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Absence of evidence is not evidence of absence: Feral pigs as vectors of soil-borne pathogens

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Abstract Invasive soil-borne pathogens are a major threat to forest ecosystems worldwide. The newly discovered soil pathogen, *Phytophthora* 'taxon Agathis' (PTA), is a serious threat to endemic kauri (*Agathis australis*: Araucariaceae) in New Zealand. This study examined the potential for feral pigs to act as vectors of PTA. We investigated whether snouts and trotters of feral pigs carry soil contaminated with PTA, and using these results determined the probability that feral pigs act as a vector. We screened the soil on trotters and snouts from 457 pigs for PTA using various baiting techniques and molecular testing. This study detected 19 species of plant pathogens in the soil on pig trotters and snouts, including a different *Phytophthora* species (*Phytophthora cinnamomi*). However, no PTA was isolated from the samples. A positive control experiment showed a test sensitivity of 0–3% for the baiting methods and the data obtained were used in a Bayesian probability modelling approach. This showed a posterior probability of 35–90% (dependent on test sensitivity scores and design prevalence) that pigs do vector PTA and estimated that a sample size of over 1000 trotters would be required to prove a negative result. We conclude that feral pigs cannot be ruled out as a vector of soil-based plant pathogens and that there is still a high probability that feral pigs do vector PTA, despite our negative results. We also highlight the need to develop a more sensitive test for PTA in small soil samples associated with pigs due to unreliable detection rates using the current method.

Key words: Bayesian, invasive species, *Phytophthora*, PTA, vector.

INTRODUCTION

Invasive species are a prominent threat to forest health worldwide through competition, predation, hybridization and disease (Falk-Petersen *et al.* 2006; Hansen 2008). Although invasive vertebrates and pest plants are a serious threat, many forests of the world are also being attacked by microscopic soil-borne invaders (Hansen 2008). Microbes are generally understudied in invasion biology especially in natural ecosystems, perhaps because of their complex and cryptic life cycles. However, these alien pathogens may impact communities and ecosystems by reducing species diversity, changing environmental conditions and altering ecological processes (Chapin *et al.* 2000; Gurevitch & Padilla 2004). Lodge (1993) has highlighted the importance of studying the community interactions that determine invasion success, instead of studying communities or colonist species in isolation. This is of particular importance with soil-borne pathogens, as assisted movement of soil-borne disease is required for rapid spread over large distances because natural spread is slower and localized. Therefore, for effective management of invasive species, such as soil-borne pathogens, the pathways of spread must be investigated.

Lockwood *et al.* (2007) split the invasion process into four stages: the first stage involves the transport of the invader to a new location, the second stage involves invader establishment and the third stage, population increase and spread, after which the impact of the invader is perceived (the fourth stage). These stages of invasion can be affected by a vector (Ruiz & Carlton 2003) which is defined as the conveyance that moves a non-native propagule to its novel location (Lockwood *et al.* 2007). Vectors may influence the number of individuals transferred to a new area and the number of transfer events initiated (related to propagule pressure) (Colautti *et al.* 2006). Faster transport may also improve the survivorship of the invader (Ruiz & Carlton 2003). However, the vector may also increase chances of establishment through targeted vectoring to an optimum environment (increasing niche opportunity) (Shea & Chesson 2002; Ruiz & Carlton 2003). Therefore, understanding vector pathways is crucially important to adequate response and management of soil-borne pathogen spread.

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New Zealand's iconic kauri tree, *Agathis australis*, is known to host two invasive species of soil-borne *Phytophthora* pathogens that cause ill health, *Phytophthora cinnamomi* and *Phytophthora* 'taxon Agathis' (PTA) (Beever *et al.* 2009). *Phytophthora cinnamomi* is found widely in natural kauri stands and has been linked with some tree deaths, especially in poorly drained sites (Podger & Newhook 1971). However, PTA is now of greater concern to kauri health and has been spreading throughout kauri's natural range, causing canopy thinning, defoliation, large resin-exuding lesions on the lower trunk and eventual tree death (Beever *et al.* 2009). Based on the relative recency of the first report of this pathogen (Gadgil 1974), indications that it has spread since then into previously uninfected areas of kauri (Beever *et al.* 2009) and its similarity to *Phytophthora katsurae* (native to Taiwan), Beever *et al.* (2009) theorized that PTA is invasive and was introduced to New Zealand.

The genus *Phytophthora* in Greek means 'plant destroyer'. Members of the genus *Phytophthora* are among the most serious threats to agriculture and cause devastating diseases in hundreds of plant hosts (Judelson & Blanco 2005). *Phytophthora* species have been studied extensively in agricultural systems, although little study has been conducted on the effects and pathways of spread of these pathogens in natural forest habitats. *Phytophthora* species are eukaryotic, microscopic 'fungus-like', soil-borne organisms. They are taxonomically classified in the class Oomycota (commonly termed oomycetes) and owe much of their pathogenic success to the combination of asexual and sexual production of spores (Judelson & Blanco 2005). *Phytophthora* species persist in the soil and infected plant tissue, predominantly as dormant resting spores (oospores and chlamydospores), but reproduce through the production of motile, biflagellate, infective zoospores (Wilcox 1992). *Phytophthora* can be dispersed in soil either via water movement on the soil surface or via the movement of soil by vectors, such as humans and other animals (Ristaino & Gumpertz 2000). Keast and Walsh (1979) found that *P. cinnamomi* could be successfully transported through the gastrointestinal tracts of termites (*Nasutitermes exitiosus*) and birds (*Pachycephala pectoralis* and *Pachycephala rufiventris*) in Australia. Similarly, Li *et al.* (2010) found that *P. cinnamomi* could survive gut passage in pigs and viable spores were excreted up to 7 days post ingestion. Kliejunas and Ko (1976) confirmed other vectors of *P. cinnamomi* when they isolated the pathogen from human boots, vehicle tyres and also from the trotters of feral pigs (*Sus scrofa*). However, PTA is a newly discovered species in New Zealand and no previous work has been conducted on the potential vectors of this pathogen. Feral pigs have a known association with soil through the disturbance caused when they forage for invertebrates and fungi below ground. This close association with soil and the relatively high abundance of feral pigs in the North Island of New Zealand (King 2005) make them a prime suspect in the investigation and subsequent management of PTA vectors. However, evidence of vectoring is required to justify the expensive and often controversial culling of feral pigs in northern New Zealand forests.

The primary aim of this study is to determine whether pigs do indeed vector PTA. More specifically, we investigate whether snouts and trotters of feral pigs carry soil infected with PTA. This was achieved by obtaining the trotters and snouts from culled pigs from a forest infected with PTA, and screening for PTA in the associated soil. We then used the data in a Bayesian modelling approach to determine the probability that pigs vector PTA. This Bayesian approach (Box & Tiao 1992) is based on expert opinion in the absence of empirical data (due to the recent discovery of PTA). Bayesian modelling is useful in this situation as it allows a direct interpretation of probabilities and is a valid alternative to classical statistical methods when empirical data are lacking (Crome *et al.* 1996; Wade 2000; Ellison 2004; Martin *et al.* 2005).

METHODS

Pathogen detection in soil associated with pigs

The Auckland Council contracted hunters for pig culling operations in the Waitakere Ranges Regional Park, Auckland, New Zealand (extending from 36°53′ to 37°03′S and from 174°27′ to 174°34′E). In this area of conifer/broadleaf-dominated temperate rainforest, pigs are present in substantial, but unquantified numbers. No other mammalian ungulates are found there. The culls started in October 2008 and occurred approximately every 3 months. Nine culling operations were sampled in total with the last cull sampled in August 2011. Where the pig kill was accessible, hunters collected one trotter chosen at random, and the snout of the culled pig. Each was stored separately in a labelled bag. The snout and trotter were then refrigerated (4°C) for a maximum of 7 days until the soil sampling could take place.

Overall, 457 individual pigs were sampled, with 364 snouts sampled (snout presses), 189 trotter samples swabbed (swabbed trotters) and 268 trotter samples washed (washed trotters).

The snout samples were directly pressed onto an agar plate containing clarified V8 P_5ARPH agar (a selective media for *Phytophthora*, containing vegetable juice, antibiotics and antifungals to select against bacteria and true fungi). The agar plates were incubated in the dark at 18–20°C and checked every 2 days for 10 days. Subcultures were taken of any *Phytophthora*-like growths and placed onto a general potato dextrose agar (PDA) medium to induce production of aerial mycelia. After incubation of these plates for 10 days,

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Phytophthora-like cultures were then DNA sequenced. Samples for DNA extraction were obtained by scraping mycelia from the agar plates using a pipette tip. The pipette tip was then put into an Eppendorf tube containing 420 μL of tissue extract buffer and 4.2 μL of protease K enzyme from the Corbette robot DNA purification kit. After a 30 s vortex, the tubes were incubated at 56°C for 1 h, and centrifuged at 16 000 rpm for 3 min. Two hundred and twenty microlitres of the supernatant was then removed and loaded onto an X-tractor Gene robot (Qiagen) for DNA extraction. The robot was run according to manufacturer's instructions (Qiagen). The ITS gene was targeted for amplification using ITS6 (Cooke & Duncan 1997) and ITS4 (White *et al.* 1990). Successful amplifications were then confirmed by running the PCR products on 1.5% agarose gel stained with ethidium bromide at 150 V for 30 min. A sequencing PCR reaction was then completed and the completed reaction was then cleaned using Applied Biosystem's Big Dye Xterminator purification kit and loaded onto an ABI Genetic Analyser 3031XL sequencing machine (Applied Biosystems). Sequencing results were compared against GenBank using BLAST search for identification.

For the swabbed trotters, soil was swabbed from the trotters using sterile cotton buds. The soil was swabbed into a sterile Petri dish; any hair on the trotter matted with soil was also shaved into the dish using a scalpel blade. The Petri dishes were then sealed to keep in any moisture and were stored at 10°C until baiting was ready to commence. To isolate *Phytophthora* from a soil sample the soil has to be flooded with water and 'baited' with plant tissue to induce the production of infective motile zoospores. Baiting involved a variation of the needle baiting technique used by Dance *et al.* (1975) which was developed as the national standard operating procedure (SOP) by three separate Crown Research Institutes in New Zealand as part of the National Kauri Dieback Biosecurity Response (Beever *et al.* 2010). Collected and stored soil (from the swabbing method) was macerated within the Petri dishes to eliminate clumps, and then air dried for 2 days. These samples were then spray-moistened with reverse osmosis (RO) water, until the soil surface was shiny. They were left for 1 h and then re-sprayed to target any clods of soil. They were left for a further 4 days at room temperature to stimulate any *Phytophthora* oospores. After 4 days, the soil was transferred to a 600 mL container, flooded with RO water and immediately baited using lupine radicles (*Lupinus* spp.) and Himalayan cedar (*Cedrus deodara*) needles which were floated on the water surface. The baited samples were incubated for 2 days at 20°C. After incubation, the bait tissues were removed and rinsed in sterile RO water. They were then transferred to a 70% ethanol solution for 30 s, and rinsed in sterile RO water for a second time. The bait tissues were blotted dry with a paper towel and placed onto clarified V8 P_5ARPH agar plates. All plates were incubated at 18°C for 10 days. After 10 days of incubation any growths that looked similar to *Phytophthora* mycelia were then subsampled to clarified V8 P_5ARPH to obtain a pure culture, and then onto PDA media. These were incubated again at 18°C for 10 days and then DNA sequenced (using the methods previously described) to obtain a species level identification. Fungal by-catch on plates was identified by light microscopy where possible.

For the washed trotters, each trotter was placed in a separate plastic container with approximately 270 mL of RO water. The trotter was then washed of all soil, until the hair was as clean as possible. The water in each container was then baited immediately using the above methods.

Sensitivity testing

To assess the ability of the techniques used to detect PTA, a positive control experiment was undertaken, testing all the techniques used. *Phytophthora* 'taxon Agathis' (sourced from the Landcare Research ICMP 18403 culture) was grown on PDA media at 20°C. From the growing edge of cultures, 6.5-mm-diameter plugs of agar were placed into clarified V8 (vegetable) juice broth and incubated at 20°C for 56 days. *Phytophthora* 'taxon Agathis' was harvested from the V8 juice broth and macerated in a Waring blender for 20 s. Viable oospore concentrations were estimated by staining a sample of this maceration with a 0.1% solution of tetrazolium chloride, and then counting a known volume using a haemocytometer. The solution used for the positive control was calculated to have approximately 2.5×10^5 viable oospores per millilitre. This concentration is thought to be 100 times higher than naturally present in soil (S.E. Bellgard, pers. comm., 2011). Sterilized soil was sieved to remove any large clumps. Then 66 600-mL containers were filled with 100 g of the sieved sterilized soil, 10 mL of the oospore suspension and 20 mL of sterile RO water, and thoroughly mixed. A separate container was used for each pig sample; 66 trotters and 40 snouts were used, which had been sterilized using 95% ethanol. Each sample was pushed into the spiked soil to simulate a pig stepping in, or rooting in, infected soil. The samples were then refrigerated for 7 days. The snout samples were then pressed onto clarified V8 P_5ARPH plates. Thirty-three trotter samples were then washed and baited and the remaining 33 samples were swabbed following the methods above.

Analysis of pig vectoring probabilities

To calculate the probability of feral pigs carrying PTA, a Bayesian probability model was constructed using the test sensitivity data and expert prior probabilities. The analysis calculates the probability of PTA being present given a negative test.

We aimed to establish the probability that pigs carry PTA given that all the tests are negative ($[PTA^+|Tests^-]$). Using Bayes theorem we obtain:

$$[PTA^+|Tests^-] = (1 - SeGroup) \times [PTA^+] / ((1 - SeGroup) \times [PTA^+] + (1 - [PTA^+])).$$

$[PTA^+]$ denotes the *prior* probability that pigs carry PTA (what we perceive before we conducted the study). This was set at 90% based on known association of pigs with soil through their natural foraging behaviour, anecdotal evidence of extensive pig disturbance under infected kauri trees in the Waitakere ranges and Kliejunas and Ko (1976) and Li *et al.* (2010) who found that pigs vector *P. cinnamomi* in Hawaii and Australia.

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SeGroup is the sample group sensitivity which is calculated using $SeGroup = 1 - (1 - SeTest \times P^{\star})^{n}$, where *SeTest* is the test sensitivity data from the positive control tests and *n* is the number of pig samples tested. P* is the design prevalence, the minimum prevalence we could detect. For this value we used expert opinions elicited following Kuhnert *et al.*'s (2010) guidelines where possible. Twenty-six experts were asked to contribute their opinion, of which 22 replied. They were asked: 'in your opinion what is the probability that any snout or trotter collected in the Waitakere ranges would carry PTA'. The experts were split into groups according to occupations and research interest with means created for each group. These groups included mycologists ($n = 4$), pig hunters ($n = 3$), land managers ($n = 13$) and ecologists ($n = 3$) (one manager was also a hunter).

We explored the variability of the results using the differing *SeTest* values from the test sensitivity experiments and also the range of means for design prevalence from the different groups of experts.

RESULTS

Pathogen detection in soil associated with pigs

A number of oomycete and fungal species were isolated from the soil carried on pig trotters and snouts (see Appendix S1 for more details). Many of these species were 'by-catch', as the methods used were optimized to isolate *Phytophthora* species from soil samples and to select against true fungi (through the use of specific antifungals in the media); therefore, the percentages of trotters carrying these pathogens are not presented as it would provide a false representation of the prevalence of these pathogens. While we found a related species of *Phytophthora* (*P. cinnamomi*) and 18 other plant pathogens, we did not isolate any PTA from the soil collected from the trotters or snouts.

Sensitivity testing

Phytophthora 'taxon Agathis' was detected from only 3% of the trotters ($n = 33$) that were 'walked' through soil with known concentrations of PTA oospores and then washed and baited. No PTA was detected from the swabbed trotters ($n = 33$) 'walked' through PTA-positive soil or when snouts were pressed into the PTA-positive soil ($n = 40$) and then pressed directly onto agar.

Analysis of pig vectoring probabilities

The expert opinion means were variable between occupational groups (Table 1). The expert opinion of pigs as vectors from the 'managers' was the highest and the 'ecologists' the lowest. The overall mean of all 22 expert opinions was 0.26, which may be used as a reasonable estimate of design prevalence.

Table 1. Mean and standard error of expert prior opinions for each occupational group of the probability that any sample would be *Phytophthora* 'taxon Agathis' (PTA)-positive, including a mean of all opinions gathered ($n = 22$; one manager was also a hunter)

Occupational group	Mean	Standard error	*n*
Overall mean	0.26	0.0597	22
Ecologists	0.05	0.0282	3
Hunters	0.20	0.20	3
Managers	0.35	0.079	13
Mycologists	0.23	0.160	4

The posterior value (end product) of the Bayesian model varied greatly depending on the sample size of pigs tested, the test sensitivity and also design prevalence (expert opinion) values (Figs 1,2). Using a test sensitivity value of 0 for the swabbed trotters and pressed snouts yielded a constant posterior probability of 0.90 for any number of pigs tested (this is based on the original prior probability of 0.90); therefore, these tests were not displayed in Figure 1. With a test sensitivity of 0.03 (268 washed trotters) there is a 0.35–0.86 posterior probability that pigs carry PTA depending on the expert prior opinion used.

With a design prevalence based on the overall mean of the expert opinions, there is a 53% probability that feral pigs vector PTA using the washed trotters, and a 90% probability for the swabbed trotters and pressed snouts. Extrapolations of the data used in Figure 1 show that a sample size of over 1000 pigs would have been needed to be confident in a negative test result. These data show that the greater the test sensitivity values the lower the probability of pigs as vectors of PTA (more confidence that there is no type II error) (Fig. 1). Larger sample sizes also lower the probability of pigs as vectors of PTA because a larger proportion of the pig population has been sampled (Fig. 2). Larger design prevalence (expert opinion) values also lower the probability of pigs vectoring PTA as this value contributes to group sensitivity (*SeGroup*) value (Figs 1,2).

DISCUSSION

This study has shown that feral pigs are a potential vector of a large number of soil-borne plant pathogens. We found 19 species of known plant pathogens on the

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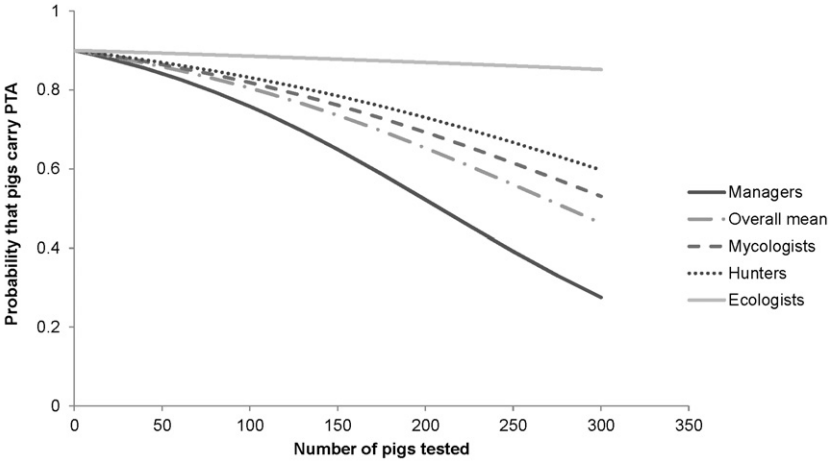

Fig. 1. Relationship between the number of pigs tested and the posterior probability that pigs carry *Phytophthora* 'taxon Agathis' (PTA) based on a *SeTest* (test sensitivity) value of 0.03 for different design prevalence (minimum detection) values based on expert group opinion.

trotters and snouts of feral pigs, some of which are particularly aggressive (e.g. *Sclerotinia*, *Fusarium* and *Botrytis*). Several plant pathogenic oomycete species including *Pythium heterothallicum* and *Pythium vexans* were identified and further demonstrated proof of concept that soil-borne spores of disease agents can be routinely vectored by feral pigs in forests. For example, *P. vexans* has been previously associated with plant decline and diseases in forest ecosystems in Australia, China, Hawaii and North Carolina (Kliejunas & Ko 1975; Vawdrey *et al.* 2005; Zeng *et al.* 2005; Ivors *et al.* 2008). The method used in this study, however, was not optimized for collecting genera other than *Phytophthora*, and while a number of fungi were found on the selective media, many more may have been found using other methods of isolation. Many species of invertebrates are known vectors of plant pathogens (Evans 1973; El-Hamalawi & Menge 1996; Louis *et al.* 1996; Nault 1997), but few studies have investigated vertebrates as vectors. Feral pigs should be of particular interest due to their foraging habits and transport of soil. Therefore, more targeted research into the extent of the role feral pigs play in vectoring would benefit our understanding of vector pathways of other plant diseases.

While no PTA was found in the soil associated with the pigs sampled, *P. cinnamomi* was isolated from the soil collected from a pig trotter. This adds to the results of Kliejunas and Ko (1976) and Li *et al.* (2010) in identifying feral pigs as vectors of *P. cinnamomi*. *Phytophthora cinnamomi* is found widely in natural kauri stands and has been linked with tree mortality especially in poorly drained sites (Podger & Newhook 1971). *Phytophthora cinnamomi* has also been linked with seedling mortality in nursery beds (Newhook & Podger 1972) and has been found in both regenerating and mature kauri (Beever *et al.* 2009). However, Beever *et al.* (2009) also concluded that *P. cinnamomi* plays a minor role in the health of adult kauri, and that abnormal conditions at some sites lead to disease.

We elicited opinions from a variety of experts to gain a representation of the varying opinions on pigs as vectors of PTA. Expert opinions elicited in the Bayesian modelling process varied widely and managers made considerably higher estimates than ecologists. The ecologist, mycologist and hunter groups also had higher variance in their estimates than the managers. The hunter and manager groups may have been influenced more by the politics of the pig hunting (and the hunters' desire to continue hunting), whereas the ecologist and mycologist groups may have focused more on the complexities of the question asked. The Bayesian modelling shows the importance of developing a sensitive test to isolate PTA from animal tissue. A test sensitivity of 0.2 would have increased our ability to reject pigs as a vector of PTA. However, given the current test sensitivity, a sample size of over 1000 pigs would be needed to be confident in a negative result. The results from modelling the current values for test sensitivity and design prevalence estimated that there is a 35–90% probability that pigs are a vector of PTA. The test used in the isolation of PTA is the national SOP developed by three Crown Research Institutes in New Zealand as part of the National Kauri Dieback Biosecurity Response and is based on widely accepted methods of isolating *Phytophthora* species (Dance *et al.* 1975; Beever *et al.* 2010). The test sensitivity of between 0 and 3% found in this study was

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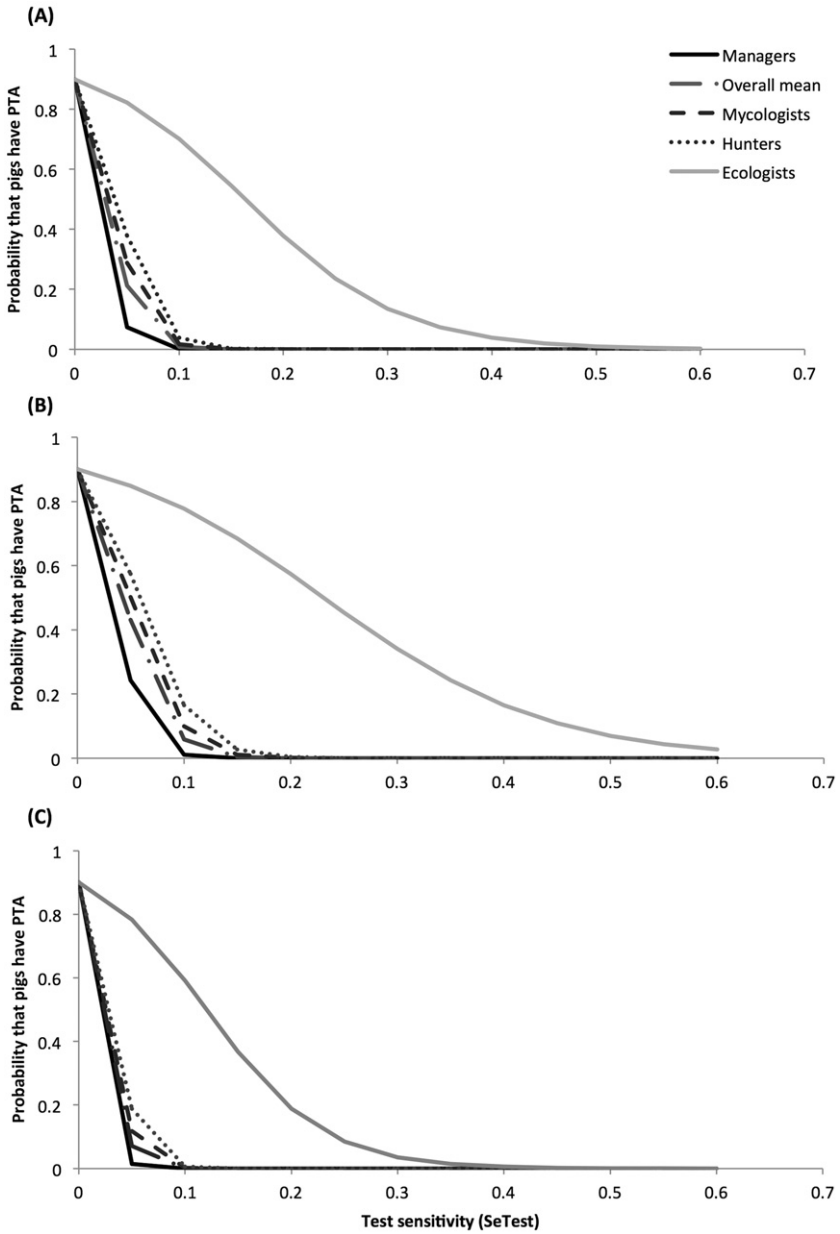

Fig. 2. Relationship between test sensitivity and the posterior probability that pigs carry *Phytophthora* 'taxon Agathis' (PTA) based on a sample size of (A) 268 washed trotters, (B) 189 swabbed trotters and (C) 364 pressed snouts with differing design prevalence (minimum detection) values based on expert prior opinion.

low compared with other studies using the same methods (Beever *et al*. 2010). A recent report has calculated and compared PTA detection probabilities between the three New Zealand Crown Research Institutes currently using the standard baiting SOP and these probabilities ranged between 0.22 and 0.56 when testing kilograms of soil from around kauri trees known to be infected with PTA (Beauchamp 2011). At best, PTA can only be detected in the soil directly below infected trees one out of every two times it is tested. As soil samples from the pigs were between 0.5 and 5 g, a decline in test sensitivity was expected, although not to the extent shown here. This may be explained by differences in the detection of spores in artificially spiked soil in the sensitivity testing in this study, compared with naturally infected soils used in other studies (Beever *et al*. 2010; Beauchamp 2011), or that some factor associated with the soil from pig tissue is interfering with the test (perhaps bacteria from the decomposing

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flesh). Obtaining a higher test sensitivity result would have substantially decreased our posterior probability results and thus increased the confidence of a true negative test.

Studies have shown unequivocally that PTA is present in the soil around kauri trees, and that this disease is killing kauri (Beever *et al.* 2009, 2010; Dick & Bellgard 2010; Bellgard *et al.* 2011). The association of pigs with soil is clear through their natural behaviour of rooting (below-ground foraging). Observational evidence has also recorded feral pigs rooting beneath infected kauri: Hill and Davis (2011) noted 98 occurrences of pig rooting below known PTA-infected trees. Through this study we have also discovered that soil naturally adheres to pig's trotters and snouts with approximately 0.5–5 g of soil collected from each sample and that pigs have the ability to transport soil-borne pathogens, including *Phytophthora*. We therefore conclude that, although this study failed to detect PTA, there is still a high probability that PTA is transported in soil associated with pig snouts and trotters, and that either the test methods used failed to detect it, or given the sporadic distribution of PTA in the environment, the pigs tested did not happen to pass through PTA-infected soil before culling. The destructive sampling method used in this study (the culling of the pig) provided a single sample of each individual; a capture–sample–recapture method may have increased the chances of PTA encounter. Other studies have also failed to detect PTA from likely sources including a survey of *Phytophthora* species within streams in catchments with infected kauri stands (Randall 2011) and also a survey of soil carried on human boots that had walked around infected trees (Pau'uvale 2011). No detection probabilities were carried out for these studies, although the results highlighted here could suggest that there is a chance that these studies also failed to detect the presence of PTA, and it cannot be ruled out that PTA may be carried in soil on human boots, pig trotters and in stream water. Due to the recent discovery of PTA, Beever *et al.* (2009) have provided the only published information on this pathogen, and little is known about PTA survival as a soil saprophyte and the optimum conditions for PTA growth in natural situations, which makes interpretation of negative results difficult. However, PTA has been detected throughout the northern North Island of New Zealand since the first record on the mainland (previously only found on Great Barrier Island) was noted in 2007 (Beever *et al.* 2009). If indeed pigs, humans and water are not the vectors of PTA, then what could be the mechanism for spread of this organism over large distances (further than neighbouring trees)? It is therefore important to develop improved methods for isolating PTA from vectors. Managing the spread of this disease is crucial to preserving the kauri forests of the North Island, and to do this the vectors of this disease must first be established. *Phytophthora* species are notoriously difficult to isolate from soil (Tsao 1990; Davidson & Tay 2005); however, Davidson and Tay (2005) isolated *P. cinnamomi* from 100% of their positive controls, in comparison with 0–3% in this study. It may be that soil artificially inoculated with PTA responds differently to the current baiting technique, that bacteria from the decomposing pig tissue have a negative effect on PTA zoospores or that there is a structural difference with PTA spores (as yet unknown) causing the difficulty with isolation. Therefore, we conclude that optimization of the baiting technique needs to be conducted specifically for isolation of PTA from animal-associated soil and serological or DNA test methods should also be pursued as an alternative to baiting when trying to isolate PTA from pig trotters. DNA test methods that allow immediate testing of the soil may also be more successful as they would bypass the baiting technique and any problems of competition with bacteria from the decomposing animal tissue.

Feral pigs may also enhance the susceptibility of kauri trees to the PTA disease indirectly through disturbance. Diseases are recognized to attack weakened systems; Schoeneweiss (1975) stated that any disturbance may predispose plants to disease. Therefore, regardless of whether pigs are vectors of PTA, they may potentially facilitate the establishment of this disease due to the disturbance they cause around kauri root zones. Kauri ill health is known to occur when their root zones are compromised even without PTA (Sando 1936). Feral pig disturbance around these root zones could also lead to the destruction of the pūkahukahu (mound of decaying litter surrounding the base of the tree) and destroy any beneficial mycorrhizal associations. Pig introduction of foreign bacteria and other plant pathogens could also compromise kauri health making PTA infection more likely.

We cannot rule out feral pigs as a likely vector of PTA. Although this study failed to isolate PTA from the soil associated with pigs, this may be due to the sporadic distribution of PTA in the forest and that none of the trotters sampled had come into contact with the disease, or due to the sensitivity of the test, we failed to detect PTA. Bayesian modelling showed a 35–90% chance that pigs may vector PTA. Future research should focus on developing an appropriate test to isolate PTA from small soil samples associated with animal tissue, with reliable test sensitivities, before any vectors of PTA are investigated in the future. This study has shown that feral pigs are capable of carrying up to 5 g of soil on each trotter, and are a vector of *P. cinnamomi* and other plant pathogens. Based on this circumstantial evidence

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and the Bayesian modelling results, we deduce that feral pigs have the ability to vector PTA. This study highlights the impact introduced vertebrate species may have on enhancing the vectoring of endemic and exotic soil-borne plant pathogens. In particular, very few studies have examined the role of introduced ungulates in soil-borne pathogen spread and we hope our conclusions will provide stimulus for further research on a range of vertebrate species.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Microbial species isolated from soil associated with feral pigs.

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