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CASE REPORT

The use of detector dogs in the diagnosis of nematode infections in sheep feces

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Abstract This study was conducted to assess a dog's ability to differentiate between nematode-infected and uninfected sheep feces. Two German shepherd bitches were trained for scent detection over a 6-month period using operant/clicker conditioning. On completion of the training, testing was undertaken with 9 paper bags containing uninfected and 1 with infected feces, placed randomly around a circle. The dog and handler were not able to observe the placement of the bags. The 10th bag contained feces from sheep infected with either *Teladorsagia circumcincta, Trichostrongylus vitrinus, Haemonchus contortus,* or a mixed infection of all 3 species. Over 80 trials the dog had a mean success rate of greater than 80% in the detection of *T. circumcincta*- or *T. vitrinus*-infected feces and *H. contortus*-infected feces was detected with a slightly lower reliability of 76%, but mixed infections were detected at 92% reliability (one-proportion binomial analysis, $P < 0.05$). Trials were then undertaken to determine the time after administration of infective larvae that the dog was first able to differentiate *T. circumcincta* infection in sheep feces. At 7 days postinfection (dpi), the dog was capable of identifying *T. circumcincta* at least 85% of the time. These trials demonstrate that dogs are able to detect the common parasite infections in sheep with reliability equal to immunological assays. The results suggest that diagnostics based on odor detection using dogs to identify signature odors which could then be used to create sensitive detection devices might allow parasite detection on-farm and on all sheep in a flock.

Introduction

Gastrointestinal nematodes cause major health problems for the Australian sheep industry with losses in productivity and the associated costs of control (McLeod, 1995). The 3 major pathogenic nematodes of sheep and goats in Australia are the abomasal worms *Haemonchus contortus* and *Teladorsagia circumcincta* and *Trichostrongylus spp.*, which infects the small intestine of its host (Beiser and Love, 2003). These nematodes cause anemia, edema, diarrhea (scouring) and anorexia, impaired weight gain, decreased fertility, reduced wool growth, and eventually death if not treated (Sykes, 1994; Eysker and Ploeger, 2000; Yu et al., 2000). Currently, the industry relies heavily on chemicals from a limited number of chemical groups for nematode control. However, this type of control is threatened by widespread and increasing levels of drench resistance in all the major nematode species (Sangster, 1999), leading to

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the view that current anthelmintics may become virtually useless within the next 10 years (Beiser, 2003).

Traditional methods of diagnosis have relied on detecting animals with clinical signs of infection such as scouring, inappetance, and behavioral changes. However, symptoms of nematode infection in living animals are not always evident, and relying on visual inspection limits farmers to finding infections that have already caused substantial damage to the animal. The alternative is the fecal egg count (FEC), usually involving lab-based analysis of farm-collected samples, which is expensive and results in at least a few days delay between sampling and treatment. The FEC is ineffective prior to the appearance of eggs at patency, a period of at least 3 weeks, at which the nematode has reached reproductive maturity. At patency the infection is firmly established, and damage to the host has begun (Johnson and Behnke, 1996; Schallig et al., 1995). Furthermore, the FEC is less effective in older animals, where egg production may be suppressed by the host immune system or when large numbers of non-egg-laying arrested parasites are present (Cole, 1986).

There is a marked variation among individual sheep in response to parasites, based on genetically determined effects on both resistance to worm infection (ability to limit the size of the worm burden) and resilience to the worm burden (the ability to tolerate worms with minimal adverse effects) (Beiser, 2003). As a result, drenching is usually required for only part of the flock, and some animals can be left untreated, to minimize the buildup of drench resistance. Consequently there is a need for a more rapid and reliable test that will allow farmers to test large numbers of animals on-farm to decide the need for treatment and to treat only those animals that are carrying clinical levels of infection.

Most organisms emit odors, which, because of their complexity and low rates of emission, may be difficult to characterize chemically (Wallner and Ellis, 1976). The domestic dog, *Canis familiaris,* has an excellent ability to discriminate specific odors at concentrations down to 1 part per trillion (Johnson, 1977). As a result, domesticated dogs have been widely exploited to detect a variety of materials, including explosives (Duhaime et al., 1998; Gazit and Terkel, 2003), land mines, drugs (Adams and Johnson, 1994b), smuggled agricultural products (Eastwood, 1990), accelerants at fire scenes (Kurz, 1994), brown tree snakes (Engeman et al., 2002), bladder cancer, (Willias et al., 2004), bear feces (Wasser et al., 2004), screwworm (Welch, 1990), cows in estrus (Hawk et al., 1984), and gypsy moth eggs (Wallner and Ellis, 1976).

To date, there is no published information on the use of dogs to detect nematode infections. The purpose of this study was to determine whether dogs could be trained to detect gastrointestinal nematodes in sheep. The authors also wanted to determine whether dogs could detect the 3 species of nematode *Haemonchus contortus, Trichostrongylus species,* and *Teladorsagia circumcincta,* and at what stage of the infection the dog might first recognize the infection.

Method

Experimental infections: animals and parasitic strains

All work with sheep was carried out under the approval of the La Trobe University Animal Ethics Committee. All sheep used in this study were part of a larger project conducted by La Trobe University, Department of Agricultural Sciences and funded by the sheep Cooperative Research Centers to determine new diagnostic techniques for gastrointestinal nematodes in sheep. One-year-old Merino and Merino-cross Border Leicester wether (castrated male) lambs were obtained from commercial farms. The lambs were quarantined on arrival and were administered ivermectin at the manufacturer's recommended dose of 0.2 mg/kg body weight, to eliminate any current parasite infections. The sheep were housed indoors in individual pens 1.25 m $\times$ 1.5 m at ambient temperature and fed a mixture of lucerne and oaten chaff plus protein pellets. Water was available ad libitum. The sheep were divided into 4 groups: group 1 contained control animals (no nematode infection), group 2 were orally infected with approximately 10,000 *Haemonchus contortus* L3 larvae, group 3 were orally infected with approximately 15,000 *Trichostrongylus vitrinus* L3 larvae, and group 4 were orally infected with approximately 20,000 *Teladorsagia circumcincta* L3 larvae. The degree of infection was monitored by FEC carried out weekly during the patent period. Feces were collected when necessary from canvas collection bags (45 cm $\times$ 25 cm) strapped to the torso of the lambs.

Dogs

Two German shepherd bitches (*Canis familiaris*) were used in this study. Both dogs (Seb and Elle) were bonded with handlers from 8 weeks of age. The dogs were purchased privately specifically for this study and remained companion animals on its completion. They were selected for their scenting ability and a strong play drive.

Basic obedience training

Basic obedience training commenced at 10 weeks of age, and food lures were used to entice the dogs into the basic positions of sit, drop, and stay. Each dog was trained by its own handler throughout the experiment to ensure a close bond between handler and dog and to minimize anxiety and stress. Once the dogs were taught the basic commands, they advanced to more intensive training for scent detection. This training commenced when the dogs were approximately 3 months of age and was completed at 9 months of age.

Scent detection training

Scent detection involved operant conditioning (clicker training) as described by Kaplan et al. (2001). Mixed infections were used initially, as the dogs were required to recognize an unknown odor signature only present in infected feces, to first determine if the study was achievable. Initial training involved both control and infected (mixed infection) fecal samples, where dogs were asked to differentiate between the two by sitting next to the infected sample. After several attempts it was clear the dogs were not identifying the infected sample by smell, but merely sitting next to the sample that produced a reward response. Therefore the training method changed, and the dogs were taught to associate an object with the scent of nematode-infected sheep feces. This was achieved by placing contaminated feces of a mixed infection into a plastic canister with a length of 30 cm and diameter of 15 cm, capped at both ends, and with 3-mm holes drilled at 5-cm centers across the end caps. Fecal egg counts of the infections varied between 200 eggs per gram (epg) and 30,000 epg, and for several weeks the dogs were trained to locate and to sit next to the canister containing infected feces, which had been hidden around the training area. The container was hidden by an assistant while the handler and dog were absent from the area. These "controlled finds" were used to ensure the handler did not influence the dog and forced the dog to rely on its sense of smell rather than vision to locate the canister. Following each successful find, clicker training was used, and the dogs were given a toy reward (a play of tug-of-war with a rope toy). Training was conducted at different locations and at different times of the day throughout the week. The dogs were then taught to scent single-species infections placed randomly in a group of uninfected feces. Approximately 200 g of sheep feces infected with *Haemonchus contortus, Trichostrongylus vitrinus,* or *Teladorsagia circumcincta* was placed in an opaque paper bag among 9 bags of uninfected feces. Again the dogs were asked to "find it," and successful selection resulted in a toy reward. At this stage of training, one of the dogs was omitted from further testing as she was easily distracted and was not reliable in differentiating between infected and uninfected feces (mixed or single infections).

Field testing—reliability of detection

Eight trials of 10 replicates, 80 in total over the 14-day testing period, were conducted on the La Trobe University Agriculture Reserve. Twelve grams of feces during a patency period (28-42 dpi) with known FEC (*H. contortus* 1400 epg, *T. vitrinus* 400-600 epg, *T. circumcincta* 300-500 epg) were placed in opaque paper bags (length: 20 cm, width: 15 cm). Ten bags were placed approximately 0.5 m apart in a large rectangle. Nine of these bags contained feces collected from 9 uninfected sheep. The 10th bag contained feces collected from a sheep that was infected with *H. contortus,* *T. vitrinus,* or *T. circumcincta,* or a mixture of all 3 at 1:1:1 respectively. To avoid scent matching, 3 sheep were infected with *H. contortus,* 3 with *T. vitrinus,* and 3 with *T. circumcincta* and were used randomly during the 80 tests.

The infected bag was randomly placed among the 9 control bags by an assistant, and the dog was told to locate the infected sample and sit next to it. These trials were repeated 10 times each day over 14 days, ensuring that the infected bag was moved randomly around the square among the control bags. Each trial day was interspersed with at least 1 rest day to maintain the dog's enthusiasm for the task, so the full trial period was over 14 days.

Field testing—stage of detection

Trials were conducted as outlined in the previous section, except that the infected feces were selected from infected sheep at 7, 14, 21, or 28 days postinfection. Each infected feces sample was randomly located among the control bags. The dog was given only 1 opportunity to determine the infected bag from the controls. These trials were repeated 20 times in the morning and 20 in the afternoon on the day of fecal collection for the relevant stage of infection.

Environmental conditions

The trials described above were conducted over a 2-month period. The temperature, humidity, wind direction, and speed were recorded from the Bureau of Meteorology weather station located on the La Trobe University Agriculture Reserve.

Statistical analysis

Scent detection trials were statistically analyzed using 1-proportion binomial analysis, where n equaled the number of tests and x equaled the number of correct responses. A P value of < 0.05 was considered significant. Statistical analysis for weather conditions during the trials and for the time of day was by 1-way analysis of variance (ANOVA). Temperatures ranging between 12°C and 17°C were compared to those between 18°C and 21°C, wind speeds between 2 km/h and 6 km/h were compared with 7 km/h to 13 km/h, and times before and after 12 PM were compared. These variations were considered to capture the bulk of the variation in conditions over the period of experimentation.

Results

Detection of 3 species of gastrointestinal nematodes

The dog was given the opportunity to detect fecal preparations of known egg concentration in a number of

Table 1 Statistical analysis using 1-proportion binomial analysis of positive indication of different nematode species

Species	Correct response (X)	Number trials (N)	Raw %	95% confidence interval for mean: Lower bound (%)	Significance (*P*)
T. circumcincta	72	80	90	83	0.036
T. vitrinus	73	80	91	84	0.046
H. contortus	68	80	85	77	0.035
Mixed	78	80	97	92	0.040

different trials (Table 1). Egg concentrations determined using the FEC were as follows: *Teladorsagia circumcincta* 300-500 epg, *Trichostrongylus vitrinus* 400-600 epg and *Haemonchus contortus* 1400-1500 epg. The overall detection performances for each of the different nematodes were trialed. The mean rate of successful detection was 90% for *T. circumcincta*, 91% for *T. vitrinus,* 85% for *H. contortus,* and 97.5% for mixed infection (Table 1). The dog correctly gave a positive indication for *T. circumcincta* and *T. vitrinus* on 72 and 73 of 80 occasions, respectively. At a 5% level of significance, "Seb" would be expected to maintain this success rate at least 83% of the time for *T. circumcincta* (1-proportion binomial, CI = 82.6795, $P = 0.036$), and 84% for *T. vitrinus* (1- proportion binomial, CI = 84.1928, $P = 0.046$). She correctly identified *H. contortus* infection on 68 of 80 occasions and would be expected to maintain this rate greater than 77% of the time (1-proportion binomial, CI = 76.8324, $P = 0.035$). "Seb" performed best when detecting mixed infections of all 3 species and found the positive infection on 78 of 80 occasions, maintaining a success rate greater than 92% (1-proportion binomial, CI = 92.3389, $P = 0.040$) (Table 1).

Detection of T. circumcincta at various stages of infection (dpi)

Using the same statistical analysis, *T. circumcincta* infections were assessed at various times postinfection (Table 2). The mean success rates for detection of the infection at 7, 14, 21, and 28 dpi were 95%, 90%, 100%, and 97.5%, respectively. Thus at 7 dpi, the dog was capable of identifying *T. circumcincta* on 38 of the 40 occasions. At a 5% level of significance ($P < 0.05$), "Seb" would be expected to maintain this success rates at least 85% of the time (1-proportion binomial, CI = 85.1, $P = 0.049$), 78% of the time at 14 dpi (1-proportion binomial, CI = 78.6, P = 0.042), 92% of the time at 21 dpi (1-proportion binomial, CI = 92.8, $P = 0.036$), and 88% of the time at 28 dpi (1-proportion binomial, CI = 88.7, $P = 0.039$) (Table 2).

Response to weather conditions

Weather conditions (Table 3) were shown to have no significant effect on the dog's ability to detect infections. Thus, temperatures below 17°C (12°C to 17°C) were compared to those above 18°C (18°C to 21°C) and showed no significant effect (ANOVA, $P > 0.05$). Wind speeds below 6 and above 7 km/h were also without effect (2 to 6 vs 7 to 13 km/h) (ANOVA, $P > 0.05$). There were no significant (ANOVA, $P > 0.05$) differences between trials conducted before 12 PM and after 12 PM for each of the species (*T. circumcincta*, F = 0.55, $P = 0.5$; *T. vitrinus*, F = 0.16, $P = 0.7$; *H. contortus*, F = 0.13, $P = 0.7$; and mixed infection, F = 3.0, $P = 0.13$).

Discussion

Scent-detection dogs are defined by their ability to locate and respond to a specific odor and not respond when the target odor is not present. Several studies have used dogs in attempts to differentiate a range of target odors. For example, Wallner and Ellis (1976) trained German shepherds to locate egg masses of the gypsy moth with a 95% success rate. Similarly, dogs were trained to successfully distinguish milk of pre-estrous cows from milk of estrous

Table 2 Statistical analysis using 1-proportion binomial analysis for correct responses of *T. circumcincta* at different stages of infection

Days postinfection (dpi)	Correct response (X)	Number of trials (N)	Raw %	95% confidence interval for mean: Lower bound (%)	Significance (*P*)
7	38	40	95	85.08	0.049
14	36	40	90	78.56	0.042
21	40	40	100	92.78	0.036
28	39	40	97.5	88.68	0.039

dpi, days postinfection.

Table 3 Raw data showing various times and dates the dog trials were performed and the temperature and wind speed on those days. Data was obtained from Bureau of Meteorology (www.bom.gov.au)

Date	Time	Temperature C	Wind speed Km/h
2/5/2005	10:00–11:00	12.9–15.6	6
3/5/2005	12:00–1:00	18.9–19.6	0.6–13
4/5/2005	10:00–11:00	18.7–20.9	0.6–11
4/5/2005	11:00–11:30	20.9–20.2	0.9–11
6/5/2005	10:00–11:00	14.5–13.2	0.6–4
11/5/2005	10:30–11:00	14.8–15.7	0.6–7
16/5/2005	4:00–5:00	15.4–14.8	0.4–0
17/5/2005	9:30–10:00	10.3–10.1	2
18/5/2005	3:30–4:00	18.5–17.8	9
19/5/2005	10:00–11:00	12.9–14.1	0.2–7
19/5/2005	4:30–5:00	17–16.6	0.6–7
20/5/2005	12:30–1:30	17–18.3	0.6–4

or luteal-phase cows with greater than 76% accuracy (Hawk et al., 1984). Furton and Myers (2001) used dogs as chemical detectors to detect explosives, and Jack Russell terriers were used to locate brown tree snakes in shipping cargo (Engeman et al., 1998; Engeman et al., 2002). Finally, Willias et al. (2004) proved that dogs were capable of identifying bladder cancer on the basis of urine odor in people. We have now shown that a German shepherd can detect gastrointestinal nematodes with an accuracy greater than 76% via fecal odors.

"Seb" and "Elle" were required to recognize an odor signature that was present only in the feces of sheep infected with gastrointestinal nematodes among a number of uninfected (control) fecal samples. It was difficult to determine a precise training procedure that could be used in this project without the knowledge of compounds involved in the odor signature. The trainers believed operant conditions (clicker training) would be the most rapid form of training (Kaplan et al., 2001; Laule et al., 2003; Dinsmoor, 2004). This form of training is used to pinpoint the exact moment the dog has carried out a task correctly. During the initial stages of training, attempts were made to use both control and infected (mixed infection) fecal samples to teach the dog to differentiate the two. However, it was thought that "Seb" was merely determining the sample that would receive a reward through trial and error and then remembering its position and sitting next to it rather than identifying the infected sample by smell. Therefore, more conventional training methods were used whereby the dogs were taught to find a hidden sample (Brooks et al., 2003).

Previous analyses of training methods, length of trialing time, and environmental influences (Wallner and Ellis, 1976; Johnson, 1977; Welch, 1990) were used as guides throughout this study. For example, there was a possibility that both dogs were merely scent matching (Willias et al., 2004) the sheep samples, rather than learning to pick out the distinctive odor common to the individual parasites. This possibility was overcome by using samples from different sheep infected with the same parasite and changing between sheep during each training period.

During this study, puppies began training at 10 weeks of age. Puppies were chosen for this study because of time constraints. It was easier to purchase a puppy with a strong play drive and scenting abilities than to purchase a dog that showed these qualities. However, a study by Wallner and Ellis (1976) found that there was a difference in detection abilities between older and less experienced dogs. The older dog, previously trained in scent discrimination with other odors, started and remained at a high level of accuracy throughout the testing period, whereas the younger dog started poorly and significantly increased in accuracy by the end of the study. This finding suggests that if the present study were repeated with an older dog (no more than 2 years old) already trained to detect another unrelated scent, it would make a more rapid transition to fecal detection. An older dog might also be more beneficial, as it would not display puppy development characteristics such as growth spurts and short attention spans (Adams and Johnson, 1994a, 1994b; Simpson, 1997; Peachey, 2001; Adams et al., 2004).

Wallner and Ellis (1976) also found that there was a decrease in efficiency of detection of gypsy moth eggs when trials were conducted in the field compared to those conducted within the laboratory. Wallner and Ellis (1976) believed that interference of odors from plant materials, soil, and other sources not encountered in the laboratory may have influenced this decrease. This suggestion led to training being conducted exclusively under field conditions in areas where a number of odors existed (including old sheep feces) to maximize the resolution of the dog's scent discrimination.

A study conducted by Szetei et al. (2003) showed that a dog's behavior depends greatly on the relationship with its handler. This knowledge was used to ensure that the handler kept the dog interested and motivated throughout the trialing process. Incorporating a positive control trial (a trial where the dog was guaranteed to be rewarded) such as search scenarios between trialing periods seemed to reduce the number of mistakes. In addition, Kurz (1994) found that not all dogs are equally adept in scent detection and had varying sensitivity and/or discriminatory powers. This finding was confirmed in the present study by the differences in scent ability between "Elle" and "Seb." "Elle" was capable of detecting the infections in the sheep feces, but she lacked motivation and concentration and did not reliably work on demand.

Differences in weather conditions (temperature, wind speed, wind direction, and humidity) did not seem to impact Seb's performance during the trials. However, other studies have reported that on warm days, dogs become less effective as they start panting (Schmidt-Nielsen et al., 1970;

Goldberg et al., 1981; Kurz, 1994). Gazit and Terkel (2003) identified a direct correlation between the rate of sniffing and efficiency of olfaction. Following extreme exercise there was a decrease in sniffing frequency and an increase in panting rate, resulting in reduced detection of explosives. Other studies (Thesen et al., 1993; Steen et al., 1996) have suggested that under "normal circumstances" without exercise, dogs found scent tracks with 90% to 100% accuracy. Therefore, further studies need to examine the effect of both increased temperature and exercise on the dogs' detection rate.

Finally, few studies have been conducted concerning the effect of multiple-odor discrimination on detection performance (Brisbin and Steven, 1991; Stitzel et al., 2002; Williams and Johnston, 2002). Results of this study showed that there was a significant difference between the accuracy of detecting mixed infections to single infections, with *H. contortus* having the lowest detection rate at 76% (Table 1). This difference might be explained by the fact that "Seb" began training at 10 weeks of age on mixed infections, and she was not introduced to *H. contortus* infections until 1 month before the trial period. However, Williams and Johnston (2002) examined multiple-odor detection and concluded that responses to previously learned odors did not decrease with the addition of novel substances (odors) in the training regime. In addition, the amount of training required when introducing a new odor decreased as more odors were added.

Current diagnostics for nematodes in sheep depend on the detection of patent infections (more than 21 dpi) by FEC or on clinical symptoms that occur only in high-level infections and usually after patency. The authors were interested in the ability of the dog to detect infections prior to egg production and at levels of infections where other symptoms were also absent. Trials showed that "Seb" was capable of detecting *T. circumcincta* as early as 7 dpi with greater than 85% accuracy (CI = 85.08, $P < 0.05$) and was then similarly capable until well after patency (Table 2). Thus, the odors being detected are not only associated with egg laying, and changes are obviously occurring in fecal composition as early as 7 days postinfection.

The actual limit of sensitivity of the dog was not determined, but "Seb" detected infections to below 100 epg (data not shown). This level is well below the suggested trigger levels (500 epg in general or 1000 epg for *H. contortus*) for anthelmintic treatment of infected sheep to reduce the development of anthelmintic resistance in nematodes (McKenna, 1986; Jorgensen et al., 1998; Sykes and Greer, 2003). However, the sheep infections used in this study were controlled for age of infection and for sheep diet, and no other significant gut infections were present (low levels of coccidian oocysts were present in most infected and control animals). Thus, further work with field-infected animals kept under standard grazing management conditions would be necessary to obtain realistic sensitivity data.

The composition of the odor that "Seb" was detecting in the feces is unknown. Further investigation using high-performance liquid chromatography (HPLC) and gas chromatography/mass spectroscopy (GC/MS) may determine the chemical composition of these odors. Evidence from studies on the pathogenesis of nematodes suggests that feces could contain elevated levels of blood, mucus, and various microbial products, as well as excretory and secretory products (ES) from the nematodes themselves or other pathogenic changes caused by the infection (Symonds and Steel, 1978; Abbott et al., 1986; Lightowlers and Rickard, 1988).

The present study has shown that dogs can be trained to reliably detect feces from sheep infected with 3 species of gastrointestinal nematodes with reliability rates of greater than 85% from as early as 7 dpi. This study has proved sheep feces infected with gastrointestinal nematodes have a different smell than uninfected sheep feces. Such knowledge allows future studies using HPLC and GC/MS to determine the chemical composition of these odors in the attempt to create sensitive detection devices. Such a device might allow parasite detection on-farm and on all sheep in a flock to minimize the sheep industry's reliance on drenches.

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