

Field assessment of dog as sentinel animal for plague in endemic foci of Madagascar

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Abstract

The epidemiology of *Yersinia pestis*, the causative agent of plague, involves vectors and reservoirs in its transmission cycle. The passive plague surveillance in Madagascar targets mainly rodent and fleas. However, carnivores are routinely surveyed as sentinels of local plague activity in some countries. The aim of this study is to assess the use of domestic dog (*Canis familiaris*) as sentinel animal for field surveillance of plague in a highly endemic area in Madagascar. Cross-sectional surveys of plague antibody prevalence in *C. familiaris* were conducted in endemic areas with contrasting histories of plague cases in humans, as well as a plague free area. Rodent capture was done in parallel to evaluate evidence for *Y. pestis* circulation in the primary reservoirs. In 2 sites, dogs were later re-sampled to examine evidence of seroconversion and antibody persistence. Biological samplings were performed between March 2008 and February 2009. Plague antibody detection was assessed using anti-F1 ELISA. Our study showed a significant difference in dog prevalence rates between plague-endemic and plague-free areas, with no seropositive dogs detected in the plague free area. No correlation was found between rodents and dog prevalence rates, with an absence of seropositive rodents in some area where plague circulation was indicated by seropositive dogs. This is consistent with high mortality rates in rodents following infection. Re-sampling dogs identified individuals seropositive on both occasions, indicating high rates of re-exposure and/or persistence of plague antibodies for at least 9 months. Seroconversion or seropositive juvenile dogs indicated recent local plague circulation. In Madagascar, dog surveillance for plague antibody could be useful to identify plague circulation in new areas or quiescent areas within endemic zones. Within active endemic areas, monitoring of dog populations for seroconversion (negative to positive) or seropositive juvenile dogs could be useful for identifying areas at greatest risk of human outbreaks.

Key words: *Canis familiaris*, Madagascar, rodent, serology, *Yersinia pestis*

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INTRODUCTION

Plague, caused by *Yersinia pestis*, is a flea-borne zoonotic disease. It induces severe disease in rodent hosts which is characterized by epizootic periods that cause widespread rodents die-offs followed by quiescent period with little or no evidence of disease in rodent (Gage *et al.* 1995). Although most commonly associated with rodents, nearly all mammals can become infected with *Y. pestis* (Pollitzer 1954; Gage & Kosoy 2005). In 1898, rat-infested steamships from India brought plague to the seaport of Toamasina in Madagascar (Brygoo 1966). In the 1920s, plague reached the central highlands where it became endemic at altitudes above 800 m where the black rat (*Rattus rattus*), the most abundant small mammal, is the main plague reservoir. In the highlands, high human plague season extends from October to April (hot and rainy) when rat population are low due to low reproduction and plague epizootics while low human plague season occurs from May to September (cold and dry season) when rat reproduction is high. Maximum abundance of rodents in the field is observed in July and August, followed by the maximum abundance of fleas from September to November. Conversely in the west coastal plague focus of Mahajanga, which experienced 4 successive plague outbreaks from 1995 to 1998, outbreaks of human plague occurred during the dry and cold season (Andrianaivoarimanana *et al.* 2013).

In Madagascar, plague surveillance (in humans and rodents) is a key priority of the Plague National Control Program (PNCP) established since 1993 (Chanteau *et al.* 1998). Among the objectives of the PNCP are the determination of plague activity in rodent populations in endemic areas and implementation of control measures to reduce human plague (Chanteau 2006). Rodent and human serology has become an important component in plague surveillance but is not necessarily representative of *Y. pestis* transmission since it is performed on surviving individuals. After an epizootic event, rodent populations will only be composed of resistant rodents and newly born susceptible rodents, while plague is a fatal disease in humans without prompt and appropriate treatment (Andrianaivoarimanana *et al.* 2019). Therefore, neither rodent serology nor human serology can represent the true extent of plague transmission. In such cases; identifying a suitable alternative surveillance approach is necessary. Animal sentinels may be used to detect pathogens or disease outbreaks in a new area, monitor changes in prevalence or incidence, or track expansion of a pathogen over time and space. The ideal sentinel would be susceptible to but also survive infection, and would develop

a detectable and measurable response, whether clinical or immunological (Schmidt 2009). Dogs have been proposed as excellent sentinels for certain infectious-disease pathogens in Canada and are recommended for California serogroup viruses, other viruses, bacteria, and parasitic diseases surveillance (Bowser & Anderson 2018).

Most studies of carnivores as potential sentinel animal for the detection of plague are done opportunistically (Salkeld & Stapp 2006). Carnivores are able to acquire *Y. pestis* infection by multiple routes of infection. They may become infected by bites from infected fleas or by ingesting infected prey (Barnes 1982; Thomas *et al.* 1989), but tend to develop asymptomatic or low severity disease. Dogs seem to have low susceptibility to plague and develop antibodies against *Y. pestis*, which may persist for several months (Rust *et al.* 1971). Thus, the serologic study of dogs can give an indication of plague circulation in the surveyed area. In Madagascar, people in remote areas usually have pet dogs but the animals are free-roaming and mainly follow the owner in the field. In these ways, dogs may be exposed to infection by exploring the surroundings, by feeding on garbage which may contain infected rodent carcasses and ectoparasites, and hunting domestic, peri-domestic, and/or wild small mammals. In this study, we assessed the use of domestic dogs (*Canis familiaris*) in plague surveillance in the context of Malagasy foci. We conducted a cross-sectional survey to assess the *Y. pestis* seroprevalence among *C. familiaris* and a serology follow-up (resampling) to assess the seroconversion among surveyed *C. familiaris*. We compared plague circulation among rodents and dogs populations.

MATERIALS AND METHODS

Study design and setting

This study was conducted from March 2008 to February 2009. A cross-sectional survey of seropositivity of antibody against plague in dogs was conducted in 4 sites with a follow-up serology survey of dogs in 2 of these sites. For the sites with serology follow-up, initial sampling (session-1) occurred in April to May 2008 during the quiescent period of plague transmission to humans, with the follow-up sampling (session-2), conducted in September 2008 to March 2009, during the high human plague transmission period. Our surveys included 3 sites in plague endemic areas which differed in their plague endemicity levels. Site-1 (Fokontany Ambohitsararay, Rural Commune of Anjoma Betoho, District of Manjakandriana, Lat 18.82°S and Long 47.7°E) is a re-emerging focus with no plague activity in the 20 years

preceding sampling, but which declared new human cases in February 2008. Site-2 (Fokontany and Rural Commune of Inanantonana and surrounding area, District of Betafo, Lat 19.64°S and Long 46.62°E) is an active plague focus in the central highlands and had reported human cases repeatedly in the years preceding this study. Two areas were surveyed in Site 2, the village of Inanantonana (Site 2a) and the surrounding areas (Site 2b). Serology follow-up surveys were carried out for dogs sampled in Sites 1 and 2a to evaluate the persistence of *Y. pestis* antibodies in free-roaming dogs. Site-3 (Fokontany Abattoir/Marovato, Urban Commune of Mahajanga, District of Mahajanga I, Lat 15.721°S and Long 46.325°E) is a coastal plague focus which has not reported a confirmed human case since 2000. A single cross-sectional survey was conducted at this site to evaluate whether plague is circulating in this area. Site-4 (Rural Communes of Zazafotsy Lat 22.20°S and Long 46.36°E and Sahambano Lat 28.17°S and Long 48.89°E, District of Ihosy) is a plague-free area located outside the limit of plague foci and included as the negative control site (Fig. 1).

In Madagascar, the administrative breakdown is divided into 3 sub-units starting with the Fokontany (basic administrative sub-units), Commune, and District.

Animal sampling

Blood samples were collected from the saphenous vein on the hind leg of each dog with verbal consent and assistance from the owner. In the serology follow-up site, the same dogs were re-sampled during session-2. In parallel, rodents were captured according to our standard protocol (Rahelinirina *et al.* 2010). All captured rodents were euthanized and morphologically identified to the species level. Rodent handling was done in accordance with the directive 2010/63/EU of the European Parliament and of the Council (Official Journal of the European Union 2010) and the American Society of Mammalogists for the use of wild mammals guidelines (Sikes *et al.* 2016). Blood samples were collected either on sterile Eppendorf tube or on dried blood spot filters, and rodent spleen samples were stored in Cary–Blair transport medium for *Y. pestis* isolation.

Laboratory analysis

Y. pestis expresses a specific capsule-like surface antigen, the fraction 1 protein or F1 antigen which is highly immunogenic. Anti-F1 Ig G antibodies have been used for serological diagnosis of plague infection in animals (Rajerison *et al.* 2009; Tollenaere *et al.* 2010;

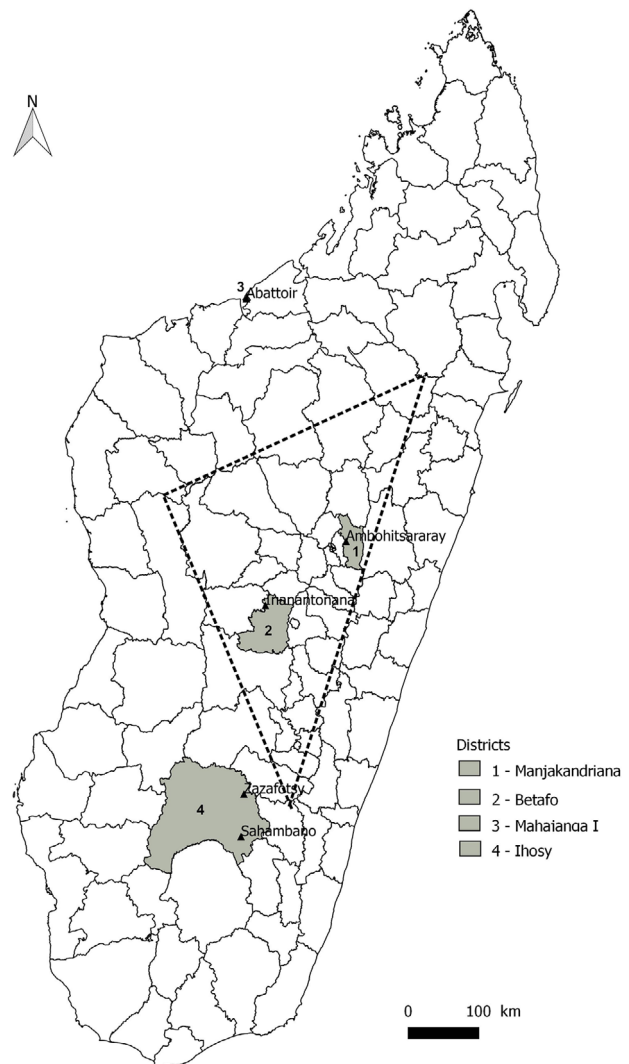


Figure 1 Location of the districts (grey color) including the study sites in Madagascar. Dashed line: limits of the main plague-endemic area in the central highlands of Madagascar (altitude > 800 m).

Andrianaivoarimanana *et al.* 2012). An enzyme linked immunosorbent assay (ELISA) for anti-F1 IgG detection was performed on dog sera as previously described (Andrianaivoarimanana *et al.* 2012) with modifications. Briefly, anti-F1 IgG detection was assessed on a plate previously coated with F1 antigen diluted in carbonate buffer and in parallel with a plate coated with carbonate buffer alone (for background identification). Dog sera were diluted 1/100, 1 negative, 2 positive control sera (high and low titers), and 2 well control (without sera) were included in each series of experiments. An anti-dog IgG

Table 1 Seroprevalence of anti-F1 antibodies in domestic dogs and captured rodents per study site

Serology	Dogs		Rodents	
	Prevalence rate % [95% CI]		Prevalence rate % [95% CI]	
Sites/Periods	Session 1	Session 2	Session 1	Session 2
Site 1 (reemerging focus-center)	8 50 [22–78]	9 56 [27–81]	35 11 [4–26]	54 19 [10–31]
Site 2 (active focus-center)-a	22 55 [34–73]	31 48 [32–65]	42 [†] 26 [15–41]	57 28 [18–41]
Site2-b	20 95 [76–99]	n/a	175 0 [0–2]	n/a
Site 3 (inactive-coastal)		17 6 [1–27]		51 0 [0–7]
Site 4 (plague free)	25 0	n/a	0/87 0	n/a

[†] *Y. pestis* was isolated on rodent; n/a: not applicable.

peroxidase conjugate (Byosis, 1:4000) was used for the revelation step. The mean optical density (OD) obtained against the coating buffer alone was subtracted from the OD against F1 antigen (delta OD). The threshold of positivity was set at 0.450 and samples were considered positive when the mean OD was above the defined threshold.

Detection of anti-F1 IgG antibodies in rodent was conducted using modified (Dromigny *et al.* 1998) and previously described protocols (Andrianaivoarimanana *et al.* 2012). Rodent's spleen samples were tested using the rapid diagnostic test (RDT) for F1 antigen detection based on lateral flow immunochromatography (Chanteau *et al.* 2003) and those yielded positive result for RDT were subsequently assessed on bacteriology for *Y. pestis* isolation (Rasoamanana *et al.* 1996).

Statistical analysis

Prevalence rate, defined as the number of animals positive for anti-F1 IgG antibodies divided by the total number tested, was determined for dog and rodent from each site. Other indicators were studied, such as *Y. pestis* infection rate which is the ratio of captured rodents in which *Y. pestis* was isolated among the total tested. Correlation between rodent serology and dog serology was evaluated by Spearman's rank correlation. For sites 1 and 2, changes in the anti-F1 seropositive rate for dogs between the 2 sampling periods were evaluated using chi-square test. Significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

A total of 107 dogs with a median age of 3 years (range: 1 month–15 years) and 414 rodents were sampled in the plague endemic study sites during the study period. Twenty-five (25) dogs with median age 0.67 years (2 months–10 years) and 87 rodents were sampled in the control site (Table 1). As expected, no seropositive dog was observed in the plague-free control site (Table 1). Within the plague endemic area, prevalence rates ranged from 6% (95% CI = 10–27) in the coastal focus, to 48% (95% CI = 32–65) to 95% (95% CI = 76–99) in the central highland sites (Table 1). The difference in dog prevalence rate between endemic areas and the plague-free area was strongly significant (chi-square test, $\chi^2 = 14.01$, $P < 0.001$). As expected no seropositive rodents were detected in the plague-free control site. There were also no seropositive rodents observed from the inactive coastal focus site (Table 1). Rodent prevalence rate ranged from 0% (95% CI = 0–2) to 28% (95% CI = 18–41) for the sampling events in the central highlands.

No correlation was found in plague prevalence rate between rodents and dogs (Spearman's rank correlation $r = 0.07$, $P = 0.84$, Fig. 2). Our results highlight the value of using dogs as sentinels for detecting plague circulation in an area, compared to surveillance of the reservoir rodent population directly. As infected rodents usually die of infection during epizootics, surveillance of rodents can yield low prevalence rates, even in areas of recent

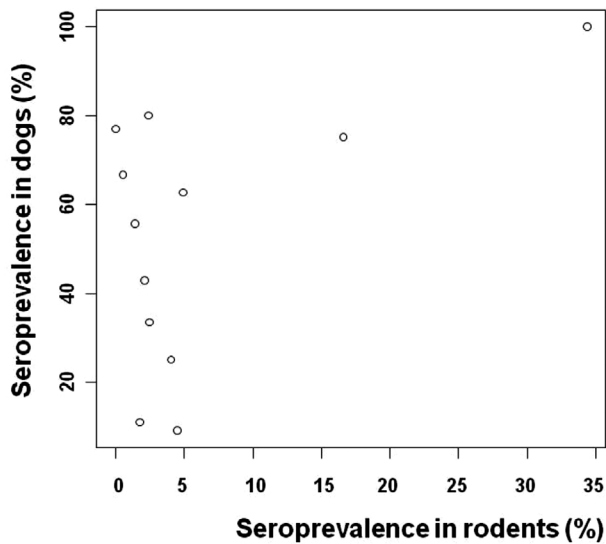


Figure 2 Comparison between rodent and dog prevalence rates

active plague circulation (e.g. Site 2b), because sampled rodents will mostly be rats that escaped infection or newly born individuals. In contrast, as dogs typically survive after plague infection and develop antibodies, prevalence rates tend to be higher than for rodents, and seropositive dogs can therefore provide evidence of plague circulation, even when rodent sampling yields no seropositive individuals. This is highlighted by our data from the inactive coastal focus of Mahajanga (Site 3), where no seropositive rodents were detected but a seropositive dog, aged 4 months old, indicates a recent circulation of *Y. pestis*. Indeed, since sampling for this study was conducted, further sampling in this focus has isolated *Y. pestis* from a rodent (Rahelinirina *et al.* 2017), confirming that *Y. pestis* continues to circulate despite a lack of confirmed human plague cases since 2000.

For the serology follow-up, among 14 seropositive dogs on session-1 sampling, 5 became seronegative on session-2, and 4 out of the 10 seronegative on session-1 became seropositive on session-2 (Table 2). The seroconverted negative to positive dogs are likely to indicate recent local transmission of plague in the rodent population during the time between the 2 sampling sessions. For Site 1, an increase in the prevalence rate of *Y. pestis* antibodies against plague among captured rodent was identified between session-1 and session-2 although not significant (Table 1). For Site 2, *Y. pestis* was isolated from captured rodents (one *Y. pestis* strain isolated from spleen culture among 2 RDT positive rodents). In this case, surveillance of dogs for seroconversion negative–

Table 2 Serological status follow-up of paired blood sampled dogs from site 1 and 2

Session 1	Session 2		Total
	Pos	Neg	
Pos	9	5	14
Neg	4	6	10
Total	13	11	24

positive would be a valuable predictive marker to estimate the risk of plague in humans.

The finding that some dogs are seropositive in both sampling sessions is consistent with previous studies of other carnivores (Hopkins & Gresbrink 1982; Brinkerhoff *et al.* 2009). For seroconverted positive to negative dogs, 3 dogs from Site 1 were highly seropositive during the first sampling and became negative 9 months later. The naturally infected dogs in our study may be exhibiting persistence of anti-F1 IgG, on a time-scale consistent with previous studies of experimentally infected dogs, where antibodies persisted for at least 300 days after infection (Rust *et al.* 1971). Indeed, Site 1 is an isolated focus with human plague case observed on February 2008 after 20 years of silence (Rajerison 2008, unpublished data) suggesting that dogs' seroconversion from positive to negative status might be explained by the absence of antibody boosting production due to a low exposure of animals to plague.

Alternatively, as some dogs in our study go from seropositive to seronegative between the 2 sampling occasions, some of the dogs with persistent antibodies may have been re-exposed to plague infection.

CONCLUSION

Dogs are useful as sentinel animals for plague surveillance as they typically survive *Y. pestis* infection, produce detectable levels of anti-F1 antibodies, and are longer lived than rodents. In contrast, as rodents often die following infection, surveillance based on rodents may yield “false negatives,” where plague circulation goes undetected. Moreover, as effective surveillance could be achieved sampling fewer individuals, dog blood-sampling could be more cost-effective.

In Madagascar, dog surveillance for anti-F1 antibodies could be useful in 2 ways. First, dogs could be used as sentinels in plague-free areas thought to be at risk from plague emergence or areas that are quiescent in terms

of human plague occurrence. In such areas, plague may be present but at relatively low levels of transmission in the reservoir community, so that dog surveillance may be useful for picking up increased plague circulation before human cases occur. Secondly, to detect increased plague activity within active plague endemic areas, surveillance during the quiescent period of human plague could target juvenile dogs (<4 months or those born during the period between human plague seasons). Seropositivity among this population could indicate plague circulation among the rodent population a few months before sampling, and highlight areas at increased risk. Such surveillance could be focused on areas which experienced relatively low numbers of plague cases in the preceding plague season, but were close to areas with outbreaks of human plague and could therefore be at higher risk in the following season. This would provide an early warning of risk and allow in-time implementation of appropriate control measures.

Further study using antibody titration in combination with ELISA anti-F1 IgG detection as well as an evaluation of maternal antibody persistence would help us to better understand persistence of antibodies in dogs and further optimize the use of older and younger dogs as sentinel for plague.

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