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Rabies serosurvey of domestic dogs in Kigali City, Rwanda

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In Rwanda, rabies is a threat to public health and the control is mainly done by vaccinating pet dogs annually. However, it is unknown whether dogs that received rabies vaccination achieved protective antibody levels. This study assessed factors influencing rabies antibody titres in vaccinated and non-vaccinated pet dogs in Kigali City, Rwanda. A questionnaire was used to gather information on 137 study dogs and clotted blood samples were collected from 93 healthy pet dogs. Enzyme-Linked Immunosorbent Assay (ELISA) was used to quantify rabies antibody titres. Frequency and geometric mean titres, as well as chi-square and regression analysis, were used to study the data. The results showed that 35% of the vaccinated dogs had antibody titres below 0.5 EU/ml while 53.8% of the non-vaccinated dogs had detectable antibodies varying between 0.133 and 0.238 EU/ml. All types of rabies vaccine (A, B, C, D, and E) used elicited diverse antibody levels and the overall mean titre was 1.071. Vaccinated dogs had a higher mean number of rabies antibodies (11.776059735) than non-vaccinated dogs (1.41579378). Mean titres decreased with time between vaccination and sampling, that is, 1.559, 0.949, and 0.934 in dogs sampled 1-5, 6-9 and 10-12 months following vaccination, respectively. The mean titres increased steadily from the first to the fourth vaccination times, namely 0.608, 1.320, 1,395, and 1.787, respectively. Mean titres increased with dogs' age and varied between 0.638 and 1.515. Factors including vaccination status, number of vaccination, time elapsed between vaccination and sampling, and age at vaccination influenced rabies antibody titres. Irrespective of the type of rabies vaccine applied, 99% of vaccinated dogs produced rabies antibodies though not all had protective levels. Considering the high number of vaccinated dogs that were poor responders to rabies vaccination, further studies should be undertaken to investigate and understand the phenomenon.

Key words: Rabies, vaccination, domestic dogs, seroconversion, Kigali city, Rwanda.

INTRODUCTION

According to World Health Organization (WHO, 2013), rabies is a fatal zoonotic disease that can affect all mammals. It is mainly transmitted through the bite and virus-containing saliva of an infected host. Other

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transmission routes are contamination of mucous membranes, aerosol transmission, and corneal and organ transplantations (Leung et al., 2007). Human rabies is mainly transmitted by dog-bites (WHO, 2012). According to Rwanda Biomedical Centre, on average, 54 cases of human dog-bites are reported across Rwanda (Rwanda Focus, 2016). Rabies is caused by Rabies virus (RABV), which belongs to the Lyssavirus genus of the Rhabdoviridae family (World Organisation for Animal Health [OIE], 2011b). According to Malerczyk et al. (2014), 15 Lyssavirus species are known to exist. There is no recognised treatment for rabies, and once it manifests (Rupprecht et al., 2017), it leads to a deadly acute encephalitis or meningoencephalitis (Crowcroft and Thampi, 2015). Vaccination of dogs can help eliminate terrestrial rabies (Crowcroft and Thampi, 2015). To accomplish immunity in dogs, at least 70% of the population should receive rabies vaccination (WHO, 2013). The failure of rabies vaccination in animals might be higher, but it was rated at 0.025% (Oboegbulem et al., 1987; Tepsumethanon et al., 2016). A case-control study carried out in Finland on dogs received rabies vaccination over five years, revealed that 10.7% of the dogs had antibody levels lower than 0.5 IU/ml (Nokireki et al., 2017). According to OIE (2013), the cut-off point for antibody response to both canine and feline rabies vaccination is 0.5 IU/ml.

A study by Berndtsson et al. (2011) indicated that factors such as dog's breed and breed size, age at vaccination, brand of utilised vaccine and rabies vaccination number can influence outcome of canine rabies vaccination. Other factors such as nutrition status, sex and proper vaccine preservation (Kennedy et al., 2007; Jibat et al., 2015) as well as animal health status, amounts of antigens and way of application can also impact antibody response (Moore and Hanlon, 2010). Indirect enzyme-linked immunosorbent assay (ELISA) using rabies glycoprotein can be used to confirm whether dogs and cats which received rabies vaccination have seroconverted (Quinn et al., 2011). ELISA can replace neutralization tests for demonstrating antibodies (Moore and Hanlon, 2010).

The control of rabies disease in Rwanda involves vaccinating owned dogs annually and culling stray dogs (OIE, 2011a). In 2010, the number of both canine and feline population in Rwanda was approximated to 31,448 including 8,650 dogs vaccinated against rabies (Southern and Eastern African Rabies Group, 2011). The vaccination coverage for canine and feline rabies vaccination was at 27.5%. The report by Rwanda Agriculture Board revealed that dog population in Rwanda in 2016 was estimated to be 18, 117 and that only 11,375 were vaccinated against rabies and 2,870 culled. The rate of rabies vaccination coverage was at 62.7% (The New Times, 2017). There is no published data on dogs' response to rabies vaccines in field condition in Rwanda. Thus, this study assessed factors influencing rabies antibody titres in vaccinated and non-vaccinated pet dogs in Kigali City, Rwanda. The hypothesis assumed that dogs vaccinated against rabies in Kigali city, Rwanda seroconverted to protective antibody levels regardless of type of applied vaccine.

MATERIALS AND METHODS

Ethics standards

The University of Nairobi, Faculty of Veterinary Medicine's Biosafety, Animal Use, and Ethics Committee (FVM BAUEC/2017/126) and the Rwanda National Ethics Committee (Review Approval Notice: No. 115/RNEC/2017) approved this study. Before collecting samples from dogs, owners signed certificates of consent.

Study area

The present study was conducted in the city of Kigali, in Rwanda between September 2016 and March 2017. Administratively, Kigali city is divided into 3 districts and 35 sectors which are also divided into cells and villages (National Institute of Statistics of Rwanda, 2014). During data collection, the records of rabies vaccination of dogs at the district level were used. The map illustrating the study sectors is indicated in Figure 1.

Figure 1 illustrates 9 study sectors, namely Niboye, Gatenga, Kicukiro, Gisozi, Kacyiru, Kimironko, Kigali, Mageragere, and Nyamirambo. Blue points indicate households where dogs were identified. Three administrative sectors were chosen per district based on distribution of dogs and vaccination history.

Study design

This cross-sectional study involved collecting dogs' individual information as well as clotted blood from vaccinated and non-vaccinated pet dogs across Kigali city. Cluster sampling was used to select study sectors while snowballing sampling was used to identify households owning dogs. Computation of study dogs was done in accordance with a formula by Chomel et al. (1987).

Collection of individual dog data

Administrative local leaders helped the investigator in identifying and reaching households owning dogs and a total of 137 households owning at least a dog were identified, that is, a dog per household was recruited in the study.

A questionnaire was used to collect information on 137 dogs including each dog's rabies vaccination history (age, sex, date of vaccination, number of vaccinations and utilised vaccine type). Some dogs were aggressive while others died, thus, only 93 dogs including 80 received rabies vaccination and 13 non-vaccinated were sampled.

Collection of samples

Clotted blood samples were collected from restrained dogs. Figure 2 shows how dogs were restrained.

Clotted blood samples were collected in plain vacutainer collection tubes. The samples were preserved in a cooler box without ice packs. Laboratory analysis was held at Rwanda

Figure 1. Map of the study area.
Source: Generated from GPS data by authors.

Figure 2. Restraining dogs during collection of blood samples.

Table 1. Summary of general information on study dogs.

Dog's information	Percentage
Sex	
Male	75.3
Female	24.7
Rabies vaccination status	
Vaccinated	86
Non-vaccinated	14
Ages of the vaccinated dogs	
Younger than 1 year old	10
1-2.5 years old	32
>2.5-4 years old	29
At least 5 years old	29
Used types of rabies vaccines	
A	60
B	11
C	4
D	14
E	11
Number of vaccination	
Once	31
Twice	28
Three times	11
Four times	12
At least five times	18
Time elapsed between vaccination and sampling	
10-12 months	59
6-9 months	15
1-5 months	26

National Veterinary Laboratory in Kigali. In the laboratory, the samples were centrifuged at 1,500 × g for 10 min at room temperature and the obtained sera kept frozen at -80°C until used.

Detection of rabies antibodies

Quantification of rabies antibody titres in dog sera involved a commercial indirect ELISA test. The assay was performed in accordance with the manufacturer's protocol (Platelia™ Rabies II; Bio-Rad, France). Optical densities in the microplate were read with a spectrophotometer at 450 nm, copied and transferred to the Bio-Rad conversion tool to obtain serum titres. A unit equivalent to the international units defined by seroneutralization (Equivalent Units per milliltre: EU/ml) was used to express the sera titres. The cut-off point for antibody was 0.5 EU/ml (OIE, 2013). The titres were interpreted based on four seroconversion levels which included undetectable (<0.125 EU/ml), insufficient (<0.5 EU/ml), sufficient (0.5-4 EU/ml) and high level of sero-conversion (>4 EU/ml).

Data analysis

Statistical Package for Social Sciences (SPSS) Statistics version 20 was used to analyse computerised data. Both descriptive (frequency and geometric mean titre) and inferential (Chi-square tests and regression analysis) statistics were used to interpret impact of various factors (type of utilised rabies vaccine, number of rabies vaccinations, time elapsed between vaccination and sampling, dogs' age) on antibody titres in the studied dogs. The level of significance was set to 5%.

RESULTS

General information on study dogs is summarised in Table 1.

Table 1 summarises study dogs information regarding sex, rabies vaccination status, ages of the vaccinated dogs, number of vaccination and time elapsed between vaccination and sampling. The dogs were vaccinated by both private and public veterinarians using five types of rabies vaccines. Vaccines were coded as A: Rabies Veterinary Vaccine Inactivated B.P. (Vet.) by Indian immunological limited, India; B (Vaxipet R: inactivated vaccine, Laprovet, France); C: (Vaxipet DHPPi+LR:

Table 2. Levels of sero-conversion in dogs that received rabies vaccination.

Sera titre	Interpretation	Frequency	Percentage
> 4 EU/ml	Highly seroconverted	18	22.5
0.5 - 4 EU/ml	Sufficiently seroconverted	34	42.5
0.125 - 0.5 EU/ml	Insufficiently seroconverted	27	34.0
< 0.125 EU/ml	Did not seroconvert	1	1.0
Total	-	N= 80	100

Table 3. Percentage of protection for vaccines' type and vaccination frequency.

Vaccination frequency	Rabies vaccine type and protection status				
	A (n=48)	B (n=9)	D (n=11)	C (n=3)	E (n=9)
	Protected	Protected	Protected	Protected	Protected
Once	4 (8.3)	2 (22.2)	1 (9)*	0	5 (55.6)
Twice	10 (20.9)	1 (11.1)*	4 (36.7)	0	1 (11.1)*
Three times	4 (8.3)	0	0	1 (33.3)*	1 (11.1)*
Four times	5 (10.4)	1 (11.1)*	1 (9)*	1 (33.3)*	0
At least five times	5 (10.4)	3 (33.4)	1 (9)*	0	1 (11.1)*
Percentage	28 (58.3)	7 (77.8)	7 (63.7)	2 (66.6)	8 (77.8)

*****Cases that were not considered for trend analysis.

Laprovet, France); D (Rabisin: Merial, France); and E (Nobivac® Rabies: Intervet India Pvt. Ltd). The 5 types included monovalent (A, B, D, E) and polyvalent (C) vaccines.

Serological analysis findings

Table 2 shows that, of the vaccinated dogs, 65% had sufficient protective antibody levels (≥ 0.5 EU/ml), while 34 and 1% had inadequate and undetectable antibody levels, respectively. Ninety-nine percent of the vaccinated dogs seroconverted. Seven out 13 (53.8%) non-vaccinated dogs had detectable antibody titres varying between 0.133 and 0.238 EU/ml, while 46.2% did not have detectable antibodies (<0.125 EU/ml).

Impact of various variables on production of antibodies

Vaccination status was found influential to rabies antibody response, that is, on average, the number of rabies antibodies per dog received vaccination (11.776059735) was eightfold than that of non-vaccinated dog (1.41579378). Considering other factors, the five vaccine types elicited diverse antibody levels and varyingly impacted the immunity in dogs received rabies vaccination. The highest mean titre (2.115) was attained by vaccine type E (Nobivac Rabies) while the mean titres for types B (Vaxipet R) and C (Vaxipet DHPPi+LR) were 1.850 and 1.261, respectively. The overall mean titre (1.071) was higher than 0.897 and 0.814 that were the lowest mean titres and produced by vaccine A (Rabies Veterinary Vaccine Inactivated) and D (Rabisin), respectively. Percentages of the dogs that were protected per vaccine are shown in Table 3.

Table 3 indicates that, irrespective of sampling time, 41.7 and 36.3% of dogs which received vaccine types A and D were not protected while 33.4 and 22.2% of those vaccinated with vaccine types C and B were not protected. Approximately 11.1% of dogs vaccinated with vaccine type E were also not protected (Table 3). In terms of sampling time, the difference between protected and non-protected vaccinated dogs, $\chi^2(9) = 10$, $p = 0.350$, was not statistically significant. This study found that time elapsed between vaccination and sampling influenced dogs' sera titres and the titres decreased with time, that is, 1.559, 0.949, and 0.934 in dogs sampled at 1-5, 6-9 and 10-12 months after vaccination, respectively. Approximately 42, 40 and 19% of the vaccinated dogs that were not protected (<0.5 EU/ml) were sampled at 6-9, 10-12 and 1-5 months following vaccination, respectively. The difference between sero-conversion levels, $\chi^2(9) = 12$, $p=0.213$ was not statistically significant. Also, the difference between elapsed vaccination-sampling time and sero-conversion implication, $\chi^2(9) = 12$, $p=0.213$ was not statistically significant. Antibody titres increased by number of vaccinations (Figure 3).

Figure 3 shows that the mean titres gradually augmented from the first to the fourth rabies vaccination while on at least fifth time, it slightly declined.

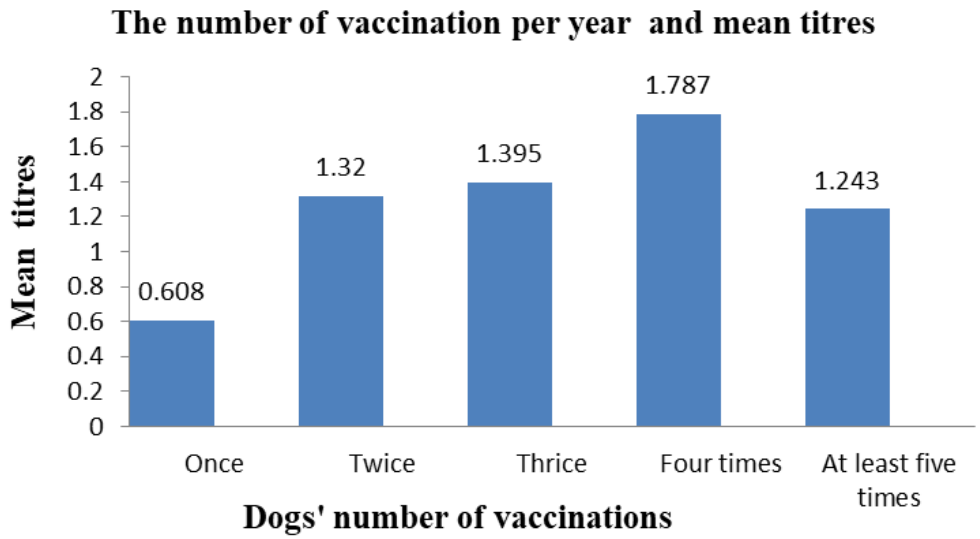

Figure 3. Applied number of vaccinations and mean titres.

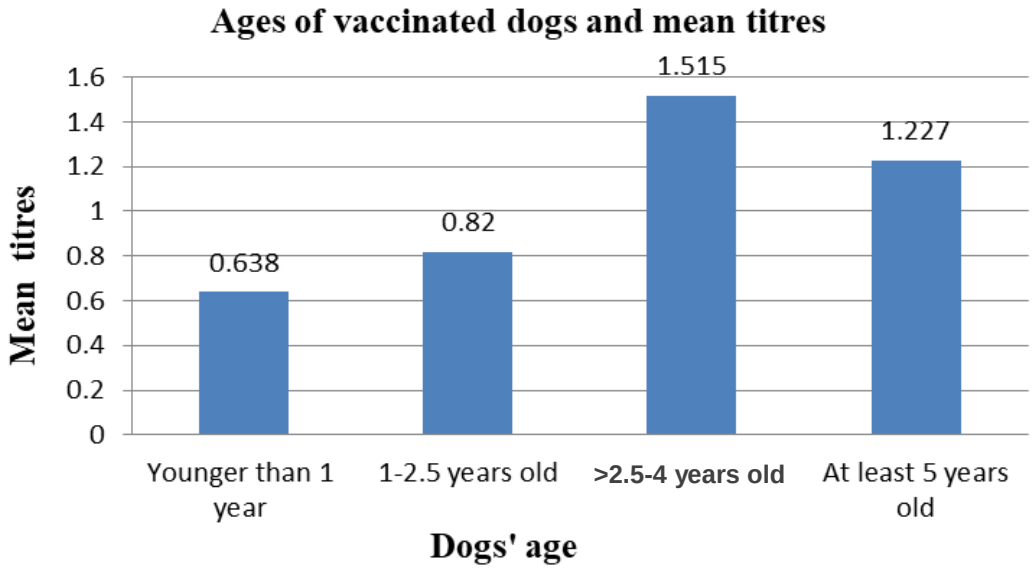

Figure 4. Influence of dogs' age on production of antibodies.

Statistics of rabies protection status of the vaccinated dogs versus vaccination frequency are indicated (Table 4).

Table 4 shows that, the highest percentage (52%) of the vaccinated dogs that were not-protected was recorded in those vaccinated once. In terms of number of vaccinations, the difference between protected and non-protected dogs, $\chi^2(25) = 30$, $p=0.224$ was not statistically significant. The mean titres increased by dogs' age (Figure 4).

Figure 4 shows that, the lowest mean titre (0.638) was recorded in dogs that were under 1 year of age. The dogs aged at least five years had the mean titre of 1.227 and the mean was 1.515 in dogs aged >2.5 to 4 years old. Chi-square tests of association indicated that the relationship between seroconversion levels and ages of dogs, $\chi^2(9)=11.509$, $p=0.242$ was not statistically significant. Pearson's correlations coefficient showed that vaccination number at $\alpha=0.05$, $r=0.255$, $p=0.013$, vaccination status at $\alpha=0.001$, $r=0.528$, $p<0.001$, time elapsed between vaccination and sampling at $\alpha=0.001$, $r=0.391$, $p<0.001$ positively correlated with antibody titres. Dogs' age negatively correlated with antibody titres at $\alpha=0.001$, $r=-0.281$, $p=0.006$. At both $\alpha=0.05$ and $\alpha=0.001$, type of rabies vaccines did not correlate with antibody titres, $r=-0.008$, $p=0.939$.

Table 4. Statistics of non-protected dogs versus vaccination frequency.

Frequency of vaccination	Total number of dogs	% of protected (n=52)	% of non-protected (n=28)
Once	25	48	52
Twice	22	73	27
Three times	9	67	33
Four times	10	80	20
At least five times	14	71	29
-	80	-	-

DISCUSSION

The present findings show that 35% of vaccinated dogs had rabies antibodies below the protective levels (<0.5 EU/ml) while 53.8% of non-vaccinated dogs had measurable rabies antibodies varying between 0.133 and 0.238 EU/ml. Such dogs whether vaccinated or not were at risk of contracting rabies infection. According to Oboegbulem et al. (1987) and Tepsumethanon et al. (2016) rabies vaccination failure in animals might be higher, but it was approximated to 0.025% in rabies vaccinated dogs. The 35% of vaccinated dogs that were not protected in this study was higher than 4.62, 12 and 30% which were reported by Ondrejková et al. (2015), Wallace et al. (2017) and Fernandes et al. (2017). The high number of dogs that were not protected in this study could be related to time elapsed between vaccination-sampling. In the present study, dog blood samples were collected between 1 and 12 months after the dogs received vaccination while Ondrejková et al. (2015) sampled dogs on the 30th day following rabies vaccination.

In the study by Wallace et al. (2017), sampling interval varied from at least 3 days to over 270 days following rabies vaccination. The results are consistent with those of Cliquet et al. (2003), Kennedy et al. (2007), Minke et al. (2009), and Berndtsson et al. (2011) who reported that time elapsed between vaccination and sampling influenced rabies antibody titres. One of the other possible reasons behind the high number of vaccinated dogs that were not protected would be number of vaccination. The results were compatible with those of Cliquet et al. (2003), Berndtsson et al. (2011), and Watanabe et al. (2013) who reported that the number of antirabies vaccinations impacted rabies antibody titres. The present findings were also compatible with those of Kennedy et al. (2007) and Berndtsson et al. (2011) who found that age impacted on dogs' rabies antibody titres. However, the study disagrees with that of Salinas et al. (1992) who reported that number of vaccinations did not influence rabies antibody response. We think that the difference would be related to how the immune response was quantified. The study assessed the immune response of study dogs based on antibody titres, while Salina et al. (1992) evaluated the immune response of sample dogs based on optical densities. This could have influenced the cutoff point.

This study indicates that, of non-vaccinated pet dogs, 53.8% had detectable antibodies lower than the protective levels (<0.5 EU/ml). Probably, these dogs might have had an abortive infection. Ondrejková et al. (2015) reported 13.04% of non-vaccinated pet dogs that had measurable antibody titres while Cleaveland et al. (1999) found rabies antibody titres in 7.4% of non-vaccinated pet dogs. Detection of rabies virus antibodies in healthy animals was reported by Prager et al. (2012), though it was not known whether the animals had an abortive infection or recovered from rabies. A study by Watanabe et al. (2013) found maternal rabies antibodies in 15.3% of non-vaccinated study puppies younger than three month old; however, none of unvaccinated dogs involved in the present study was younger than three months old. A report by El-Sayed (2018) indicated that abortive animal and human rabies may be caused by non-encephalic rabies strains and such an infection does not result in health abnormalities.

Study limitations

The impact of dogs' breeds and breed size on rabies antibody titres could have been assessed, but the investigator had trouble recognising study dogs breeds, thus breed size data of the studied dogs was also not collected.

Conclusion

This study found that the vaccinated dogs produced diverse rabies antibody levels regardless of type of rabies vaccine applied. Some vaccinated dogs were poor responders to rabies vaccines and did not have protective rabies antibody levels. Some non-vaccinated studied dogs had detectable rabies antibodies below the cutoff point. Different factors including rabies vaccination status, number of rabies vaccination, age at vaccination, time elapsed between vaccination and sampling influenced rabies antibody titres of the studied dogs. Considering the high number of vaccinated dogs that

were poor responders to rabies vaccination, further studies should be undertaken to investigate and understand the phenomenon.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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