AUSTRALIAN STANDARD DIAGNOSTIC TECHNIQUES FOR ANIMAL DISEASES

STANDING COMMITTEE ON AGRICULTURE AND RESOURCE MANAGEMENT

ANIMAL HEALTH COMMITTEE

SUB-COMMITTEE ON ANIMAL HEALTH LABORATORY STANDARDS

Elaphostrongylus in Deer

Detection of Larvae in Faeces

P. J. A. Presidente

Victorian Institute of Animal Science, Department of Agriculture, 475–485 Mickleham Road, Attwood, Vic. 3049, Australia.

Contents

1. Introduction

The tissue worm of deer, *Elaphostrongylus cervi*, has not been found in Australia and is considered an exotic parasite to this country (Presidente, 1984). In red deer, the normal host, the nematodes live in the fascia over muscles in the forequarters; granulomatous lesions associated with degenerate worms and discolouration of regional lymph nodes may result in condemnation of carcasses (Mason, 1989). However, the parasite is a potential pathogen to other species of deer and possibly other ungulates. Infection may cause posterior paresis and paralysis as larvae migrate up the spinal cord; nervous disorders, blindness and death may occur if worms locate in the meninges (Watson, 1983). As a chronic form of the disease, ill-thrift and verminous pneumonia occur.

Deer with mature infection may be detected by recovering first-stage larvae that pass out in the faeces. However, there is a long prepatent period of up to 125 days when infection with developing worms cannot be detected. Larval output is often low and shedding may be intermittent. Moreover, treatment with anthelmintics suppresses output of larvae for several weeks (Watson, 1986).

From 1975 to 1984, importation of deer to Australia was not allowed because animals infected with *E. cervi* are difficult to identify and there was, and still is, no effective treatment to kill the worms. Importation of live deer from New Zealand began in 1986 to accelerate expansion of the deer industry in Australia. After *E. cervi* infection was first detected (Presidente, 1986), quarantine regulations were progressively tightened to ensure that introduction of important pathogens, such as tuberculosis and *E. cervi*, did not occur.

There was a need for standardised testing of imported deer for *E. cervi* while held in quarantine in Australia. The Baermann test, a procedure used to recover nematode larvae from faeces or herbage, was used but modified to increase the likelihood that small numbers of larvae could be detected in deer samples. In addition, each deer was tested three times during the quarantine period of 100 days.

Since 1986, nearly 10 000 deer have been imported from New Zealand and tested by the procedures described herein. The modified Baermann test has proven sufficiently sensitive to detect infection in eight deer while held in quarantine in Australia. Post mortem inspection of carcasses from these animals were unsuccessful in finding the mature worms.

2. Tissue Worm

2.1. Taxonomy

The tissue worm is a Nematode, or roundworm, and therefore is classified as belonging to the CLASS NEMATODA. It is assigned to the Metastrongyloidea, a group that consists mostly of lung parasites of a variety of mammals. The parasite was first described from a red deer (*Cervus elaphus*) in Scotland by Cameron (1931). Since that time two other similar worms have been identified. Recently, the three nematodes have been re-examined and their validity as distinct species confirmed (Gibbons *et al.*, 1991). The full classification of the tissue worms is:

CLASS NEMATODA
ORDER STRONGYLIDA
SUPER FAMILY METASTRONGYLOIDEA
Family Protostrongylidae
Subfamily Elaphostrongylinae
E. alces
E. cervi
E. rangiferi

2.2. Hosts and Geographic Distribution

Red deer are the normal host for *E. cervi* and the parasite has been reported in this host in Scotland, throughout Europe and occurs in maral (*C. elaphus sibericus*) and sika deer (*Cervus nippon*) in Western Russia. Infection has also been identified in roe deer (*Capreolus capreolus*) (English *et al* ., 1985; Mason, 1989) but not in fallow deer (*Dama dama*).

In 1976, *E. cervi* was first identified in red deer and wapiti (*C. elaphus canadensis*) in the south-western region of the South Island of New Zealand. A survey of deer farms in 1981 revealed a prevalence of 35% in faecal samples of red deer on 115 farms (Mason and Gladden, 1983).

Infection in moose (*Alces alces*) in Sweden is caused by *E. alces* while reindeer (*Rangifer tarandus*) in Sweden and Norway and caribou (*R. tarandus caribou*) in Newfoundland have *E. rangiferi* (Gibbons *et al.*, 1991).

2.3. Life Cycle

Mature parasites are thin, thread-like worms up to 6 cm long that live in the connective tissue between the muscles over the shoulder region, or on the meninges of the brain or spinal cord. Eggs are carried to the lungs in the blood, larvae hatch in the lungs, migrate into the airways, are swallowed and pass out in the faeces (Watson, 1981).

The parasite has an indirect life cycle; i.e., an intermediate host is required for *E. cervi* to complete its life cycle (Watson, 1981). Development to infective stage larvae takes about two months in the intermediate host. Three slugs (*Arion hortensis, Deroceras reticulatum* and *Lehmannia flava*) and one land snail (*Helix aspersa*), were identified as potential intermediate hosts for *E. cervi* in New Zealand (Watson and Kean, 1983). These species are common introduced species in Australia as well and are widespread throughout the eastern states.

The larvae are accidentally ingested in infected snails by deer while grazing. They burrow

into the wall of the stomach and migrate up nerves into the spinal cord. Then, the larvae proceed up to the brain or out into connective tissue between muscles over the shoulders, rib cage or forelimbs.

The time required for the worms to mature is variable and depends on the numbers of larvae ingested. Watson (1983) reported that larvae were first detected in faeces 107–125 days after infection when 200 infective larvae of *E. cervi* were inoculated, but only 86–98 days if 400–500 larvae were given (Watson, 1986).

3. Infection

3.1. Clinical Signs and Pathology

No clinical signs in red deer have been attributed to light infection acquired naturally in the wild (Mason, 1989). In red deer calves experimentally infected with 200 *E. cervi* larvae, exercise intolerance was associated with verminous pneumonia; blindness, hindlimb incoordination, nervous disorders and deaths also occurred (Watson, 1983). Diffuse interstitial penumonia, focal consolidation, emphysema and severe pulmonary haemorrhage result from an inflammatory reaction to first-stage larvae of *E. cervi* (Watson 1981, 1983). Nervous disorders were caused by focal reactions to worms, eggs and larvae located in the subarachnoid space or leptomeninges of the brain.

Chronic granulomatous lesions that encapsulate degenerate worms in the fascia result in discoloration of the fascia and lymph nodes. Treatment with anthelmintics may promote formation of these lesions. For example, Watson (1986) observed that the carcass of an experimentally infected deer, killed four months after treatment with ivermectin, was condemned at slaughter because of numerous nodules on the muscles.

3.2. Other Hosts

In cervids other than red deer, *E. cervi* often migrates to the brain and causes central nervous signs; the parasite is also a potential pathogen of sheep, goats and cattle (Watson, 1981). Three clinical syndromes have been identified: an acute form when posterior paralysis occurs; a chronic form of illthrift; and verminous pneumonia (Mason, 1989). It is not known whether *E. cervi* is pathogenic to the tropical deer species, chital (*Axis axis*), hog (*Axis porcinus*), rusa (*Cervus timorensis*) and sambar deer (*Cervus unicolor*), that occur in Australia if they were to become infected with this parasite. The response of fallow deer to artificial infection is unknown.

Limited experimental infection studies suggest that *E. cervi* is less pathogenic than the meningeal worm, *Parelaphostrongylus tenuis*, a parasite of white-tailed deer (*Odocoileus virginianus*) in North America. Populations of moose and wapiti have died out in areas where the white-tailed deer has extended its range during the past 100 years. Recent attempts to reestablish moose and wapiti in their original habitats, or introduce new deer species where there are white-tailed deer now, have all been unsuccessful. It was not until the mid 1960s that wildlife researchers identified the meningeal worm as the cause of the deaths (Anderson, 1972).

4. Prevention

4.1. Treatment

There is no anthelmintic that kills all mature worms, although after treatment larval output in faeces often declines. When given at elevated dose rates for several days, the benzimidazole anthelmintics, thiabendazole and mebendazole, were reported to be highly efficacious against both larvae and adults (Mason, 1989). Other investigations indicate that treatment with oxfendazole given for three consecutive days, or a single subcutaneous injection with ivermectin, only reduced larval output for several weeks (MacKintosh *et al.*, 1985, Watson, 1986).

4.2. Quarantine Regulations

A protocol has been developed for the importation of live deer from New Zealand. The current conditions for importation are summarised below.

(a) The farm must remain 'closed' for one year and deer for export isolated for a six months . Prior to export, the deer are held off pasture on sand for a preembarkation quarantine period of 42 days.

(b) The farm must have no history of *E. cervi* infection. During that 'closed' period, two whole herd tests for *E. cervi* are undertaken. If infection is detected in any deer, the farm is declared ineligible.

(c) To improve the likelihood that the test for *E. cervi* undertaken during the pre-embarkation period in New Zealand will detect infection, deer are not treated with an anthelmintic 80 days prior to entry. This is beyond the time when larval shedding would be suppressed by anthelmintics.

(d) To improve the likelihood that testing in Australia will detect infection, deer must not be treated for nematodes at the end of the preembarkation quarantine period.

The regulations that apply to imported deer on arrival in Australia are as follows.

(a) The imported deer are held in quarantine off pasture for 100 days in a Government approved quarantine station.

(b) Each deer is tested three times for *E. cervi* at 30 day intervals, beginning no earlier than 28 days after arrival.

(c) After three negative tests and before leaving the quarantine station, the deer are treated twice with ivermectin with an interval of four to five days between treatments.

5. Diagnosis

Detection of infected animals with mature *E. cervi* is possible by recovering larvae that pass out in the faeces. Infection with immature worms during the long prepatent period cannot be detected by this means. Output of larvae is often low, <1 larva per gram of faeces (lpg), particularly in deer that have previously been tested at least four times. Stress is known to affect larval output which increases among males during the rutting season and among females during lactation (Halvorsen, 1986). The transport and shipment of deer, their period of quarantine including the sampling required, all provide additional stressors that should increase the likelihood that larval shedding will occur and detection of infection possible.

5.1. Sensitivity of Test

To achieve the sensitivity required to detect light infections when larval output is likely to be <1 lpg, a sample size of 20 g faeces is used. The animal is retested when the sample submitted is <15 g. This achieves a theoretical sensitivity of 0.05 lpg (Mason, 1989). Tests in New Zealand indicate that in deer with low larval counts (<1 lpg), detection of larvae is inconsistent when sample size is < 8 g (P.C. Mason, pers. comm. 1988).

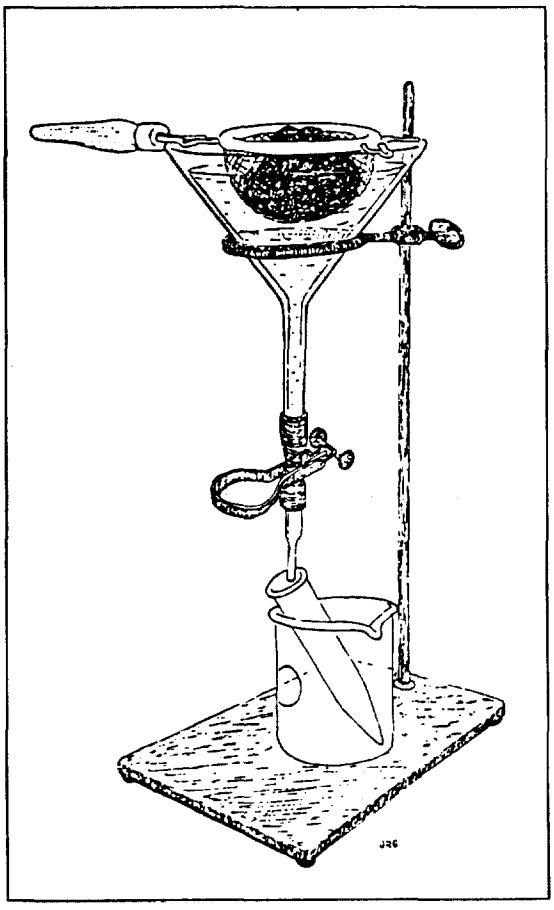

Figure 1. Components of the Baermann technique: a funnel, clamp, strainer and stand (Georgi and Georgi, 1990).

5.2. The Baermann Apparatus

The basic components of the apparatus are a funnel, a piece of tubing to which a pinch clamp is attached, a stand to support the funnel and a small sieve or tea strainer (Fig. 1). The basic principle is that when faeces are wrapped in gauze or cheesecloth, set in the tea strainer and suspended in lukewarm water, larvae in the faecal sample will become motile, escape from the faeces and slowly drop to the bottom of the funnel where they accumulate above the clamp. It is important that the sample collected is as free of debris as possible to ensure that larvae are detected on microscopic examination. Too much debris decreases the likelihood of detecting the larvae.

The cheesecloth and strainer are used to reduce the amount of debris that accumulates at the clamp. The time required for larvae to settle depends on the size and activity of the larvae, as well as the distance that they must drop. The fluid at the clamp is then collected, concentrated by centrifugation, the supernatant fluid discarded and the sediment examined under the microscope.

To standardise the method for use in Australia, the size of faecal sample, incubation temperature, sedimentation time and speed of centrifugation have been set. All materials to use, screen type and size, type of funnel, tubing, tissue paper and tissue culture plate have been specified (see 5.3.). The funnel stand and racks were designed to allow for a high through put of samples, with minimal chance for error.

5.3 Specifications for the Basic Apparatus

5.3.1. Funnels

Plastic funnels (Selby Anax) [11 cm outside diameter (o.d.)] are sufficiently large to accommodate faecal samples weighing from 15 to 25 g. A 10 cm length of silastic tubing (Dow Corning) (13 mm o.d.) is pushed onto the stem of the funnel so a clamp can be attached.

The funnels may be rinsed quickly with hot water if they have to be reused immediately. Periodically, the funnels are soaked in hot soapy water for several hours, then rinsed before reuse. Growth of algae may occur in the silastic tubing if the funnels are not washed well every three weeks.

5.3.2. Clamps

A variety of metal clamps was tested and leakage proved to be a significant problem with them. When silastic tubing (10 cm long, 13 mm o.d.) and snap-on plastic clamps (Nalgene Labware) were used, leakage declined to <1 % of samples tested (Fig. 1).

5.3.3. Screens

To ensure good recovery of larvae from the sample, an adequate surface area above the screen is necessary so that the sample is not 'packed' in

Figure 2. Technician collecting sediment from funnels. Funnel stand showing construction of the racks to support funnels, the funnels with clamps and splash trays. 80 samples could be tested in a stand like this.

the funnel. A fine metal screen (Swiss Screens), pore size 100 µm, 150 mesh/in, 45 ga, with a diameter of 73 mm placed in the funnel (20 mm from the rim) was used to support the faecal sample. There was an adequate area above the screen to cover the faeces with water and the surface area sufficient for larvae to migrate through the fine screen. The amount of debris that dropped down to the clamp was minimal.

The screens proved to be durable and could be quickly soaked in hot soapy water, rinsed in hot water, then put back in the funnels for immediate use again, if required.

5.3.4. Funnel Stands and Racks

To process large numbers of samples and enable rapid cleaning for immediate reuse, moveable metal stands of 80 funnel capacity were constructed to support 16 metal racks that each held five funnels (Fig. 2). Each funnel rack measured 65 x 15 cm to allow a 1 cm gap between each funnel and a lip to fit along brackets in the stand. Each rack had five holes 8 cm in diameter to support the funnels. A slot 1 cm wide in the front of each hole was used to aid in removal of the funnels. Each rack was labelled clearly to identify the sample numbers it contained.

With a structure two racks deep, two racks wide and two tiers high, the capacity was 40 funnels on one side of the stand. The stand was built on wheels, so once samples were set up on one side of the stand, it could simply be turned around and the other side loaded similarly.

Removable metal splash trays were made to slide into the stand below each tier to prevent contamination of samples from above, identify any samples that leaked and allow rapid clean up after samples had been removed from the funnels.

5.4. Collection of Faecal Samples

Faecal samples (20 g) are collected from the rectum of each deer. Each sample is placed in a plastic container that is clearly labelled with the identification number of the animal. The identification number of the animal is checked off a list of the imported animals to ensure that all animals in the consignment are sampled. The container should be filled to the top to exclude air and the lid screwed tightly to retard the embryonation of nematode eggs. Samples are submitted in styrofoam coolers containing crushed ice or freezer blocks to keep them cool in transit.

Sampling and delivery schedules should be established with the collectors to ensure that samples are freshly collected and delivered to the laboratory within four hours of collection and set up immediately.

5.5. Set up of Baermann Test

(a) The faecal sample is placed on a single piece of tissue paper (Kimwipes) and weighed. A sample size of 20 g should be used for each test, a second test is required if the available sample weighs <15 g.
Animal identification number, weight of faecal sample and faecal consistency, as well as the funnel site for the test are recorded.

(b) The tissue is wrapped over the faeces and the sample placed on the screen, set in the top of the funnel.

(c) The clamps are closed and lukewarm tapwater is carefully added to each funnel when each rack is completed. Samples are set up for 20–24 hours at a temperature of 18–22°C. Each sample is lifted and the water poured down the side of the funnel to ensure that the water does not wash directly over the faeces and air is not trapped under the sieve. The tissue holding the sample is then submerged in the water to ensure that the faecal sample is completely wet.
The time that water is added to each stand is recorded. Funnels are checked to ensure that no clamps leak.

5.6. Centrifugation and Examination

(a) After standing for 20–24 hours, any larvae that have accumulated in the plastic tube above the clamp are recovered by releasing the clamp and collecting 10 mL of fluid into a labelled centrifuge tube (10 mL, free-standing, conical base — Hardie Health Care). Control of the clamp is essential to ensure that overflow of the tube does not occur.

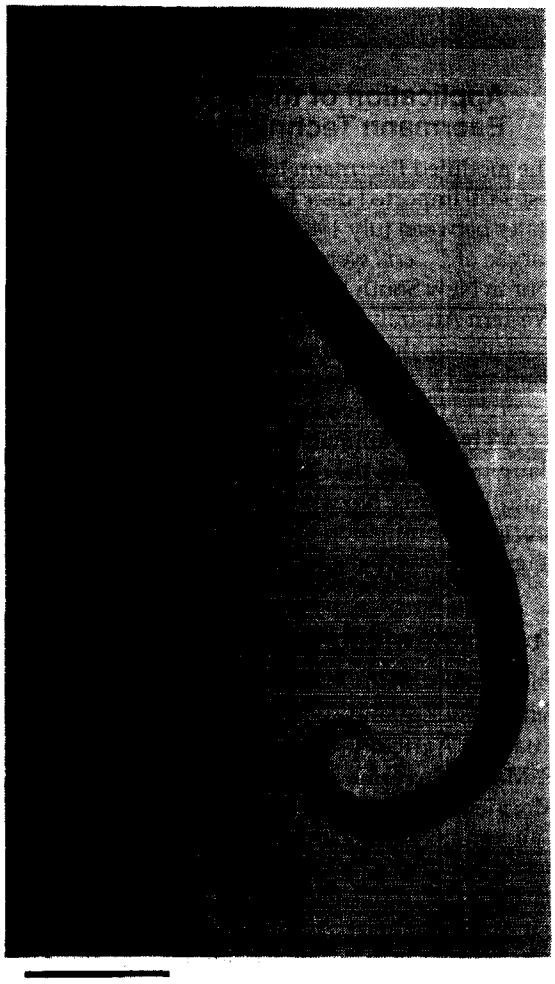

Figure 3. A larva of *Elaphostrongylus cervi*. Note the characteristic spiked tail that is coiled. (Bar length = 19

(b) Centrifuge tubes are spun at 1000 *g* for five minutes.

(c) The supernatant fluid (9 mL) is removed by suction pump.

(d) The sediment in each tube is then transferred to one of the wells in a six-well tissue culture plate. The tube is agitated before the content is poured into the well; the tube is rinsed twice with small amounts of water and this is added to the well for examination.
The tissue culture plates (Crown Scientific) are carefully labelled in units of 1–5, etc. and only five of the wells are used. This corresponds to the basic structure of the entire system — five funnels in each funnel rack, five samples to examine in each culture plate. On the bottom of each plate, lines are etched across each well at about 1 cm intervals to provide 'tracks' so that overlap of fields does not occur.

(e) Each well is carefully examined under the good quality, zoom Stereomicroscope (Wild Leitz) at x15 magnification, or an inverted microscope (Selby Scientific) with an mobile stage at x40 (R. Wroth, pers. comm. 1992) and the numbers and types of larvae recorded.

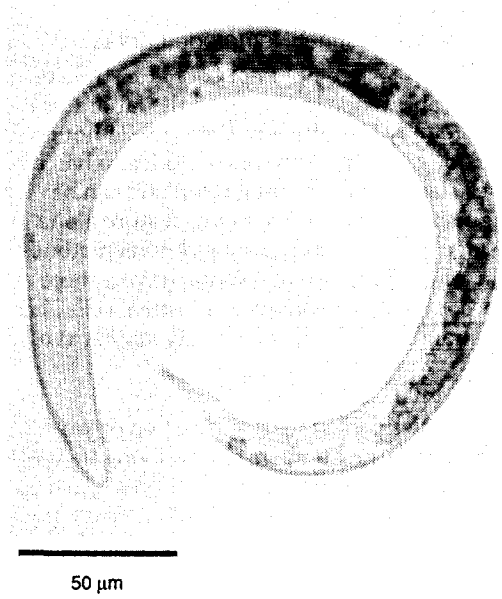

Figure 4. A larva of *Dictyocaulus viviparus*. Note the characteristic clear oesophagus with granules in the intestine. (Bar length = 21 mm).

5.7. Differentiation of Larvae

At x15 magnification, larvae can be counted easily, but differentiation between larvae of *E. cervi* and the lungworm, *Dictyocaulus viviparus*, requires confirmation under the compound microscope at higher magnification (x400).

5.7.1. *Elaphostrongylus cervi*

Larvae of *E. cervi* are about 400 µm in length (mean length, 410 µm; range 360–450 µm), are quite transparent, have a long oesophagus (mean length, 180 µm; range 162–220 µm) and a characteristic spiked tail that is usually coiled (Fig. 3). Larvae of *E. cervi* are active and do not moult; consequently, 95% migrate down within 24 hours.

Larvae of *E. cervi* can be recovered from faecal samples that have been refrigerated for several days. However, differentiation of *E. cervi* from gastrointestinal nematode larvae will then become a problem. Unless the samples have been kept refrigerated the entire period, the nematode eggs will have sufficient time to embryonate and hatch.

5.7.2. *Dictyocaulus viviparus*

Larvae of *D. viviparus* (Fig. 4) in red deer are about 400 µm long (mean length, 411 µm; range 370–450 µm) with dark granules and a clear oesophagus (mean length, 113 µm; range 90–130); they may be rather sluggish in activity and usually remain straight. If samples are not fresh, the larvae may go (or have gone) into first lethargis, and remain inactive for up to 24 hours. Consequently, only 60–80% of the larvae may be recovered in a test set up for 20–24 hours.

5.7.3. Gastrointestinal Nematode Larvae

If faecal samples are not kept cool after time of collection and while in transit to the laboratory, then there may be sufficient time (18–24 hours) for eggs of gastrointestinal nematodes to hatch.

These larvae have a straight tail and can be distinguished from *E. cervi* on this feature alone. First-stage larvae of trichostrongyloid nematodes vary in length from 300 to 450 µm, have a short clear oesophagous, prominent intestinal cells and a long straight tail. They are usually very active.

5.7.4. Free-living Nematodes

Faecal samples picked up from the pen or yard often contain large numbers of free-living nematodes, both larvae and adults. Their presence may make identification of *E. cervi* larvae difficult, particularly when large numbers are present. They may range in size from 300 µm as immature forms to over 1 mm as adults. All free-living larvae are characterised by a rhabditiform oesophagus, one that has a prominent bulb in the organ.

The requirement for providing freshly collected rectal samples is essential. With freshly collected rectal samples problems with free-living nematodes or gastrointestinal nematode larvae should not be encountered.

5.8. Report of Findings

Faecal samples (minimal weight 20 g) are requested from each deer with the intent of setting up 15–20 g. If <15 g are submitted, the sample will be set up, but no result given until after a second sample is tested. For calves, the minimal sample size is 10 g. Counts of *E. cervi* are expressed as lpg of faeces .

Any suspect *E. cervi* larvae in Australia may be sent to Dr P. Presidente or Dr A. English for confirmation before a positive result is reported. Specimens should be killed in 5% formalin and sent by air. A second faecal sample has usually been requested to confirm the identity of the infected animal. Specimens of *E. cervi* larvae for reference material may be obtained from Dr P. Presidente or Dr A. English (Department of Animal Health, University of Sydney, PMB 3, Camden, NSW 2570, Australia).

Although not all *D. viviparus* larvae are recovered in the incubation period used, results are expressed as lpg counts with the numbers of animals infected at the various levels recorded: very low counts (<1 lpg); low counts (1–20 lpg); low to moderate counts (21–50 lpg); moderate counts (51–200 lpg) and high counts (>200 lpg).

Treatment with a benzimidazole anthelmintic at the dose rate recommended for sheep is indicated if the lungworm larval count is >50 lpg at the first test. This is to reduce the level of mature lungworm infection and reduce the contamination in the quarantine yards. Suppression of larval output is generally three to five weeks, so the animals will again be shedding larvae by the time the third test is conducted.

6. Application of the Modified Baermann Technique

The modified Baermann technique was used to test 9870 imported deer held in quarantine in five States between July 1989 and December 1990. Larvae of *E. cervi* were identified in seven animals, four in New South Wales, and one in each of Western Australia, South Australia and Victoria. In each case, the identity of the infected deer was confirmed by request for a second sample which proved to be positive for *E. cervi* larvae.

All but one animal, a valuable stag, were slaughtered and the carcasses examined for mature worms. No worms were found on post-mortem examination of any of these deer. The stag was flown back to New Zealand.

7. Acknowledgments

Discussions about *E. cervi* and test procedures with New Zealand colleagues, Drs T. Watson, Ruakura Agricultural Centre, Hamilton; P. Mason, Invermay Agricultural Centre, Mosgiel; and P. McKenna, Animal Health Laboratory, Palmerston North, are gratefully acknowledged. Constructive comments of an earlier draft of this paper by Dr R. Wroth, veterinary parasitologist, Animal Health Laboratories, Western Australian Department of Agriculture, South Perth, were greatly appreciated.

8. References

Anderson, R.C. (1972). The ecological relationships of meningeal worm and native cervids in North America. *Journal of Wildlife Diseases* **8**, 304–10.

Cameron, T.W.M. (1931). On two new species of nematodes from the Scottish red deer. *Journal of Helminthology* **9**, 213–16.

English, A.W., Watt C.F., and Corrigall, W. (1985). Larvae of *Elaphostrongylus cervi* in the deer of Scotland. *Veterinary Record* **116**, 254–6.

Georgi, J.R., and Georgi, M.E. (1990). 'Parasitology for Veterinarians.' (W.B. Saunders: Philadelphia.)

Gibbons, L.M., Halvorsen, O., and Stuve, G. (1991). Revision of the genus Elaphostrongylus Cameron (Nematoda, Metastrongyloidea) with particular reference to the species of the genus occurring in Norwegian cervids. *Zoologica Scripta* **20**, 15–26.

Halvorsen, 0. (1986). Epidemiology of reindeer parasites. *Parasitology Today* **2**, 334–9.

Mason, P.C. (1989). *Elaphostrongylus cervi* — a review. *Surveillance* **16**(1), 3–10.

Mason, P.C., and Gladden, N.R. (1983). Survey of internal parasitism and anthelmintics used in farmed deer. *New Zealand Veterinary Journal* **31**, 217–20.

MacKintosh, C.G., Mason, P.C., Manley, T., Baker, K., and Littlejohn, R. (1985). Efficacy and pharmacokinetics of febantel and ivermectin in red deer (*Cervus elaphus*). *New Zealand Veterinary Journal* **33**, 127–31.

Presidente, P.J.A. (1984). Parasites of farmed and free-ranging deer in Australia. *In* 'Deer Refresher Course'. Proceedings **72**. pp. 623–43. (Post-Graduate Committee in Veterinary Science, University of Sydney: Sydney.)

Presidente, P.J.A. (1986). First report of the tissue worm in Australia. *The Federal Deerbreeder* 5(3), 13–17.

Watson, T.G. (1981). *Elaphostrongylus cervi* Cameron, 1931 and elaphostrongylosis: a review. *In* 'Proceedings of a Deer Seminar for Veterinarians'. pp. 94–107. (Deer Advisory Panel, New Zealand Veterinary Association: Queenstown.)

Watson, T.G. (1983). Some clinical and parasitological features of *Elaphostrongylus cervi* in *Cervus elaphus*. *New Zealand Journal of Zoology* **10**, 129.

Watson, T.G. (1986). Efficacy of drenching red deer and wapiti with particular reference to *Elaphostrongylus cervi* and *Dictyocaulus viviparus*. *In* 'Proceedings of a Deer Course for Veterinarians.' Deer Branch Course No. 3. pp. 170–82. (New Zealand Veterinary Association: Rotorua.)

Watson, T.G., and Kean, A.C. (1983). Some potential intermediate hosts of *Elaphostrongylus cervi* from *Cervus elaphus*. *New Zealand Journal of Zoology* **10**, 127.

9. Appendix

9.1. Appendix 1 — Equipment and Suppliers

Crown Scientific. 1–3 Florence Street, Burwood, Vic. 3125. Tel. (03) 808 0366; Fax (03) 808 0610. (Multiple well plates; Catalog # 25810-6).

Dow Corning Corporation. Via Selby Anax; Catalog # 453021; see Selby Anax.

Hardie Health Care. 2 Eskay Road, Oakleigh, Vic. 3125. Tel. (03) 808 0266; Fax (03) 808 0610.

Kimberly-Clark Australia. Via Metwood Trade Aust Pty Ltd, 544 Racecourse Road, Newmarket, Vic. 3031. Tel. (03) 376 3000; Fax (03) 373 1329. (KIMWIPES®, Fine Grade, # 4103, 200 wipers).

Nalgene® Labware. TBG pinch clamp, 1/2", Catalog # 6165-0002; via Selby Anax; Catalog # 253770; see Selby Anax.

Selby Anax. Catalog # 289013; 352 Ferntree Gully Road, Notting Hill, Vic. 3168. Tel. (03) 544 4844; Fax (03) 543 7295.

Selby Scientific. 21 Glassford Road, Kewdale, WA 6105; Tel. (09) 353 3577; Fax (09) 353 3120. (Olympus Model CK 2-TRC-2).

Swiss Screens (Aust). 6 Kinwal Court, Moorabbin, Vic. 3189. Tel. (03) 555 9166; Fax. (03) 553 2617.

Wild Leitz (Aust). 45 Epping Road, North Ryde, NSW 2113. Tel. (02) 888 7122; Fax. (02) 888 7562. (Wild M8).