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Short Communication

Seroprevalence of *Yersinia pestis* in dogs and small rodents in one hyperendemic plague focus of Democratic Republic of Congo

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Plague is endemic in Ituri of the Democratic Republic of Congo. In order to evaluate the role of commensal rodents and domestic dogs in the spread of the disease, we studied the seroprevalence at Rethy. Results showed that the 27/28 small rodents were seronegative (using immunoglobulin G anti-F1 enzyme-linked immunosorbent assay), but one of them, Nile rat was positive. These rodents serve as intermediaries, spreading the disease to domiciliary black rats. Of the 11 dogs tested by ELISA, 4 were seropositive (36%). Our results confirm that the serological prevalence in dogs is a reliable marker for the surveillance of plague.

Key words: Plague, *Yersinia pestis*, dog, Democratic Republic of Congo, seroprevalence.

INTRODUCTION

Plague is a zoonotic disease, primarily of rodents, with strong natural localisation. The first cases of plague were reported in 1928 in Ituri (Province orientale) of the Democratic Republic of Congo (DRC). The Ituri region is located north-east of the Ituri River and on the western side of Lake Albert. It has borders with Uganda and South Sudan. The region is noted for increased outbreaks of plague since 2004 (Bertherat et al., 2011). Thus, in the Rethy area (150000 habitants), 1624 cases (with 39 deaths) were registered between 2004 and 2009; and in 2010, 35 cases with 4 deaths (Ministry of Health of DRC) were registered. In order to evaluate better the role of commensal rodents and domestic dogs in the spread of the disease, we studied the seroprevalence of plague (in April 2010) at Rethy near Lake Albert (02°05'N-30°53'E, 2107 m altitude). Rethy is located 130 km north-east of Bunia (capital of Ituri). The dominant climate is influenced by a cooler mountain climate. The annual precipitation is 1,300 mm. The mean annual temperature is estimated at 23.9°C.

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Table 1. Serology results of the plague (ELISA) of dogs from Rethy (Ituri, Democratic Republic of Congo).

Dog	Gender	Age (Year)	Ectoparasites	Localisation	ELISA Plague
1	M	0,5	Fleas and ticks	Kanana	N
2	M	3	Ticks	Baidjo	P
3	F	10	Fleas and ticks	Kpamdroma	N
4	M	3	Ticks	Mission	N
5	F	2	Fleas and ticks	Mission	N
6	F	2	0	Baidjo	P
7	F	7	0	Baidjo	P
8	F	2	0	Baidjo	N
9	F	1	0	Baidjo	P
10	M	1	0	Lokana	N
11	M	1	0	Lokana	N

MATERIALS AND METHODS

Animal samples

Twenty eight small commensal rodents were captured in traps, identified (morphological and molecular method), anaesthetised with ketamine and killed by cardiac exsanguination. They comprised 19 black rats (*Rattus rattus*), 5 western multimammate mice (*Mastomys erythroleucus*), 2 Nile rats (*Arvicanthis niloticus*) and 2 shrews (*Crocidura* sp.). Blood samples were taken and put on Whatman filter papers No 4 (Seropad).

There were 11 hunting dogs (weighing around 15 kilos) belonging to 7 residents of Rethy. Average age of the dogs (6 females and 5 males) was 3 years (6 months to 10 years). Blood was taken from 4 dogs owned by one resident and from 2 dogs owned by another, and their sera were frozen.

Serological examination

Rat serology was carried out using immunoglobulin G anti-F1 enzyme-linked immunosorbent assay (ELISA) with minor modification of conjugate (IgG anti-rat peroxydase or anti-mouse peroxydase) (Rasoamanana et al., 1997). In brief, seropad was soaked overnight in 400 µl of PBS-0.05% Tween- 5% skim milk at 4°C prior antibody detection. A 96-well ELISA plates was first coated with purified F1 antigen; a capsular antigen secreted by *Yersinia pestis*. In parallel, an F1 uncoated plate was also tested for troubleshooting purposes. Each sample was tested in duplicate, the average optical density against F1 antigen was subtracted from the optical density obtained against the coating buffer. The threshold of positivity was set at 0.05 (Dromigny et al., 1998). Dog serology survey was conducted by ELISA (Rajerison et al., 2009). For each positive sample by ELISA, confirmation was carried out by competitive blocking with specific *Y. pestis* antigen as described by Chu (2000). Prior to antibody detection by ELISA, F1 antigen was added to each sample, followed by incubation at 37°C for 1 h wherein IgG if present in the serum gets captured by the F1 added. Only unblocked IgG will be detected by ELISA. A negative serum (non endemic area) and a positive serum (*Y. pestis* experimental infected rat) were included in each series of experiments.

RESULTS AND DISCUSSION

Results showed that 27 rats were seronegative, but one Nile rat was positive (3.6%). This was a female caught away from dwellings. Of the 11 dogs tested, 4 were seropositive (36%), and 3 of these 4 belonged to one individuals in the village of Baidjo, 2 km away from Rethy (Table 1). It is precisely in this zone that there had been regular occurrences of bubonic plague and of pulmonary plague in 2006. The 4 positive dogs (3 females and 1 male) had a mean age of 3 years, the youngest being 9 months old.

In DRC, plague is transmitted by fleas (*Xenopsylla cheopis* and other species) to black rats which may then suffer epizootic plague and die in vast numbers. Thus black rats are a potential threat wherever commensal rats come into contact with either the enzootic rodents or the epizootic plague-susceptible rodents living inside or outside habitations. The transfer of *Y. pestis* from native rodents to commensal rats by exchange of fleas occurs rather easily. The Nile rat is already a well known wild reservoir of plague. It frequents damp areas close to agricultural sites, and it can resist the disease. Females live for about two years. These rodents serve as intermediaries, spreading the disease to domiciliary black rats. Once commensal rodents become infected, the risk of human involvement is greatly increased. The finding of a reservoir of the disease in the wild can explain repeated occurrences in the Rethy region.

Dogs and cats are frequently exposed to *Y. pestis* in areas where the plague is enzootic. They can transmit it among themselves by feeding on infected rats (when the rats are ill the dogs can easily catch them) or by means of flea bites; and no attempt has been made to prevent infection by fleas. It has already been observed that the handling of infected cats which have been eating infected rodents can be the source of human cases (Doll et al., 1994). Whilst overt clinical illness in dogs and cats is rare, it can manifest itself by non specific fever and lethargy (Orloski and Eidson, 1995). Studies of seroprevalence in dogs have been carried out in Tanzania where in affected areas the seroprevalence was 5.5% (11/201) (Kilonzo et al., 2006). In a district where human involvement was not observed, it was 2% (1/43) (Kilonzo et al., 1993). In Madagascar, dogs are involved in the circulation of *Y. pestis*.

Since they do not die, they are probably less susceptible to the infection but retain the immunological content. The prevalence is estimated at 23.8% in areas of endemic plague (Rajerison et al., 2009). In Brazil, in 1988, a seroprevalence of 21.6% (53/245) was found in a focus of plague at Planalto Borborema in the state of Paraíba (Almeida et al., 2007). Finally, in China, in active areas, seroprevalence was 23.5% (162/689) (Li et al., 2008); and the most recent outbreak of human plague occurred in 2009 with 12 human cases (three of them died) (Wang et al., 2011). Seven strains of *Y. pestis* were isolated from dogs and patients, and field investigation and analysis of the isolated strains, revealed that this outbreak was started by a deceased dog.

Our results confirmed that the serological prevalence in dogs evaluated by ELISA with F1 antigen is a cheap and reliable marker for the surveillance of plague. Persistence of antibodies against *Y. pestis* has already been evaluated in dogs aged 4 to 8 months (Rust et al., 1971). The presence of seropositivity in dogs indicates recent local activity and serological studies in dogs are easier than catching rodents. Thus, they could often be used especially in villages where human plague has not yet occurred. The analysis of canine seroprevalence by age groups would determine the beginning and end of the period of transmission.

Conclusion

It is well established that dog is a good indicator of plague, but additional studies are needed on the nature of the infection (resistance, illness and bacteraemia) and on the epidemiological role of the dog in outbreaks of plague in man.

REFERENCES

Almeida AMP, Brasil DP, Melo MEB, Leal NC, Almeida CR (2007). Importância dos carnívoros domésticos (cães e gatos) na epidemiologia da peste nos focos do Nordeste do Brasil. Cadernos de Saúde Pública, 1:49-55.

Bertherat E, Thullier P, Shako JC, England K, Koné ML, Arntzen L (2011). Lessons learned about pneumonic plague diagnosis from 2 outbreaks, Democratic Republic of the Congo. Emerg. Infect. Dis. 17(5):778-784.

Chu MC (2000). Laboratory Manual of Plague Diagnostic Tests. US Dep't Health Human Services, Centers for Disease Control and Prevention, Atlanta, Georgia, USA and World Health Organization, Geneva, Switzerland, pp. 61-63.

Doll JM, Zeitz PS, Ettestad P, Bucholtz AL, Davis T, Gage KL (1994). Cat-transmitted fatal pneumonic plague in a person who traveled from Colorado to Arizona. Am. J. Trop. Med. Hyg. 51: 109-114.

Dromigny JA, Ralafiarisoa L, Raharimanana C, Randriananja N, Chanteau S (1998). La sérologie anti-F1 chez la souris OF1, test complémentaire pour le diagnostic de la peste humaine. Arch. Inst. Pasteur Madagascar. 64(1&2):18-20.

Kilonzo BS, Gisakanyi ND, Sabani CA (1993). Involvement of dogs in plague epidemiology in Tanzania. Serological observations in domestic animals in Lushoto District. Scand. J. Infect. Dis. 25(4): 503-6.

Kilonzo BS, Mbise TJ, Mwalimu DC, Kindamba L (2006). Observations on the endemicity of plague in Karatu and Ngorongoro, Northern Tanzania. Tanzan. Health Res. Bull. 8(1):1-6.

Li B, Guo Y, Guo Z, Liang Y, Zhu Z, Zhou Q (2008). Serologic survey of the sentinel animals for plague surveillance and screening for complementary diagnostic markers to F1 antigen by protein microarray. Am. J. Trop. Med. Hyg. 79(5):799-802.

Orloski KA, Eidson M (1995). *Yersinia pestis* infection in three dogs. J. Am. Vet. Med. Assoc. 207(3):316-8.

Rajerison M, Dartevelle S, Ralafiarisoa LA, Bitam I, Tuyet DT, Andrianaivoarimanana V (2009). Development and evaluation of two simple, rapid immunochromatographic tests for the detection of *Yersinia pestis* antibodies in humans and reservoirs. PLoS Neglected Trop. Dis. 3(4):e421.

Rasoamanana B, Leroy F, Boisier B, Rasolomaharo M, Buchy P, Carniel E, Chanteau S (1997). Clinical Field evaluation of an Immunoglobulin G anti-F1 Enzyme-Linked Immunosorbent Assay for serodiagnosis of human plague in Madagascar. Clin. Diag. Lab. Immunol. 4: 587–591.

Rust JH, Miller BE, Bahmanyar M, Marshall JD, Purnaveja S, Cavanaugh DC, Tin Hla US (1971). The role of domestic animals in the epidemiology of plague. II. Antibody to *Yersinia pestis* in sera of dogs and cats. J. Infect. Dis. 124(5):527-31.

Wang H, Cui Y, Wang Z, Wang X, Guo Z, Yan Y (2011). A dog-associated primary pneumonic plague in Qinghai Province, China. Clin. Infect. Dis. 52(2):185-90.