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## USE OF RELEASED PIGS AS SENTINELS FOR *MYCOBACTERIUM BOVIS*

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**ABSTRACT:** Identifying the presence of bovine tuberculosis (TB; *Mycobacterium bovis*) in wildlife is crucial in guiding management aimed at eradicating the disease from New Zealand. Unfortunately, surveys of the principal wildlife host, the introduced brushtail possum (*Trichosurus vulpecula*), require large samples (>95% of the population) before they can provide reasonable confidence that the disease is absent. In this study, we tested the feasibility of using a more wide-ranging species, feral pig (*Sus scrofa*), as an alternative sentinel capable of indicating TB presence. In January 2000, 17 pigs in four groups were released into a forested area with a low density of possums in which TB was known to be present. The pigs were radiotracked at 2 wk intervals from February to October 2000, and some of them were killed and necropsied at various intervals after release. Of the 15 pigs successfully recovered and necropsied, one killed 2 mo after release had no gross lesions typical of TB, and the only other pig killed at that time had greatly enlarged mandibular lymph nodes. The remainder were killed at longer intervals after release and all had gross lesions typical of TB. *Mycobacterium bovis* was isolated from all 15 pigs by mycobacterial culture. Home range sizes of pigs varied widely and increased with the length of time the pigs were in the forest, with minimum convex polygon range-size estimates averaging 10.7 km<sup>2</sup> (range 4.7–20.3 km<sup>2</sup>) for the pigs killed after 6 mo. A 6 km radius around the kill site of each pig would have encompassed 95% of all of their previous locations at which they could have become infected. However, one pig shifted 35 km, highlighting the main limitation of using unmarked feral pigs as sentinels. This trial indicates use of resident and/or released free-ranging pigs is a feasible alternative to direct prevalence surveys of possums for detecting TB presence.

**Key words:** Bovine tuberculosis, brushtail possums, epidemiology, feral pigs, *Mycobacterium bovis*, *Trichosurus vulpecula*, sentinels, *Sus scrofa*.

### INTRODUCTION

Bovine tuberculosis (TB) caused by *Mycobacterium bovis* is widespread in wildlife in New Zealand, with affected areas comprising approximately one third of the country (approximately 895 km<sup>2</sup>; Animal Health Board, 2000a). The introduced Australian brushtail possum (*Trichosurus vulpecula*) is the principal wildlife reservoir and is capable of sustaining the disease (Morris and Pfeiffer, 1995). Infection has also been found in a wide range of other introduced mammals but most have an inconsequential role in sustaining the disease (Cooke et al., 1999). Although eradication of TB has been successfully achieved in some countries, including Australia (Cousins and Roberts, 2001), involvement of the brushtail possum as a true maintenance host has so far prevented TB eradication from New Zealand livestock (Coleman and Caley, 2000). However, efforts during the 1990s to reduce

the incidence of TB in livestock by control of sympatric wildlife have been successful in many areas (Caley et al., 1999), and the TB control program now aims to achieve official freedom from TB in livestock before 2015 and to eventually eradicate the disease from wildlife (Animal Health Board, 2000b).

The TB control program, or pest management strategy as it is designated in New Zealand, aims to achieve eradication by intensive control of wildlife populations in infected areas in conjunction with a test-and-slaughter approach to eliminating the disease from livestock (Animal Health Board, 2000b). The wildlife control program aims, mainly, to reduce infected or potentially infected possum populations to uniformly low densities and hold them at those densities for long enough for the disease to die out, a strategy based originally on epidemiologic models (Barlow, 2000) but increasingly on empirical evidence

(Caley et al., 1999). However, the disease is now so widespread in wildlife that there are inadequate resources to implement this strategy over the entire affected area; therefore sequential allocation of resources will be required. One of the major challenges with this approach is deciding when an apparently successful operation can be stopped so those resources can be diverted elsewhere. Stopping control too soon risks reemergence of the disease; stopping too late incurs large costs. What is needed is a high degree of confidence that the disease is absent.

For areas of developed farmland, regular testing of livestock (mainly cattle and deer) prevents the long-term maintenance of disease by livestock (Coleman and Caley, 2000). The occurrence of TB in livestock therefore indicates recent transmission from wildlife—in effect, the livestock testing program provides surveillance of TB in sympatric wildlife. However, there are many areas where livestock are absent or their densities too low to provide useful surveillance. For these areas, TB prevalence in the wildlife hosts must be surveyed directly. However, identifying infection in the main reservoir, possums, is extremely difficult. Within managed areas, possum densities are usually very low, and they have small lifetime home ranges (typically averaging <1–5 ha, but occasionally up to 30 ha; Cowan and Clout, 2000). They also have short survival periods (typically 2–6 mo) for animals with clinical TB (Coleman and Caley, 2000). Prevalence of TB in possum populations is also usually low, with infection highly aggregated in small areas or “hot spots” of infection within the overall range (Coleman and Caley, 2000). This low and extremely aggregated occurrence of disease combined with the small home range sizes means almost all of the possums in an area would have to be surveyed to be 95% confident that TB was not present. That is not practical, and, as a consequence, persistent TB could go undetected for long periods. At Hohotaka in the central North Island

(New Zealand), for example, a pocket of residual infection in a controlled possum population was not detected until 5 yr after the initial control, despite regular monitoring and inspection of carcasses (Caley et al., 1999).

An alternative approach to disease surveillance involves using a secondary or spillover host as a sentinel for detecting TB in the primary maintenance host. Spillover hosts are those that become infected mainly by interspecific rather than intra-specific transmission. In New Zealand, the sentinel concept centers on changing the search for infected possums by using other wildlife such as feral pigs (*Sus scrofa*) that have larger home ranges and which appear to have a much higher per capita probability of developing detectable infection than do sympatric possums.

The strongest evidence that pigs are spillover hosts comes from the Northern Territory of Australia, where high densities of pigs were not able to independently sustain the disease once infected cattle and water buffalo (*Bubalus bubalis*) were removed (McInerny et al., 1995). More generally, we know of no studies that suggest TB has persisted anywhere in the world in feral pigs in the absence of other sources of infection. However, high prevalences are sometimes recorded in feral pigs (Corner et al., 1981), and transmission occurs between pigs in captivity (Ray et al., 1972); so it seems likely that transmission occasionally occurs between feral pigs (de Lisle, 1994). There is therefore always some question about the immediate source of infection in a particular feral pig. In addition feral pigs sometimes have very large home ranges, and males in particular may be seminomadic (Saunders and Kay, 1996). The presence of an infected feral pig can therefore provide only a very broad indication of the location of the original source of infection. To try to circumvent both these uncertainty problems, we released TB-free pigs, radiotracked them to determine their home range, and then killed them after several months or

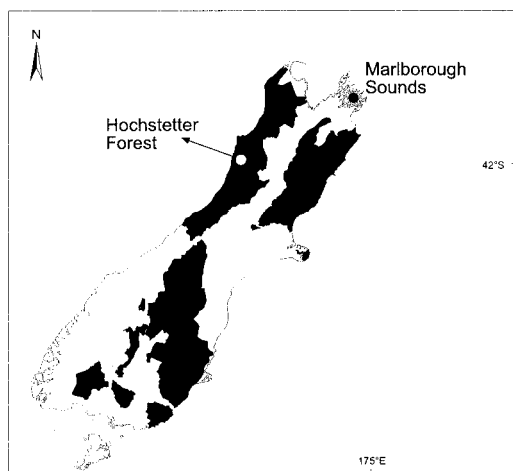


FIGURE 1. Location of the study area and the source of feral pigs used in this study, in relation to the current boundaries of Vector Risk Areas. Black areas were formally classified as having TB-infected wildlife in the South Island, New Zealand.

years to determine their TB status. The trial was conducted in an area for which we had recent evidence of widespread TB in wildlife. This paper reports the outcome of the trial, in which 15 of 17 pigs released were recaptured and necropsied, and found to be infected, demonstrating the feasibility of using released pigs (and other spillover hosts) as sentinels for routine surveillance of TB presence.

#### MATERIALS AND METHODS

Seventeen feral pigs (nine females, eight males) were captured in January 2000 in the Marlborough Sounds, New Zealand (41°10'S, 174°03'E; Fig. 1) using weldmesh panel traps, and held in captivity for 5–10 days. This area is formally classified as TB-free. As an added precaution, however, the pigs were tested for TB using an intradermal tuberculin injection in the ear (0.1 mg/ml of bovine tuberculin; AgriQuality Tuberculin Unit, Upper Hutt, NZ) that was assessed 72 hr after injection. Leslie et al. (1968) report high efficiency of this test in pigs infected with avian TB. All TB tests were negative, and three pigs from the same area that were necropsied were also free of gross lesions. Four of the males were castrated so we could later test whether mate-seeking behaviour of intact males had a large effect on home-range size and movement patterns. The weight and sex of the pigs was recorded, and

numbered eartags (with a reward offer) were attached to both ears. All of the pigs had radiotransmitters implanted subcutaneously between the shoulder blades. The transmitters were two-stage microcontroller transmitters with internal coil antenna, and weighed 30–33 g (Sirtrack Ltd., Havelock North, NZ). They were about 80 mm long with a diameter of about 18 mm and had a duty cycle of 4 days on and 10 days off. The expected operational life was 13.5 mo. The Landcare Research Animal Ethics Committee approved all of the animal manipulations (Project Code No. 99/11/1).

Once the animals had recovered from surgery they were transported by road, then helicopter, to liberation sites in Hochstetter Forest (central Westland, NZ; 42°30'S, 171°45'E; Fig. 1). Hochstetter Forest (100–500 m above sea level) is comprised of extensive gentle glacial terraces in the east, and dissected terrace hill country in the west. Large areas of Hochstetter Forest have been logged, particularly to the west and northwest, and replanted with *Pinus radiata* and other exotic species. The unmodified areas are dominated by mixed beech (*Nothofagus* spp.) forest, interspersed with small areas of pakihi (heath-like swamp land). Flagstaff Flat, the 300–400 ha of river terrace bordering the eastern boundary of the forest, is vegetated by gorse (*Ulex europaeus*), broom (*Cytisus scoparius*), and unimproved pasture. Possums on Flagstaff Flat were regularly surveyed during the 1990s to determine abundance and prevalence of TB (Coleman et al., 1999). In the most recent survey, in December 1999, a three night possum trap-catch rate of 9% was recorded, and 5% of the possums trapped were infected with TB (J. D. Coleman, unpubl. data). Between 1997 and 2000, 11% of 115 wild red deer (*Cervus elaphus*) shot in Hochstetter Forest were infected but most of the infected deer had no visible lesions and none had generalized TB (G. Nugent, unpubl. data).

Groups of four or five pigs were liberated at each of four sites, with each group comprised of at least two females, one neutered male, and one intact male. To determine whether feeding at the liberation site altered pig movement patterns, two of the groups were held in modified deer-capture pens for 7 days and fed commercial pig food (40 kg of pig pellets). The other two groups were released into small clearings within the forest, with no food provided.

After release, the pigs were periodically relocated by helicopter at approximately 2 wk intervals, from February 2000 until October 2000, except for a 1.5 mo period in July/August when the helicopter was grounded by mechanical problems. Locations were mapped using a

Geographic Information System (Arcview 3.2; ESRI, Redlands, California, USA), and home-range size was determined by the minimum convex polygon (MCP; Mohr, 1947) method. The distances between each location and all previous locations of that pig were calculated, and used to assess the likely distribution of distances between the nominal location at death and the sites at which a pig could potentially have become infected with TB.

At scheduled intervals approximately 2, 4, 7, and 9 mo after release, up to five released pigs were radiotracked and then either captured and killed by ground hunters with dogs, or shot with a high-powered rifle from the helicopter. Forty-one "resident" feral pigs (including 10 probable offspring of released pigs) seen while radiotracking or hunting within the study area were also killed, but for eight of these only the heads were available for examination. Weight, sex, and reproductive status were recorded for all whole carcasses and either a sample or all of the stomach contents were taken for analysis. Mandibles were removed and ages determined from tooth eruption patterns (Clarke et al., 1992). Twenty of the samples of stomach contents were sorted macroscopically, primarily to determine the frequency with which pigs were eating possum carrion. We used either "whole-stomach" or "sieving" methods akin to those used for possum diet analysis (Sweetapple and Nugent, 1998).

The pigs were subjected to a detailed necropsy. All of the major lymph nodes of the head, lungs, thorax, abdomen, and alimentary tract were thinly incised (1–2 mm) and inspected for gross lesions typical of TB, as were the lungs, liver, kidneys, and mammary glands. We recorded the distribution, number, size, and nature of any signs lesions suggestive of infection with TB. These were most typically found in the lymph nodes of the head and ranged from tiny (1–2 mm) petechial hemorrhages to large fibrously encapsulated abscesses several centimeters in diameter with varying degrees of liquefactive caseous necrosis and central calcification. The mandibular lymph nodes from all pigs, and up to two of any potentially tuberculous lesions or lymph nodes, were submitted to AgResearch (Wallaceville, NZ) for mycobacterial culture using the methods described by Buddle et al. (1994). The severity of infection was scored using the index in Table 1.

For the pigs that remained within the study area, the relationship between severity of infection and exposure time was explored (using time since release as the index of exposure for released sentinels and age at death for the resident feral pigs) using non-linear least squares

TABLE 1. Severity-of-infection index, adapted from Mackintosh et al. (1998) scale for deer. Because only the heads of some resident feral pigs were available, a head-only score using just the first five classes (0–4) was also recorded for all pigs. Small lesions were classified <0.5 cm, with moderate lesions 0.5–1.0 cm, and large lesions >1.0 cm.

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0—no visible lesions, culture negative
1—no visible lesions, culture positive
2—small lesion in mandibular lymph nodes
3—single moderate or multiple small lesions in head lymph nodes
4—moderate to large multiple lesions in head lymph nodes
5—multiple lesions in head and thoracic or abdominal lymph nodes
6—multiple lesions in head, thoracic and abdominal lymph nodes

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regression with the nlf function of S-Plus 2000 (Mathsoft, Seattle, Washington, USA).

## RESULTS

No major problems were encountered in the capture, surgery, and transportation of the animals. Except for one 40-kg 20 mo old sow, all of the pigs chosen for release were young (4–8 mo) and weighed between 11 and 22 kg. Many of them were from two litters captured at the same time. Where possible, these pigs were kept in the same family group when released. They settled into captivity easily, all of the pigs gaining weight during their few days in captivity.

Recovery of specific pigs at specific times proved to be more difficult than anticipated. Because the area is mostly forested, pigs were seen only rarely from the helicopter when they were being relocated, and radiotracking them on the ground in the dense understorey was fruitless on several occasions. Helicopter tracking in conjunction with a ground-based hunter with dogs was more successful, but most of the pigs were eventually shot opportunistically when seen in open areas during routine relocations or hunting forays by the helicopter operator.

All of the 17 released pigs were eventually recovered, but one is not included here because it was not recovered until March 2002, after this paper was submit-



ted. Another pig (#11) disappeared in July 2000, but was serendipitously recovered 35 km away in late May 2001, 15 mo after release. This pig was lactating and was shot with three 5 mo old juveniles.

The remaining 15 pigs were shot within Hochstetter Forest. One (#3) was not retrieved until 2 mo later so the carcass was too decomposed for necropsy. Those successfully necropsied were in average or good condition, and all of those for which both pre-release and at-recovery weight data were obtained had gained weight, typically at the rate of about 100–250 g/day (Table 2). One of the females (#22) had seven 1 mo old piglets with her when she was shot, and another (#10) was pregnant.

The first two released pigs recovered were shot 2 mo after release. One of these had no visible lesions, but the retropharyngeal and bronchial nodes had small (<2 mm) petechial hemorrhages clustered within one pole of the nodes. The other also had no visible lesions but had enlarged mandibular lymph nodes (both sides about 3 cm long and 2–3 cm in diameter) with numerous petechial hemorrhages scattered throughout, and was therefore classed as a TB suspect. Many of the other lymph nodes from this pig also had petechial hemorrhages. The remaining 13 (87%) pigs were shot 4–15 mo after release and all had gross lesions typical of TB. Lesions ranged from 1 mm creamy foci with caseation and/or calcification especially in the tonsils, to 2–4 cm-diameter whitish lesions within enlarged nodes that were typically firm, well encapsulated, with some caseation but usually extensively mineralized throughout especially in the pigs shot later in the study. The largest lesion was a 10 cm diameter mandibular abscess filled with soft caseous pus with little calcification. *Mycobacterium bovis* was isolated from all 15 pigs for which culture results of the mandibular nodes were available (Table 2).

The most common site of macroscopic infection was the head, with all 13 pigs

having lesions in the mandibular lymph nodes and six having lesions in the tonsils. Only five pigs, mostly those recovered late in the study but one after only 4 mo, had some limited generalization of the disease outside the head, with the mediastinal, bronchial, and apical nodes of the lung and ileocecal and ileojejunum nodes of the intestines involved. No lesions were found in the lungs or other organs. Although some of the mandibular node lesions in these longest-exposed pigs were large (up to 10 cm in diameter) and purulent, the number of lesions outside the head was usually low and none of them were large (<1 cm). None of the pigs appeared to be adversely affected by TB, and none of the sows had any gross signs of infection in the mammary tissue. Severity-of-infection scores for the pigs that remained within Hochstetter Forest increased with exposure (Fig. 2). As all the released pigs were infected, TB prevalence was identical in all sex and age classes.

The sample of resident feral pigs consisted of 20 piglets 1–2 mo old (from three separate litters), 17 subadult pigs <1 yr old (including one litter of eight), and four adults from 1–3.5 yr old. Three of these “resident” pigs were barrows (neutered males) that had obviously been captured or in captivity for some part of their lives. About half of these 41 pigs were shot in association with the released pigs. Of the 41, 15 (37%) had typical TB lesions, and a further seven (17%) were classed as having nontypical signs of TB.

Mycobacterial cultures were conducted for 37 of these resident feral pigs; 20 (54%) were infected. Most (87%) of the 15 pigs with typical gross lesions were culture positive. This compares with 60% of the five with nontypical lesions and 24% of those with no visible lesions. Six of seven nontypical-but-culture-positive pigs were piglets 1–2 mo old whose putative mother (shot with them) had typical TB lesions and was TB culture positive. In contrast, the seven piglets shot with a released and culture-positive sow (#22) had

TABLE 2. Sex, age at capture, period over which each pig was radiotracked, weight gain, movement data, and TB status of necropsied pigs.

Group	Pig number	Sex <sup>a</sup>	Period monitored (mo)	Number of locations	Maximum distance <sup>b</sup> (km)	Mean distance <sup>b</sup> (km)	Home-range size <sup>c</sup> (km <sup>2</sup> )	Weight at start (kg)	Age at capture (mo)	Weight gain/day (g)	Gross lesion status <sup>d</sup>	Lesion score	Culture status
1	1	F	8.3	14	5.2	2.4	17.9	18	14	94	Typ	4	positive
	2	MX	4.6	10	5.2	2.6	9.4	18	12	204	Typ	3	positive
	3	F	1.9	5	5.2	2.6	3.8	19	7		Decomposed		
	4	M	9.1	9	5.2	3.5	9.7	c. 19	17	151	Typ	5	positive
	5	F	2.0	6	5.2	2.6	3.4	21	10	195	Nvl	1	positive
2	10	F	5.0	9	4.7	2.0	26.4	18	13	226	Typ	3	positive
	11	F	15.0	10	35	5.7	23.3	18	13		Typ	4	positive
	12	M	7.3	8	12.5	3.5	20.3	22	14	179	Typ	5	positive
	13	MX	1.9	5	2.7	1.5	3.6	—	7	—	Equiv	1	positive
	20	F	7.3	14	3.4	1.3	4.7	12	18	125	Typ	4	positive
3	21	MX	4.6	9	3.4	1.7	4.1	12	9	209	Typ	5	positive
	22	F	7.3	14	3.4	1.3	4.7	22	14	105	Typ	4	positive
	23	M	7.3	14	3.4	1.3	4.8	11	12	138	Typ	6	positive
4	30	F	7.3	10	4.2	2.4	7.3	21	16		Not recovered until 2002		
	31	MX	7.3	14	6.9	1.9	12.4	22	16	241	Typ	4	positive
	32	M	4.6	9	6.9	3.2	10.6	40	25	121	Typ	3	positive
	33	F	8.3	11	4.3	2.4	14.0	20	16	52	Typ	5	positive

<sup>a</sup> MX = neutered males.

<sup>b</sup> Maximum and mean distances recorded between successive locations.

<sup>c</sup> The home range estimate for #11 does not include the single and final location 35 km outside Hochsetter Forest.

<sup>d</sup> Gross lesions status codes are Typ=typical of TB, Equiv=no typical lesions but other signs of infection, NVL=no visible lesions or other signs of TB.

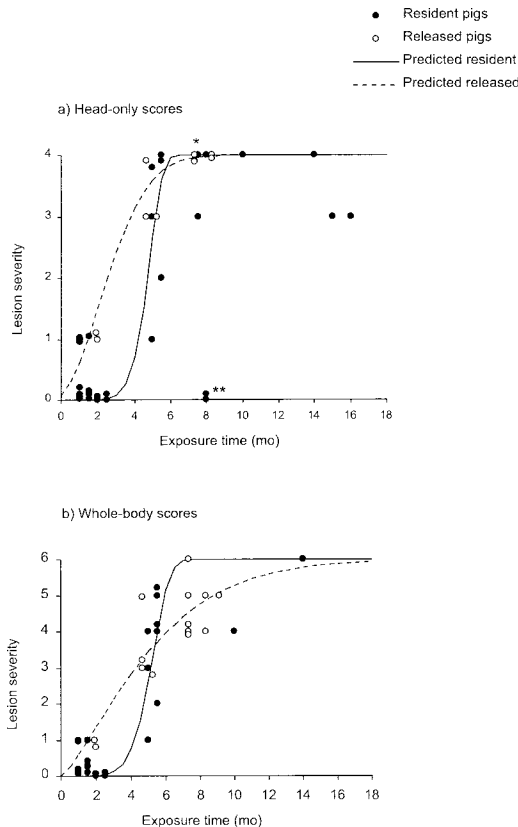


FIGURE 2. Relationship between severity-of-infection scores and exposure for released and resident feral pigs, for (a) head-only scores, and (b) whole-body scores. For released pigs, exposure is time since release, whereas for feral pigs it is age at death. The lines are the respective Weibull curves fitted to the data by nonlinear regression. The asterisks denote the three neutered resident feral pigs, and an infected 41-mo-old resident feral male is not shown. Overlapping data points have been shifted slightly to reveal their location.

no lesions and were culture negative. The prevalence of TB in resident feral pigs increased quickly with age to 100%. Apart from two barrows that were probably not born in Hochstetter Forest, the only feral pigs that were not infected were less than 3 months old (Fig. 2).

As with the released pigs, the most common site of gross infection was in the mandibular lymph nodes. Macroscopic lesions in the tonsils were found in two infected pigs (13%), and involvement of the tho-

racic and/or peripheral nodes occurred in three (19%) of the infected animals.

For both resident and released pigs, sigmoid Weibull curves (Kendall and Stuart, 1977) provided a good characterization of the relationship between exposure and severity-of-infection scores, particularly for the released pigs (Fig. 2). There was some evidence that the two curves differed in shape ( $F_{2,42}=3.3$ ,  $P=0.05$  for head-only scores;  $F_{2,38}=3.0$ ,  $P=0.06$  for whole-body scores), primarily due to an apparent lag in establishment of infection in resident feral piglets.

The relationship between lesion severity (using the head nodes only) and exposure (age) in the resident feral pigs was not as strong as for the sentinel pigs, with a greater scatter of scores (Fig. 2). The greater variability in the resident feral pigs was statistically significant ( $F_{30,12}=6.49$ ,  $P=0.0007$ ). The zero severity-of-infection scores recorded for two of the barrows, in particular, were markedly lower than for other resident feral pigs of similar age. Likewise, resident female #40 was 15 mo old, but had only five lesions of <2 mm in the mandibular and parotid lymph nodes, far less severe infection than would be predicted for a released pig exposed for that length of time.

The two groups of pigs that were held and fed at the release sites for a week were never relocated there. There is therefore no evidence that feeding had any impact on movement patterns. Following their release, the pigs mostly stayed with the groups in which they had been placed. However, some of the groups met, coalesced temporarily, and then split into slightly different groups. Because the pigs stayed within groups, there was no evidence of any sex-related differences in movement patterns.

Minimum convex polygon estimates of home-range size varied from 3.4–26.4 km<sup>2</sup> (Table 2). Excluding pigs released for fewer than 6 mo, MCP home-range size averaged 10.7 km<sup>2</sup> (range 4.7–20.3 km<sup>2</sup>). Although formal statistical analysis was pointless given the limited amount of data



and the confounding effect of changing group composition, much of the variation in home-range size appeared to result from differences between groups. Within groups, the pigs recovered last tended to have larger estimates of home-range size (Table 2).

The total distance linking all the successive locations for each pig ranged from 6–52 km, with the largest values not surprisingly tending to be for those released longest (Table 2). The single largest distance moved between successive locations was the shift of 35 km by pig #11. Of those that remained within Hochstetter Forest the largest distance was 12.5 km, by #12. Both these shifts occurred at about the time another member of the same group was caught and killed by a private hunter and his dogs, suggesting the possibility that the shifts were induced by that experience. The maximum distances shifted by each of the 15 other pigs ranged from 2.6–6.9 km. The mean distances the pigs travelled between successive locations ranged from 1.3–3.5 km and there was little apparent variation in mean distance when compared with time since release.

The pooled distribution and cumulative percentages of the distances between any one location of a pig and all of its previous locations (in 1 km classes, pooled across all pigs still present at the particular time; Fig. 3) provides an averaged indication of the radius around each pig that would encompass all or a given proportion of its previous locations. Overall, all of the curves approach the 100% asymptote at or below about 9 km, with about 95% of all distances being less than 6 km regardless of the duration of the release (Fig. 3).

Twenty of the stomach content samples collected were analyzed. The principal foods present varied widely, but items often eaten in large quantities included bracken fern (*Pteridium esculentum*) roots, soft-leaved herbaceous species, fruits of forest trees, and fungi. Vertebrate carrion was not a significant part of any of the stomach contents, but was present in trace

amounts in seven of the stomachs. A few possum hairs were found in one, but all of the remaining vertebrate carrion was mouse (*Mus musculus*), rat (*Rattus rattus*), or freshwater eel (*Anguilla* sp.).

## DISCUSSION

Animals have long been used as sentinels for monitoring environmental health hazards (National Research Council, 1991) and the use of wildlife as disease sentinels is not a new concept. Wildlife are surveyed, for example, to identify where rinderpest has persisted in cattle following widespread vaccination programs—the wildlife themselves are not considered maintenance hosts of the disease, but because they are not vaccinated they can be serologically surveyed whereas the cattle cannot (James, 1998). Deliberately exposing sentinels, as in this study, is less common, but flocks of captive chickens have, for example, been established specifically to detect viruses. (Eldridge, 1987), and Stallknecht et al., (1987) regularly recaptured wild pigs and used penned domestic pigs to document arthropod transmission of vesicular stomatitis on Ossabaw Island (Georgia, USA).

What makes a good sentinel? Guidelines issued by the Centers for Disease Control and Prevention (Centers for Disease Control, 2001) suggest the ideal avian sentinels for West Nile virus in the United States would: 1) have high susceptibility; 2) all survive but retain easily detectable signs of infection; 3) pose no risk of infection to handlers; and 4) not be capable of re-infecting the vector. We have adapted these guidelines to assess the outcomes of this trial.

All of the pigs recovered were infected, indicating high levels of susceptibility. The reasonably good fit between exposure time and the severity-of-infection scores for the released pigs that remained within Hochstetter Forest (Fig. 2) leaves little doubt that all of the pigs became infected soon after release, with subsequent generalization of the disease.

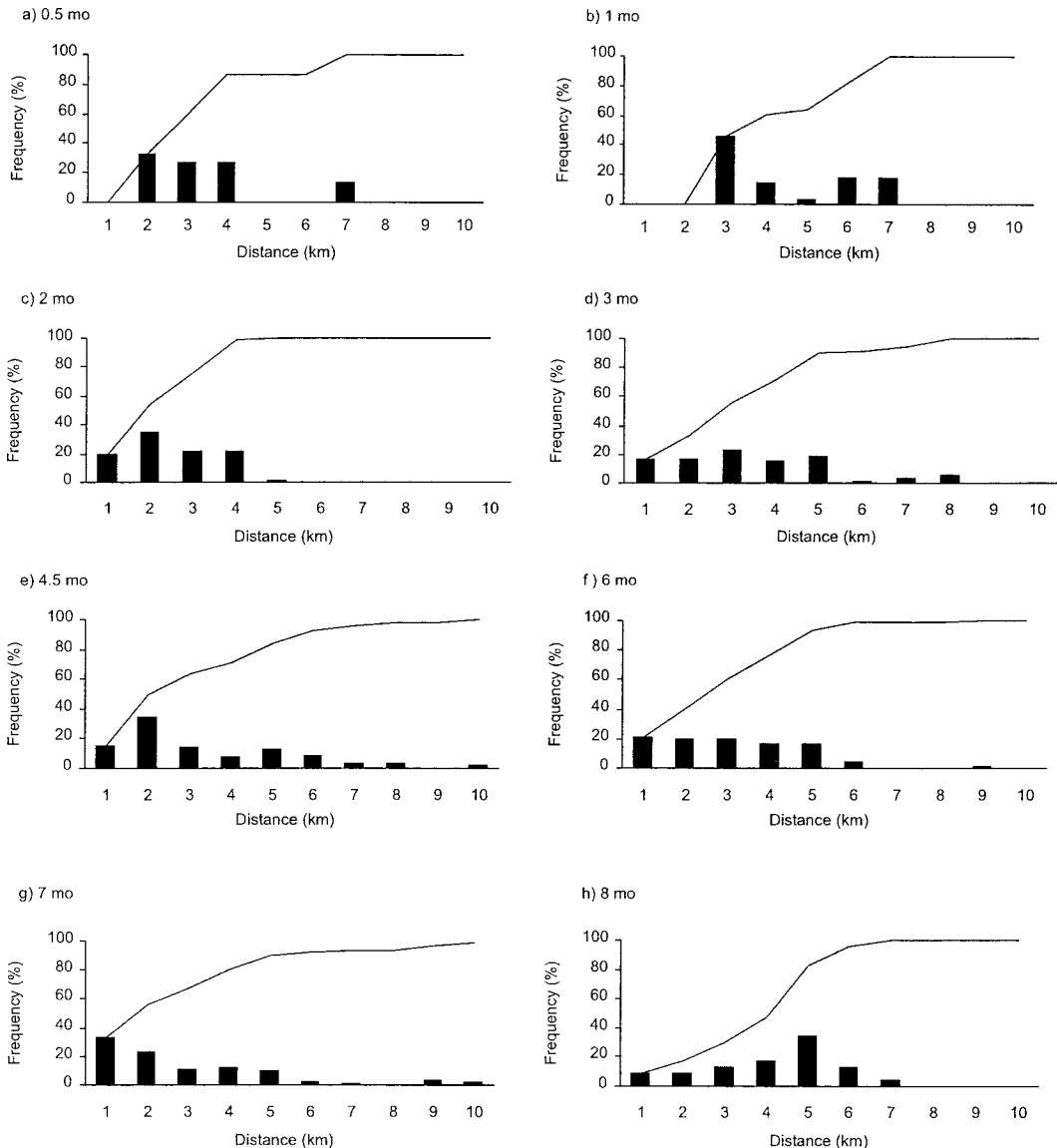


FIGURE 3. Distances between location at eight intervals during the study and all previous locations. Only pigs present at the end of each interval are included, so the number of pigs in the longest-interval graph (h; 8 mo) is low (three pigs). The two distances greater than 10 km are not shown. Data are percentages of observations falling in each of 10 1 km distance classes (bars) and cumulative percentage of observations less than or equal to each distance (lines).

None of the released pigs appeared to be adversely affected by TB. All but one had easily detectable signs of infection. However, pigs may sometimes be able to resolve lesions (Ray et al., 1972; Corner et al., 1981; McInerny et al., 1995). If so, detectability of previous infections will also decline over time.

Lesions in pigs are typically encapsulated, and contain few bacilli (Cooke et al., 1999). The risk of infection to staff involved in pig recovery and necropsy should therefore be low. Once infected, released pigs appear extremely unlikely to then contribute to TB maintenance in an area if the pigs are recovered after a few

months, long before they could potentially die of TB and be eaten by possums or other scavengers, a potential route of infection identified for red deer (G. Nugent unpubl. data) and ferrets (*Mustela furo*; Ragg et al., 2000).

In summary, pigs largely fulfill the characteristics of an ideal sentinel for TB, and we therefore advocate use of either resident feral pigs, if they are present in sufficient numbers, or released pigs, where resident feral pigs are scarce. Only two other species of wildlife (ferrets and red deer) in New Zealand are distributed widely enough and are infected with TB frequently enough (Cooke et al., 1999) to have much potential for use as sentinels. However red deer appear far less susceptible to the disease than pigs, with only 11% of the deer killed in Hochstetter Forest infected (G. Nugent, unpubl. data), and infected ferrets appear unlikely to survive as long as infected pigs. In addition, feral pig distribution most closely matches the main infected areas that are not well covered by livestock testing (Nugent, 2001).

Ultimately sentinel surveys have only two possible outcomes. Either infected animals are found or they are not. Infection in a sentinel confirms the presence of TB in the area occupied during the exposure. Identification of the most probable source of infection will always be more uncertain. In this trial, for example, two observations lend weight to the evidence from McInerny et al. (1995) that pig-to-pig transmission is an unlikely explanation: 1) universal involvement of the mandibular nodes and the scarcity of infection in lung nodes indicates an oral rather than a respiratory route of infection; and 2) no infection was found in piglets belonging to one of the most heavily infected released sows (#22). Also there was an apparent lag in disease progression at very young age for resident feral pigs compared to released pigs (Fig. 2), suggesting that piglets <1 mo old were not being exposed to the disease despite the obviously close contact

with their infected mothers. Likewise, transmission from sympatric red deer is unlikely because their densities are low, the prevalence of TB is low (11%), few infected animals have visible lesions, and most deer that die are shot and removed entirely (apart from the gastrointestinal tract) by commercial hunters (G. Nugent, unpubl. data). Possums are the only other commonly infected host present throughout Hochstetter Forest, and pigs readily eat possum carrion (Thomson and Challes, 1988). Even though we did not observe that in this study, probably because our diet sampling effectively represented less than 20 days in total of feeding whereas the pigs were released for at least 55 days, feeding on possum carrion remains the most plausible explanation for the rapid infection of the released pigs. The presence of infection in 2 mo old piglets weighing 4–6 kg is not inconsistent with that because these piglets were feeding on vertebrate carrion. Ultimately, however, identifying the source of infection is not as critically important as the simple confirmation that disease is present in wildlife.

Inferences about the timing and location of the transmission event define the scale of any management response. For unmarked free-ranging resident feral pigs, the inferences can only be based on generic information. This and previous studies in New Zealand show that the ranges of both released and resident feral pigs are hundreds of times greater than those of possums. In unforested tussock country in North Canterbury, distances between first and final location over a 25-mo period averaged 3.2 km ( $\pm 2.6$  SE) for boars (maximum 13 km) and  $0.5 \pm 1$  km for sows (maximum 0.8 km) (Martin, 1975). In similarly open habitat in central Otago (NZ), Knowles (1994) reported home range sizes of between 5.4 and 23.4 km<sup>2</sup> with distances between locations usually <3 km for sows and varying from 0–8 km for boars. In relatively undisturbed pasture and forest, near Murchison, the comparable mean distance recorded by McIlroy (1989) for

sows was 1.4 km (maximum 2.7 km) and home ranges varied between 0.3 and 2.1 km<sup>2</sup>. The range sizes recorded in this study are much higher than predicted from body weight by the regression equations developed by Saunders and McLeod (1999) but in line with predictions for the low densities that normally prevail in Hochstetter Forest. Overall, most feral pigs occupy ranges substantially less than 20 km<sup>2</sup>, although seminomadic adult boars (Saunders and Kay, 1996) and females (this study) can sometimes disperse long distances. The distribution of interlocation distances (Fig. 3) suggests a “detection radius” around kill sites of 6 km would encompass 95% of previous locations. This defines a circular 110 km<sup>2</sup> area around the kill site that has a 95% probability of containing the source of infection.

Detracting from this locational inference, pigs are sometimes illegally translocated to enhance huntable populations (Fraser et al., 2000), as illustrated by the presence of three 8 mo old “resident” barrows in this trial. Two were not infected, indicating that they had been released into Hochstetter Forest only shortly before being killed. Coupled with occasional long-distance dispersal by a few pigs, such events mean the presence of just one or two infected apparently resident feral pigs in an area does not confirm a local source of TB.

The major advantage of released radiotagged pigs is therefore the ability to delimit the area containing the source of infection with far greater certainty and precision. However, releasing pigs is costly; so released pigs should be used where they can be easily radiotracked and where feral pigs are scarce. In more open habitats, combined use of released and resident feral pigs may be most cost-effective, with the released pigs used as “Judas” animals to find and kill resident pigs. The Judas-pig method has been successfully used in Central Otago (Knowles, 1994) and Australia (McIlroy and Gifford, 1997). Potential refinements of the released-sentinels

method include use of semi-domestic or even domestic pigs on fenced farmland.

If no infection is found in the sentinels, we lack the empirical data on the relationship between prevalence in possums and the probability of infection in pigs that is needed to convert such results into statements of statistical confidence that TB is absent. This information gap is not an impediment to implementation of released pig surveys as a positive result is useful in that it provides clear management directives.

We suggest initial releases of about one pig released per 10 km<sup>2</sup>, with sequential releases at intervals of 6–12 mo to increase survey intensity and coverage. We favor use of neutered males, in the belief that this will reduce the potential for large movements, and because it reduces concerns about any additions to the pig population. The optimal time for recovery of the released animals is unclear. We suggest 6–12 mo as a starting point.

Releases are unlikely to be permitted in designated conservation areas, and farmers may object to the presence of potentially infected pigs. Where such constraints apply, feral pigs, ferrets, or deer may be available as alternative sentinels.

In summary, the potential benefit of using pigs to detect TB is ultimately one of economic efficiency or cost-effectiveness. This trial has shown that released pigs are at least moderately sensitive indicators of disease presence. Their much larger home ranges and the likelihood they would remain in a detectably infected state for longer than possums appear to be good grounds for believing the sentinel pig surveys could be more cost-effective and sensitive indicators of TB presence in possums than direct surveys of the already low possum populations in affected areas.

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