Border Collie × Labrador origin. Fifteen dogs were aged between 4 and 9 months at their first infection. A further seven dogs were existing breeding stock aged between 3 and 8 years of age. The dogs were purged and dosed with anthelmintic as required. The purgative, arecoline hydrobromide at 25 mg/7 kg body weight (Kempthorne Prosser & Co. Ltd), and the anthelmintic, praziquantel at 50 mg/10 kg body weight (Droncit, Bayer), were administered together. The purge samples were washed through a 1 mm screen and inspected for the presence of tapeworms.

All dogs had a 7 ml sample of blood taken from the cephalic vein once a fortnight into vacutainer tubes (Becton-Dickinson). The serum was separated by centrifugation and was stored at -20 °C until required for testing.

Serum analysis

The serum samples were diluted 1:40 in a phosphate buffered saline solution (pH 7.2) and assayed by ELISA for *T. ovis* antibodies[1].

The results for the test sera are expressed as a percentage of the positive cut-off OD value derived from the control sera. Each ELISA plate had three positive controls, three negative controls and was assayed in duplicate. The optical density (OD) of the ELISA reaction was determined and then the percentage of the positive cut-off value was calculated from the positive cut-off OD as follows:

$$\text{Test value as a percentage of the positive cut-off value} = \frac{\text{Sample OD}}{\text{Positive cut-off}} \times 100$$

$$\text{Positive cut-off (OD value)} = \frac{\text{Positive mean - Negative mean}}{0.5} + \text{Negative mean}$$

Values of 100% or more were considered positive.

Experimental design

Group A: Simulation of intermittent dosing and intermittent infection

Six young dogs were infected with two *T. ovis* cysts, and after 12 weeks the worms were removed with anthelmintics. After a further 26 weeks, the dogs were re-infected with two *T. ovis* cysts and after a further 4 weeks they were again treated. Their serum antibodies were monitored for 18 weeks after the second worm removal.

Group B: Simulation of 4-weekly dosing, with infections lasting 1, 2, 3 and 4 weeks, of naive or previously-infected dogs

To provide a previously infected group, four dogs were infected with five *T. ovis* cysts. Worms were removed after 4 weeks. After a further 8 weeks, the trial was started using these four dogs plus four dogs that had never been infected. Each group consisted of two adult and two young dogs.

The eight dogs were each given two *T. ovis* cysts on four occasions and worms were removed with anthelmintics after periods of 1, 2, 3 or 4 weeks as shown in Figures 2a and 2b. Their serum antibodies were monitored for 16 weeks after the last worm removal.

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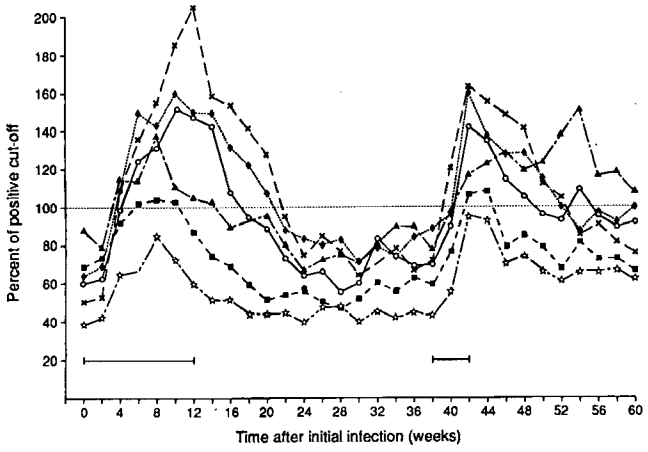

Figure 1. Simulation of intermittent dosing and intermittent infection. Absorbance values of six dogs infected with two *T. ovis* cysts, the infections being terminated after 12 weeks and then re-infected as before 26 weeks later, the second infection being terminated after 4 weeks. Group A dogs: ♦ = 1; ✖ = 2; ■ = 3; ▲ = 4; O = 5; ☆ = 6; ⊢—⊣ = infection period.

Group C: Simulation of 4-weekly dosing and intermittent infection
Three adult and three young dogs were infected with two *T. ovis* cysts each and after 4 weeks the worms were removed with anthelmintics and the dogs were left uninfected for a further 8 weeks. This cycle was repeated for a total of three infections as shown in Figure 3. Their serum antibodies were monitored for 20 weeks after the last removal of worms.

Group D: To establish whether ingestion of large numbers of dead cysts could cause an increase in ELISA absorbance
Two previously infected and two naive dogs were fed a sheep carcass containing one cyst of *T. ovis* per 2 cm^3. The meat had been frozen for 11 weeks. Serum antibodies were monitored for 8 weeks after feeding the cysts.

Results

Group A
During the first infection, five dogs showed positive absorbances to *T. ovis* by 6 weeks after infection (Figure 1). Dog 6 showed an increase in absorbance which did not reach a positive level.

The absorbance of Dog 2 continued to rise until worms were removed but the absorbances of the other five peaked at 8-10 weeks after infection, even though four out of five still had a worm when treated at 12 weeks (Figure 5). By 18 weeks after the removal of worms, the antibody absorbances were down to pre-infection levels.

With the second infection, five dogs showed positive absorbances to *T. ovis* 4 weeks after infection. The antibody levels were higher than the pre-infection levels and the absorbances of two dogs had not returned to negative values 18 weeks after the second removal of worms.

Mature worms were isolated from five of the six dogs at the first treatment (Figure 5). However, only two dogs were found to have worms at the second treatment, despite all showing increases in absorbances. The increases after the second infection became positive about 1 week earlier than after the first infection and were slower to fall after treatment (an average of 10 weeks versus 8 weeks).

Group B
In this trial, the previously infected group (Dogs 7–10) was not observed to be different from the naive group (Dogs 11–14) (Figures 2a and 2b). Dogs 7 and 12 had positive absorbances before infection. These two dogs were the oldest in the trial and were father and daughter.

After the 1- and 2-week infections, small rises in absorbances were seen in five of the eight dogs. Dog 11 was removed from the trial due to an unrelated medical condition. Five of the remaining seven dogs showed an increase in absorbances during

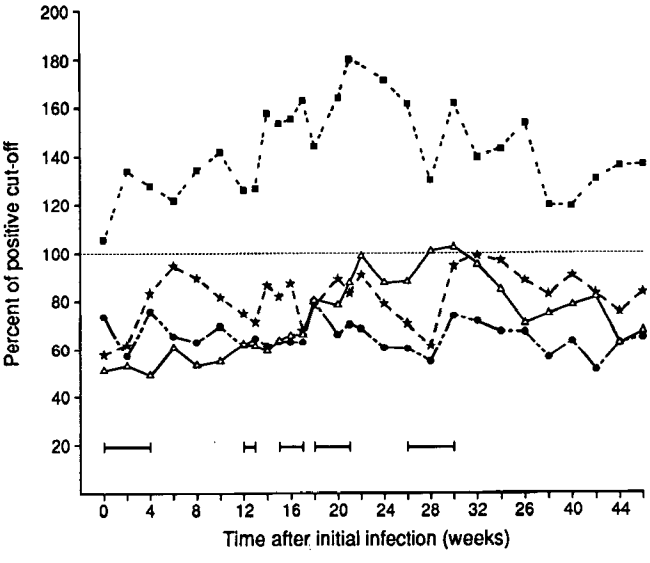

Figure 2a. Simulation of 4-weekly dosing of previously infected dogs. Absorbance values of four dogs infected with five *T. ovis* cysts, the infections being terminated after 4 weeks, following which the dogs were repeatedly infected with two *T. ovis* cysts, the second infections being terminated after 1, 2, 3 and 4 weeks. Group B dogs (previously infected): ■ = 7 (adult); △ = 8 (adult); ★ = 9 (young); ● = 10 (young); ⊢—⊣ = infection period.

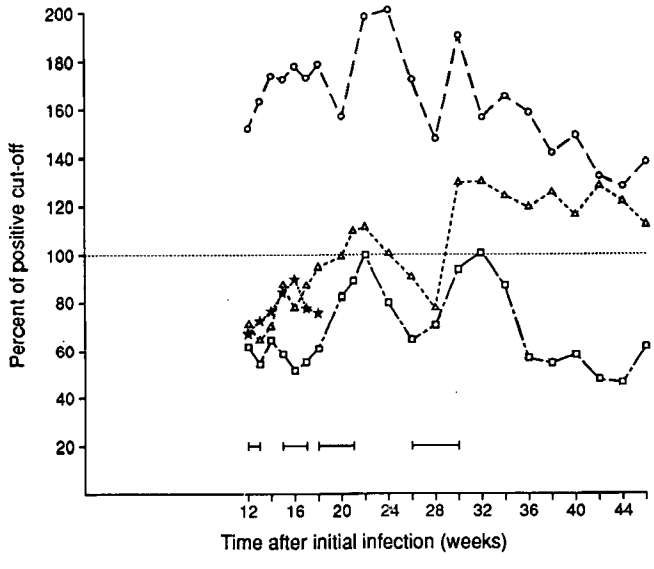

Figure 2b. Simulation of 4-weekly dosing of naive dogs. Absorbance values of four dogs infected with two *T. ovis* cysts, the infections being terminated after 1, 2, 3 and 4 weeks. Group B dogs (naive): ★ = 11 (adult); O = 12 (adult); △ = 13 (young); □ = 14 (young); ⊢—⊣ = infection period.

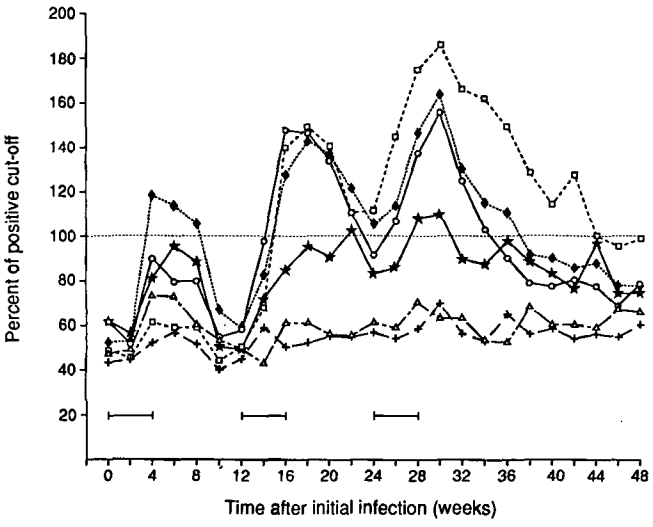

Figure 3. Simulation of 4-weekly dosing and intermittent infection. Absorbance values of six dogs infected with two *T. ovis* cysts for 4 weeks followed by an 8-week uninfected period, then the treatment repeated twice more. Group C dogs: △ = 15 (adult); ★ = 16 (adult); ✚ = 17 (adult); ▲ = 18 (young); ○ = 19 (young); □ = 20 (young); ⊢—⊣ = infection period.

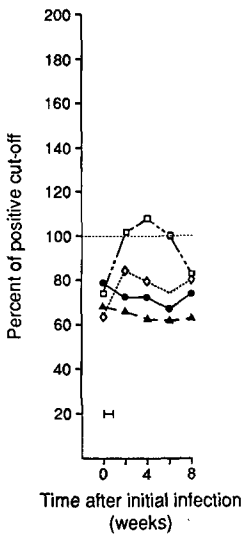

Figure 4. Absorbance values of two previously infected (Group C) and two naive dogs fed a sheep carcass containing large quantities of frozen, dead *T. ovis* cysts. Group D dogs: △ = 19 (previously infected); □ = 20 (previously infected); ● = 21 (naive); ▲ = 22 (naive); ⊢—⊣ = frozen meat fed for this period.

the 3-week infection period and six out of the seven dogs responded to 4 weeks of infection, although most did not reach the positive level.

No worms were found after sieving the purge samples of the 1- and 2-week infections. Six dogs had worms after 3 weeks and all seven dogs produced worms after the 4-week infection (Figure 5). All the worms were immature, though some of the 4-week-old worms were very close to maturation (thin shelled eggs containing immature oncospheres). The worm sizes from the 4-week infection were extremely variable – 6 cm to 150 cm (Figure 5).

Group C

After the first 4-week infection only one of the six dogs had a positive absorbance (Figure 3), although all dogs showed an elevation in absorbance.

The second infection resulted in three dogs with positive absorbances before worm removal, including one which became positive after removal of worms; the other two were non-responders. After the third infection, four dogs were positive and the two non-responders remained negative. Immature worms were isolated from five dogs each time (the same five dogs for the first two infections, with Dog 20 being twice refractory to infection). The younger dogs produced higher absorbances and the three adult dogs (15, 16 and 17) produced low absorbances (16) or became non-responders (15 and 17).

The absorbances of each positive dog increased with each infection and the absorbances were slower to fall after each successive cycle (an average of 4 weeks, then 6 weeks, then 8 weeks).

Group D

The two naive dogs (21 and 22) showed no response to the frozen cysts, but the two dogs (19 and 20) that had been previously infected showed an increase in absorbances (Figure 4). One dog gave a positive value.

Susceptibility to infection

The lengths and numbers of worms found at each treatment are shown in Figure 5. In most dogs (50%), the two cysticerci established and generally developed "normally". However, in some dogs the worms were stunted, and in some dogs no worms established. The establishment and development of worms did not appear to be related to the ELISA absorbance.

Discussion

Providing the dog receives the correct amount of anthelmintic and retains the dose, 4-weekly dosing of dogs prevents *T. ovis* tapeworms from reaching maturity. From our results, we conclude that if a dog is regularly fed infected meat while on 4-weekly dosing, the absorbance of the antibody response is likely to become higher with each infection until a positive reading is reached. If the infection period is a full 4 weeks, most infected dogs will have a positive absorbance after their second exposure. Repeated infections generally resulted in progressively higher absorbances and a longer period was required for the absorbances to fall to normal again.

Three of the 20 dogs (15%) did not show increases in antibody titres, despite the presence of worms when purged. This is consistent with the findings of Heath *et al.*[1][2] where about 20% of dogs were virtual non-responders to a primary *T. ovis* infection.

The two dogs from Group B (7 and 12) that had positive absorbances prior to infection had never previously been infected. They had, however, received frozen meat containing dead *T. ovis* cysts throughout their lives and may have developed antibodies due to repeated exposure to dead but antigenic cysts for many years. On the other hand, these dogs were related, and there may be a hereditary factor in responsiveness to *T. ovis*, because most of our breeding dogs, fed similarly, do not have high absorbances against *T. ovis* antigen.

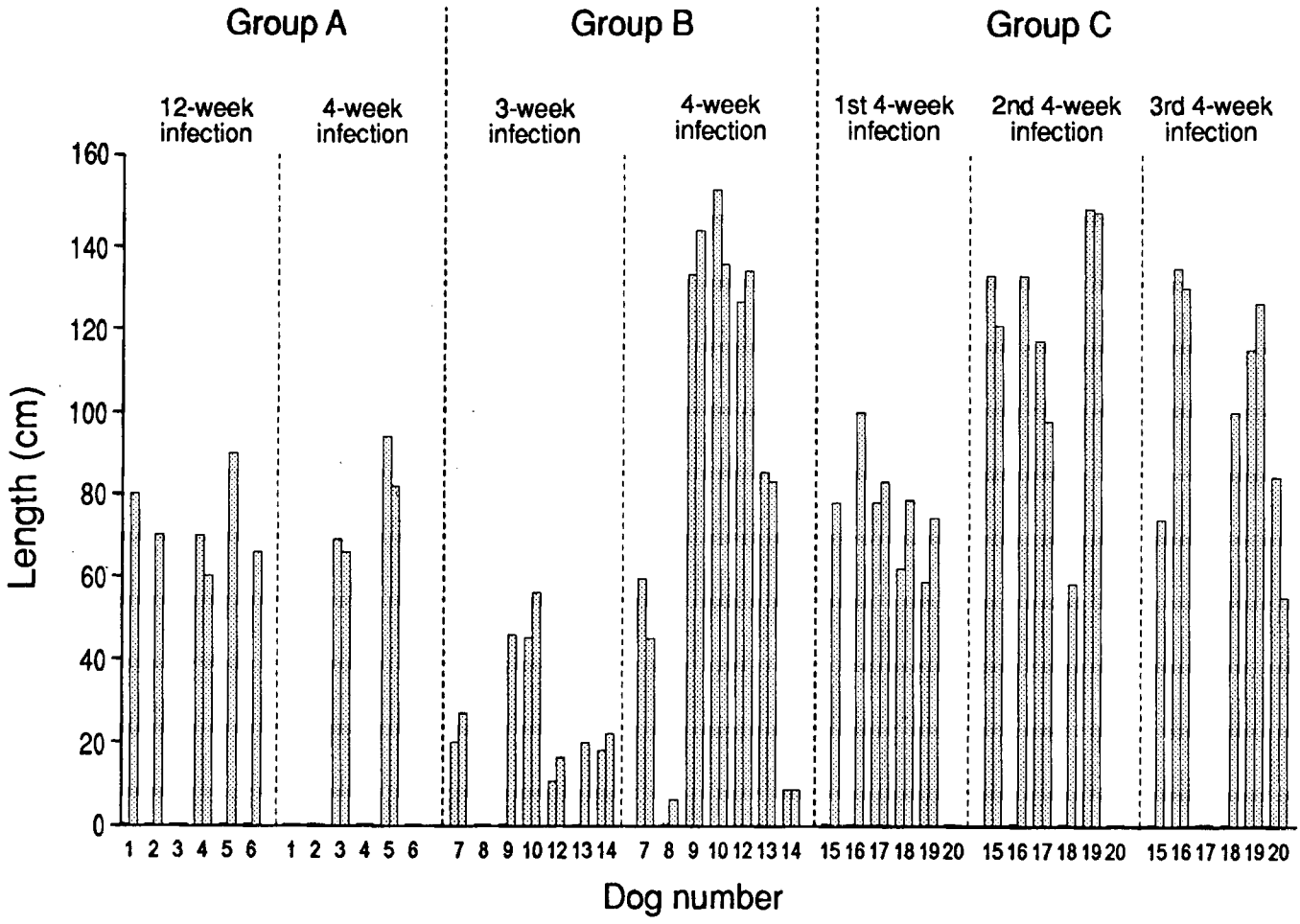

Figure 5. The length of *Taenia ovis* worms and numbers of worms obtained at purgation of Groups A, B and C dogs. All dogs received two cysts on each occasion. Lack of a histogram column indicates that one or two worms were not present at purgation. Group B dogs at 1 and 2 weeks did not appear to have any worms. However, scoleces could have been lost through the 1 mm mesh of the sieve used for washing faeces.

The two previously sensitised dogs (19 and 20) that showed an increase in absorbances provide evidence that antibodies in dog serum can be stimulated by the ingestion of dead cysts provided the level of antigenic challenge is sufficiently high (1 cyst/2 cm^3 of muscle tissue, equivalent to 500 cysts daily for 7 days compared to an estimated daily consumption by our stock breeding dogs of 1 dead cyst per day).

Dogs 8 and 14 in Group B and Dog 20 in Group C appeared to be moderately resistant to *T. ovis* worm development. Dog 20 in Group C did not respond well to the first infection, but it was the highest responder to the two subsequent infections even though it had no worms at the second purgation. Dogs 8 and 14 in Group B were also less responsive, but could not be termed non-responders. It is possible that the exposure to secretory antigen from the worm scolex is less in some dogs that reject their worms, and the antibody absorbance to this antigen would not be stimulated as much as in those dogs which retained their worms. Dog 20 became a sensitised dog for Group D, and was the only dog that produced a positive absorbance after being fed frozen cysts. It is not likely that Dog 20 was free of worms at its first two purgations by chance. As shown in Figure 5, excluding the 1- and 2-week results of Group B which were probably lost through the apertures of our sieve, out of 70 scoleces fed, 57 established, indicating 81% viability. The chance of a dog receiving 2 non-viable scoleces is 4%, whereas 20% of infections resulted in no worms being found at purgation.

The reason for non-responsiveness must lie in the type of immune response engendered. If 80% of dogs mount a systemic IgG response[1][2] and 20% do not, despite harbouring *T. ovis* cestodes, these latter dogs probably are genetically different. In the same way, two high-responder dogs, father and daughter, were identified in Group B. These dogs had systemic antibody probably stimulated by the eating of an average of one freeze-killed dead *T. ovis* cyst per day. The evidence thus points to a wide spectrum of responsiveness to *T. ovis* scolex secretory antigen in the out-bred dog population of New Zealand (about 500 000 dogs). A small proportion of dogs will respond well to *T. ovis* antigen, even if the scolex is frozen and dead. A small proportion will be non-responders or poor responders in terms of systemic antibody. The vast majority of dogs will respond well to infections that persist for 4 weeks or longer, and the decline in titres will be a function of the relative magnitude of the titre.

The series of trials described here have been carried out to elucidate various presumed false positives that have been reported to us during 6 years of serological testing of dogs for antibodies to *Taenia ovis* as a means of determining whether dogs have had access to sheep or goat meat containing live *T. ovis* cysts. The technology was requested by the National Hydatids Council in 1983, when it became apparent that arecoline purging of dogs to diagnose incorrect dog feeding was not sufficiently sensitive. This was because dog owners were able to treat their dogs with an anthelmintic

prior to presenting the dogs for arecoline purging, and thus avoid paying a penalty if a worm was produced. The difference between the observed prevalence of *T. ovis* infection in dogs using the arecoline purge, and the prevalence revealed using the serological test, enabled the National Hydatids Council to conclude that many dog owners were relying on 6-weekly treatment with praziquantel to remove any worms resulting from indiscretions in dog-control or dog-feeding. This information prompted the National Hydatids Council in 1989-90 to remove the requirement for dogs to be regularly treated every 6 weeks, except in situations where home-killing was practised, and *Echinococcus granulosus* could still possibly cycle. The rationale was that the sheep farmer had the tools available to control *T. ovis*, by freezing dog meat to kill the cysts, by controlling their own dogs' access to carcasses and by limiting the access of other dogs to their farms unless accompanied by a valid treatment certificate showing that the dog had been treated with praziquantel at least 5 days prior to entering the farm, and not more than 35 days previously. Anthelmintics to correct any breakdowns in dog control or feeding could also be used judiciously and many farmers put their dogs on to a 4-weekly anthelmintic regime as a precaution. The dog serological test, testing every rural dog once every 2 years, was retained for two reasons; it assisted in registration of dogs, and it allowed dog owners to become aware of faults in their dog-feeding practices. For monitoring of dog sera, our laboratory has received and reported on 80 000–100 000 sera annually from 1990 to 1995. The conduct of this test, and the epidemiology of *T. ovis* revealed from the analysis of the database kept on all tests, will be the subject of a further paper. The test was not meant to enforce *T. ovis* control, but to assist it. Nevertheless, some local authorities introduced a cost-recovery penalty for a dog with positive *T. ovis* serology. This inevitably increased the interest of the dog-owner in how the test worked, especially if they could not perceive how their dog could have acquired antibodies to *T. ovis*.

There appeared to be three ways in which a dog could produce a positive OD, and yet not have contaminated the environment with *T. ovis* eggs.

The first could be where a dog had had a prolonged infection previously, and was sensitised to infection. Later, when it received an infection that lasted for 4 weeks under a 4-weekly anthelmintic regime, the antibody response might be hyperstimulated by an anamnestic response, which could in some dogs keep antibody high for some time. A number of these situations were identified, and subsequent tests showed OD levels to be dropping each time. Group A dogs demonstrated this sensitisation.

The second situation was where dogs were on a carefully administered 4-weekly dosing programme, but were receiving untreated sheep meat. In time, these dogs could develop positive ODs, as shown in Groups B and C.

The third situation was where dogs were fed on meat that had been frozen to -10 °C for at least 7 days, but which often contained *T. ovis* cysts that had been live and infective until frozen. In this case, the intestinal mucosa would be exposed, regularly or intermittently, to *T. ovis* scolex antigen, which forms the antigen used in the serological test. In Group B, two of our older dogs that had regularly received frozen but infected sheep meat throughout their life had positive ODs at the beginning of the trial. None of our other breeding dogs or bitches exhibited this trait, and there may have been a genetic predisposition to hypersensitivity to *T. ovis* antigen, because the two dogs were father and daughter. We have shown that it is possible to stimulate a systemic antibody by hyperstimulation of the intestinal mucosa. In Group D, two dogs that had been infected previously also showed a response to being fed on heavily infected frozen sheep meat, with one dog showing a positive OD. However, Dog 20, which became positive, had the highest OD in its previous trial (Group C).

The overall conclusion from this work is that the serological test to detect antibodies to *T. ovis* infections in dogs generally does not produce false positives. Positive serology seems to be the result of exposure to *T. ovis* scolex secretory antigen, generally from live cysts, the scoleces of which attach to the wall of the proximal small intestine and secrete antigen to the submucosa, thus promoting a systemic antibody reaction that can be measured using the serological test. Positive serology is an indication to the dog-owner that control of *T. ovis* could be compromised at any time (for instance, by a dog rejecting its monthly anthelmintic tablet, or by being underdosed), and that other procedures, such as freezing sheep and goat meat before feeding to dogs, should not be abandoned.

Taenia ovis is a parasite of dogs in New Zealand which is likely to remain a problem. Trade barriers based on detection of caseous or calcified cysts in lamb meat have been threatened in the past, and prompted the initiation of *T. ovis* control in New Zealand from 1971 onwards. Since then, several niche markets have been lost because of *T. ovis* cysts, and extra inspection is required for mutton processed for the Japanese market.

The current *T. ovis* control procedure, where the New Zealand meat industry has accepted that *T. ovis* is a marketing problem and is therefore one belonging to them, is for the meat industry to identify lines of stock with *T. ovis* cysts and report this to the consigner for action. However, it is impossible for individual sheep meat producers to protect themselves against *T. ovis*, unless they use the *T. ovis* vaccine for lambs[5]. Otherwise, regional dog treatments are the only feasible alternative, and the serological test can play a significant role in maintaining the effectiveness of a regional scheme.

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Accepted for publication 24 June 1996.