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# A blind test of the serological response of dogs to infection with *Taenia ovis*

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## ABSTRACT

Fifty six dogs of mixed age and sex were acquired from farms in the Otago/Southland region, and maintained at the Hydatid Research Unit, Taieri, where 43 were each fed two *Taenia ovis* cysts. All were bled fortnightly for six or 12 weeks. Coded sera were sent to Wallaceville Animal Research Centre for testing using ELISA, with antigen from *T. ovis* scoleces. Dog treatments were identified after all tests were complete. A discriminant level was derived from the mean absorbance value plus three standard deviations of 56 sera taken at time zero and 78 sera from serially bled uninfected dogs. None of these 134 sera registered as a false positive using this discriminant level. The data showed no significant deviation from normality, and the expected frequency of the occurrence of false positives is therefore less than 0.14%. Four weeks after infection 63% of dogs proved to be infected were serologically positive, rising to 78% after 6 weeks. When worms were removed by anthelmintic treatment, ELISA absorbance levels decreased. Four weeks after removal 70% of previously infected dogs remained positive, decreasing to 30% after 6 weeks.

Six weeks after infection the sensitivity of the test was 78%, and the specificity 63%. However, if dogs with positive ELISA absorbance levels, but which did not purge worms, were regarded as having had worms, the respective figures would be 82% and 100%. The latter figures are similar to our previously published laboratory results. The test is of comparable efficiency to arecoline purgation for surveillance, and has the additional advantage of detecting infection in the majority of those dogs that have been infected for three weeks or more but fail to pass worms on purgation, and a substantial proportion of those infected dogs that were treated by their owners prior to presenting them for purgation in order to avoid detection of infection.

## INTRODUCTION

A serological test has been developed<sup>(1)</sup> to detect *Taenia ovis* infection in dogs. The *T. ovis* antigen cross-reacts with sera from dogs infected with *T. hydatigena*, but not with sera from dogs infected with *T. pisiformis* or *Echinococcus granulosus*. With sera collected from laboratory-bred dogs that had been infected with *T. ovis* for six weeks or longer, the test detected 87% of infected dogs, with no false positives. The test had a high repeatability, and was not greatly influenced by the number of worms carried by the dog. Higher titres were correlated with long-standing infections.

Validation of this test under field conditions in New Zealand was necessary before it could be offered as part of the hydatid control programme. Farm dogs were assembled at the Hydatid Research Unit at Taieri, and experimentally infected. The presence or absence of worms was determined by arecoline purging and necropsy.

## MATERIALS AND METHODS

### Animals

Dogs were obtained from farms in Otago-Southland, and housed in dog motels at the Hydatid Research Unit, Taieri. Dogs were of mixed breeds, sex and age, but there was a predominance of Border-Collie. On arrival they were treated for intestinal nematodes 1 tab/7 kg Canex Plus<sup>‡</sup> and cestodes 5 mg/kg Droncit,<sup>®§</sup> and vaccinated against canine distemper, hepatitis and parvovirus. When all were assembled, the minimum time from arrival of dogs to the start of the experiment was four weeks. They were randomly divided into three groups. Group A consisted of 21 dogs that were each fed two

*T. ovis* cysts, purged to remove worms six weeks later, and necropsied 6 weeks after purging. Group B consisted of 22 dogs each fed two *T. ovis* cysts and necropsied six weeks later. Group C consisted of 13 uninfected dogs that were necropsied at the same time as Group A.

### Experimental infections and purging

*T. ovis* cysts were obtained from an infected sheep at Wallaceville Animal Research Centre. They were individually excised in blocks of muscle and sent by air to Taieri. Dogs were fed cysts within 24 hours of the death of the sheep.

Dogs in Group A were purged with 1 mg/kg arecoline hydrobromide plus 2.5 mg/kg praziquantel, and one day later given 5 mg/kg praziquantel to remove any worms that resisted purgation. All dogs were purged by the treatment, but not all purges were full of mucus and it was considered that some worms may not have been removed.

### Serology

Blood was taken from the cephalic vein at the beginning of the experiment, and at fortnightly intervals thereafter until necropsy, using 5 ml vacutainer<sup>||</sup> tubes and 22 g needles. After clotting, tubes were stored at 4 °C overnight, centrifuged at 3000 r.p.m. for 30 minutes and the serum removed into 4 ml plastic tubes with caps. The tubes were sent immediately by air to Wallaceville. On arrival, sera were stored frozen at -20 °C until tested. Serological testing followed the method previously described.<sup>(1)</sup>

## RESULTS

### Worms

Number of worms found at purgation or at necropsy are shown in Figs. 1, 2 and 3. No dogs in Group A or C had worms at necropsy.

### Serology

The absorbance value above which the ELISA test could be considered positive was calculated as the mean absorbance

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||Vacutainer<sup>®</sup> Becton Dickinson, U.S.A.

‡100mg pyrantel, 380 mg oxantel/tab. Pfizer Laboratories Ltd, Manukau City, N.Z.

§Praziquantel, Bayer Leverkusen, Germany. Henry H. York & Co. Ltd, N.Z.

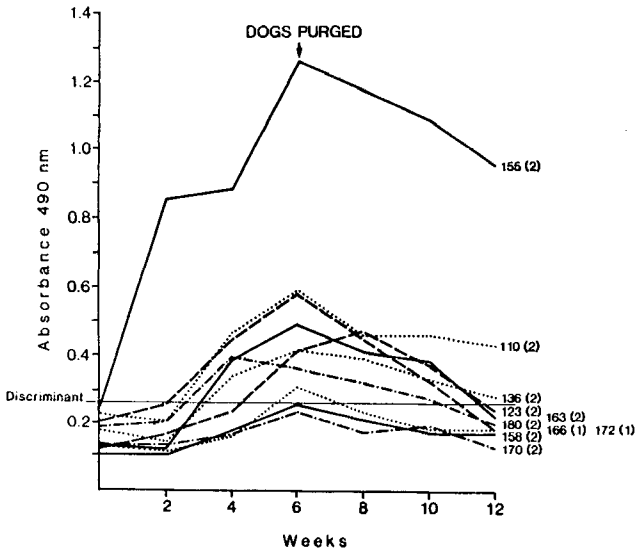


Fig. 1: Absorbance values of Group A dog sera from dogs that produced worms at purgation (number of worms in brackets).

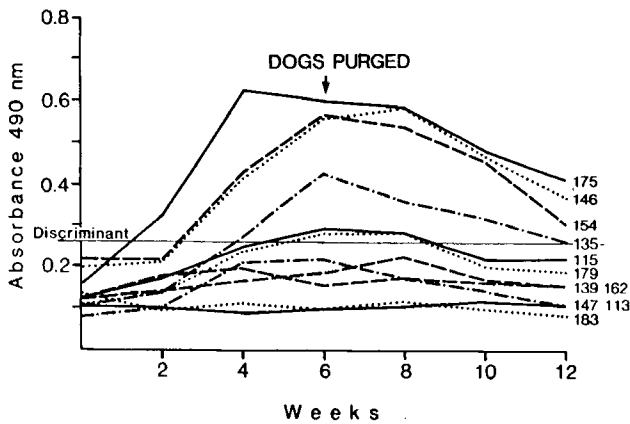


Fig. 2: Absorbance values of sera from Group A dogs that did not produce worms when purged 6 weeks after infection.

value of sera from uninfected dogs, plus three standard deviations. These sera consisted of sera from 56 dogs collected at the beginning of the experiment and 78 sera collected from 13 uninfected dogs at 2, 4, 6, 8, 10 and 12 weeks. The mean absorbance on the test day was 13.97, with a standard deviation of 4.04. The discrimination value was therefore 26.09, and titres higher than 26 were called positive. The data showed no significant deviation from normality (Shapiro-Wilk test).

In Group A, 8/10 dogs that produced worms at purgation became serologically positive within 6 weeks from infection (Fig. 1). Of those that did not produce worms, 6/11 also became positive (Fig. 2).

In Group B, worms were found at necropsy in 17 dogs, and of these 13/17 became serologically positive and four did not (Fig. 3). The five dogs that did not harbour worms remained negative and their serology is not shown.

Fig. 4 shows the percentage of dogs with worms in Groups A and B that had positive titres at each bleeding. The highest percentage of positive dogs (78%) was found six weeks after infection. Four weeks after removal of worms, 70% of dogs were still positive, but then the absorbance values declined rapidly.

In Group C, control dogs, 13/13 remained serologically negative.

**DISCUSSION**

Not all dogs were infected after exposure to two *T. ovis* cysts. Previous experience<sup>(1)</sup> suggested that about 62% of dogs might

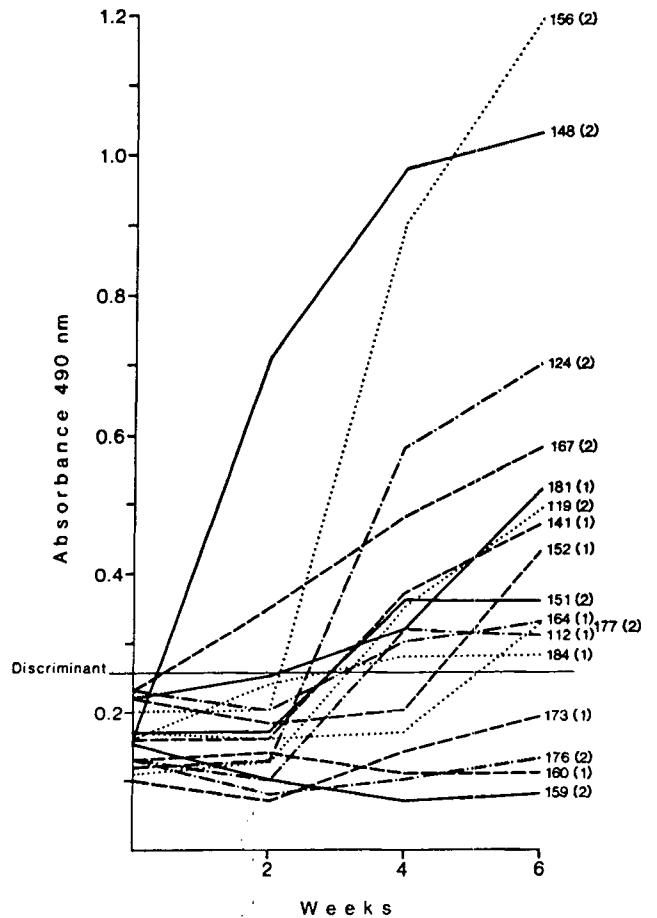


Fig. 3: Absorbance values of Group B dog sera from dogs that had worms at necropsy (number of worms in brackets).

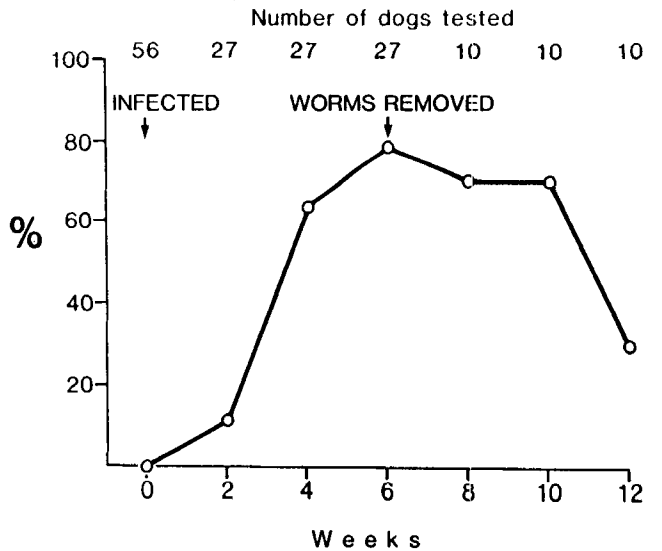


Fig. 4: The percentage of dogs harbouring *Taenia ovis* worms that gave a positive serological result when tested preinfection (o), post-infection (2, 4, 6) or after purgation of worms (8, 10, 12).

become infected. In Group B 17/22 (77%) became infected. The reason why some dogs resist infection is not clear, but appears, to some extent, to be an inherent characteristic.<sup>(1)</sup> Although only 10/21 dogs produced worms at purgation in Group A, Fig. 2 indicates that a further six were probably infected but did not produce worms when purged and were also uninfected at necropsy. Failure to produce worms after purging is one of the drawbacks of arecoline testing of dogs.<sup>(2)</sup> Alternatively, these dogs may have had infections that established for less than 6 weeks.

ELISA absorbance values rose until the period around purging, and then declined. For the identification of *T. ovis* infected dogs, the discriminant absorbance level (control mean + 3 standard deviations) has a statistical probability of demonstrating less than 0.14% false positives. The sensitivity of a test is its ability to detect infected animals, and specificity, its ability to detect uninfected animals. The present data give two different determinations of sensitivity and specificity, depending upon whether dogs with positive serology, but from which worms were not recovered, are interpreted as not-infected or infected.

1. Purge negative/seropositive interpreted as not-infected.

	Infected	Not-infected	
Test + 've	21	6	Sensitivity = $\frac{21}{27} \times 100 = 78\%$
Test - 've	6	10	Specificity = $\frac{10}{16} \times 100 = 63\%$
Totals	27	16	

2. Purge negative/seropositive interpreted as infected.

	Infected	Not-infected	
Test + 've	27	0	Sensitivity = $\frac{27}{33} \times 100 = 82\%$
Test - 've	6	10	Specificity = $\frac{10}{10} \times 100 = 100\%$
Totals	33	10	

Thus with six week-old infections the test should detect approximately 80% of infected dogs with a very low probability of false positives. In New Zealand at the present time, however, where most rural dogs are dosed with Droncit® at six-week intervals, the average age of any *T. ovis* present is likely to

be around three weeks. At this stage after infection the sensitivity is reduced to about 50% but because antibody titres can persist for several weeks after removal of the parasites, the rate of detection of dogs recently exposed to *T. ovis* infection is likely to be considerably higher than 50%. The test is thus of comparable efficiency to arecoline purgation for surveillance. It has the additional advantage of detecting infection in the majority of those dogs that have been infected for three weeks or more but fail to pass worms on purgation, and a substantial proportion of those infected dogs that were treated by their owners prior to presenting them for purgation in order to avoid detection of infection. The procedure is also arguably more humane than arecoline dosing.

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