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Full Length Research

Prevalence of rabies in apparently healthy slaughtered dogs in Southern Gombe, Nigeria

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ABSTRACT: Rabies is a fatal zoonotic disease with significant public health implications, particularly in rural areas of Nigeria. This study aimed to determine the prevalence of rabies in dogs slaughtered in Southern Gombe, Gombe State. A cross-sectional study was conducted, during which 120 brain samples from dogs were collected from slaughter slabs in Billiri, Kaltungo, and Shongom Local Government Areas. The samples were tested for rabies antigens using the Direct Fluorescent Antibody Test (DFAT) and Polymerase Chain Reaction (PCR). The results revealed a 10.83% prevalence of rabies antigen in slaughtered dogs, with 13 out of 120 samples testing positive using DFAT. PCR confirmed the presence of rabies antigens in 2 out of the 13 positive samples (15.4%). The study underscores the concerning prevalence of rabies in slaughtered dogs in Southern Gombe. It is recommended that targeted rabies education campaigns and the adoption of a One Health approach be implemented to enhance rabies surveillance and control in the region and to protect individuals who may come into contact with infected animals.

Keywords: Direct Fluorescent Antibody Test (DFAT), Nigeria, rabies, slaughtered dogs, Southern Gombe.

INTRODUCTION

Rabies is a neglected zoonotic viral disease that affects all warm-blooded mammals (World Health Organisation, 2004; Aiyedun *et al.,* 2017). Rabies is known to produce acute fatal encephalitis, caused by *Lyssavirus*, of the family *Rhabdoviridae* (World Health Organisation, 2004). Despite being 100% vaccine preventable, rabies still causes an estimated 59,000 human deaths annually in the endemic areas of Africa and Asia (Hampson *et al*., 2015; World Health Organisation, 2010; 2018). In Africa, an estimated 21,476 human deaths occur every year due to dog-mediated rabies, accounting for approximately 36.4% of global human deaths, with a loss of 1.34 million disability adjusted life-years (Hampson *et al*., 2008).

In Nigeria, conservative estimates indicate that at least 10,000 individuals receive post-exposure prophylaxis annually (Ehizibolo *et al*., 2011). The domestic dog, *Canis familiaris*, is responsible for almost 99% of human deaths from rabies (World Health Organisation, 2008). The disease has a high case fatality rate, with terrifying symptoms and only a few documented survivors (Hemachudha *et al*., 2013). The saliva of a rabies-infected dog has high concentrations of the causative agent and serves as a medium for the transmission of the disease to humans through a dog bite (World Health Organisation,

2008). However, the virus may also be found in tears, urine, serum, and other body fluids of the infected animals (Wolfang, 1999; Sani *et al*., 2019). Non-bite exposure may occur through licks or splash of infected saliva into an open wound, or splash of blood/nerve tissues into the mucus membrane or an open wound during the course of processing dogs without adequate protective measures (Audu, 2011; Dzikwi *et al*., 2013; World Health Organisation, 2020). A dog bite is considered an exposure if the infected saliva contacts a wound or mucous membrane, particularly in the absence of proper post-bite care (Beran, 1981; Ogunkoya *et al*., 2003).

Dog trade and consumption are increasing, with dog meat regarded as a delicacy in some developing countries such as Cameroon and Ghana, and Nigeria (Simmons, 1994). Dog marketing, slaughtering, distribution of raw dog meat, handling of dogs without protective measures, as well as the consumption cycle, pose serious public health risks because some of the dogs may harbour rabies (Audu, 2011; Mshelbwala *et al*., 2013). Previous studies have detected rabies virus antigen in the brain tissue of dogs slaughtered for human consumption in Maiduguri (Ajayi *et al*., 2006) and Yola (Aliyu *et al*., 2010), which share borders with Gombe State. Moreover, transboundary transmission of rabies through dog trade highlights the risk of disease spread in the study area (David *et al*., 2008; Sabo *et al*., 2008).

Reports on dog slaughter for consumption abound, with potential for RV infection, especially among individuals involved in the chain of capture, breeding, purchase, transportation, slaughtering, processing and handling (Mshelbwala *et al.,* 2013; Konzing *et al.,* 2023) The limited veterinary officers within Gombe State Government service and absence of veterinary research institute area office in Gombe State, thus there is a pressing need for surveillance of rabies antigen in the brain tissues of apparently healthy dogs slaughtered in Gombe State, Nigeria. Hence, the objective of this research is to determine the prevalence of rabies among apparently healthy dogs slaughtered for human consumption in southern Gombe State.

MATERIALS AND METHODS

Study area

The study was carried out in Billiri, Kaltungo, and Shongom Local Government Areas in the southern part of Gombe State, Nigeria. It is located between latitudes 90º30'N and 12º30'N and longitudes 8º45'E and 11º45'E in the northeastern part of Nigeria (Diary, 2012). It has an area of 737 km^2 and a population of 202,144 (Adamu, 2014). Gombe State borders Borno and Yobe States to the East and North, respectively, Adamawa and Taraba to the South and Bauchi States to the West (Diary, 2012). Residents of southern Gombe are predominantly food crop farmers, livestock herders, traders and civil servants. The study area has specialist hospital, maternity clinic, private hospitals, state veterinary clinic, private veterinary clinics, a government-approved dog market, slaughterhouse and processing slab, which attract people within and outside the State.

Sample size determination

The formula described by Thrusfield (2007) was used to determine the sample size for dogs.

$n = Z^2 P (1-P)/d^2$

n = Sample size, Z = Appropriate value for the standard normal deviation for desired confidence level= (1.96), P = Anticipated prevalence rate, d = Desired absolute precision (5%), d=0.05, P = 7.89% (Ameh *et al.*, 2014).

n = 112

However, to increase the precision, a total of 120 dog brain samples were collected.

Sample collection

One hundred and twenty (120) dogs' heads were collected from the slaughter point/slab in Billiri, Kaltngo, and Shongom Local Government Areas from December 2023 to March 2024. The dog processors stunned the dogs by striking the occipital bone with a stick before slaughter. The collected samples were transported on ice packs to the National Veterinary Research Institute, Rabies Reference Laboratory, Vom, Plateau State, Nigeria. The brain tissues were removed from the dog heads as described by Atanasiu (1975) where a portion of the hippocampus and brain stem was harvested from the brain tissue, and placed in clean sample bottles and stored in a freezer at -3ºC before the direct fluorescent antibody test (DFAT) was performed on the brain samples harvested.

Laboratory technique

Direct fluorescent antibody test

All the brain tissue samples harvested were subjected to the direct fluorescent antibody assay (DFAT) procedure as described by Dean *et al*. (1996) and CDC (2006). Rabies DFAT monoclonal antibody conjugate reagents (Fujirebio Diagnostic Inc., Malvern, P. A 19355, USA) were used, and the working reagent dilution was prepared according to the manufacturer's recommendation as described by (Flamand *et al.,* 1980). An impression smear of each brain sample was prepared on a clean glass slide, air dried and

fixed in cold acetone for one hour at -20ºC. The slide was then air-dried for 3-5 minutes, and the rabies conjugate was diluted with phosphate-buffered saline at a 1:40 ratio was applied and incubated for 30 minutes at 37ºC in a humid chamber. Excess conjugate was removed from the slides by rinsing with phosphate-buffered saline solution (pH 7.4). The slide was then placed in a coupling jar containing phosphate-buffered saline solution, which was replaced every 5 minutes with a fresh solution; this was done thrice, and the slides were air dried. The slides were examined using a fluorescence microscope within two hours after staining. Rabies virus-infected and normal brain smears were used as positive and negative controls, respectively. The presence of brilliant apple green or greenish–yellow fluorescence, graded by intensity (+ ++, +++ and ++++) against a dark background, was regarded as a positive result for rabies. If there is no specific apple green fluorescence observed, the test result is regarded as negative.

Real time Reverse Transcriptase PCR (RT-PCR)

Viral RNA from each sample that tested positive by (DFAT) was extracted using TRIzol reagent in accordance with the manufacturer's guidelines (Sigma Aldrich, USA) and subjected to Real-Time Reverse Transcriptase PCR (RT-PCR) as described by Meslin *et al*. (1996).

Ethical approval

Ethical approval was obtained from the University of Ilorin Research Ethics Review Committee before commencement of the study, with the ethical approval number UREC/FVM/PG2021/0546.

Data analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS) Version 25 (IBM Statistics). The data obtained were subjected to descriptive statistics and presented as frequencies and percentages. The prevalence of rabies was calculated as the total number of rabies antigens detected divided by the total number of dogs slaughtered and expressed as a percentage. Chi-square test was used to determine possible association of location, breed and sex with rabies infection, and p-values of < 0.05 were considered statistically significant throughout the study.

RESULTS

Fluorescence microscopy

A total of 120 brain tissue samples were subjected to direct fluorescent antibody assay (DFAT), with 13 out of 120 samples testing positive using DFAT. Fluorescence microscopy revealed green fluorescence observed in Figure 2, confirming the presence of the rabies virus antigen in the brain tissues of the dogs. The bright fluorescence signals in the image confirm rabies positivity in the brain samples are positive for rabies (Figure 2). Figure 1 displays a rabies-negative brain tissue field under fluorescence microscopy, showing no characteristic green fluorescence, indicating no presence of the rabies antigen (Figure 1).

Table 1 presents the prevalence of rabies antigen in dog brains, stratified by location, sex, and breed of the dogs. Billiri had a total of 55 samples, with a negative FAT of 47 (85.45%) and a positive FAT of 8 (14.55%). In Kaltungo, 35 samples were collected, with 32 testing negative (91.43%), positive FAT was 3 (8.57%). In Shongom, a total of 30 samples were collected, with negative FAT 28 (93.33%), and positive FAT was 2 (6.67%). The chi-square value for the location of the abattoir is 1.509 with a p-value of 0.470, indicating no statistically significant difference in rabies prevalence among the locations (Table 1).

All sampled dogs were adult (n=120), with a negative FAT of 107 (89.17%) and a positive FAT of 13 (10.83%). Chi-square analysis was not applicable for age, as there are no young dogs sampled (Table 1).

For the sex of the sampled dogs, the male has a total sample of 75 with a negative FAT of 66 (88%), and a positive FAT of 9 (12%). The female has a total sample of 45, with a negative FAT of 41 (91.11%) and a positive FAT of 4 (8.89%). The chi-square test for sex showed $\chi^2=0.282$, $p=0.596$, indicating no significant association between sex and rabies prevalence (Table 1).

Among the indigenous breed group (n=112), 13 (11.61%) tested positive with a negative FAT of 99 (88.39%). The mixed breed has a total of 8 samples, with a negative FAT of 8 (100%) and a positive FAT of 0 (0%). The chi-square value for breed is 1.041 with a p-value of 0.307, indicating no statistically significant difference in rabies prevalence between indigenous and mixed breeds (Table 1).

Overall, the table shows that there is no statistically significant difference in the prevalence of rabies detected by FAT based on the location of the abattoir, sex, or breed of the dogs. The highest rabies antigen prevalence (14.55%) was observed in dogs from Billiri, while no rabies antigen was detected in the mixed breed group (Table 1).

Polymerase Chain Reaction (PCR)

Out of the 13 tissue samples from the slaughtered dogs that tested positive for direct fluorescence antibody test, only two samples (2\13) were positive for rabies antigen by real-time PCR. This may be due to RNA degradation,

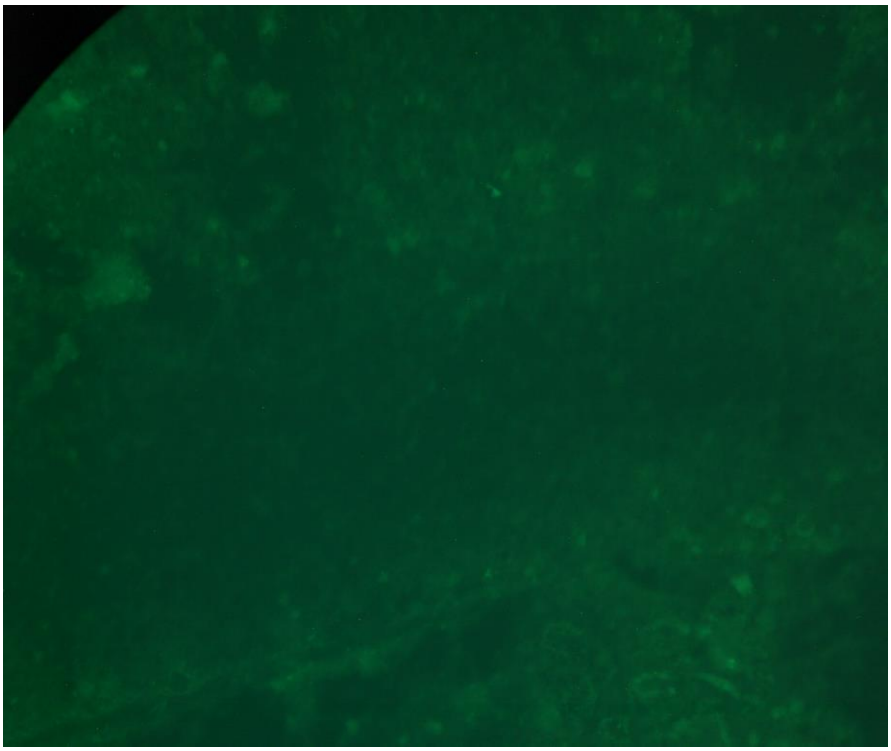

Figure 1. Brain tissue of a dog negative to rabies antigen detection using a direct fluorescent antibody test (DFAT) showing no immunofluorescence.

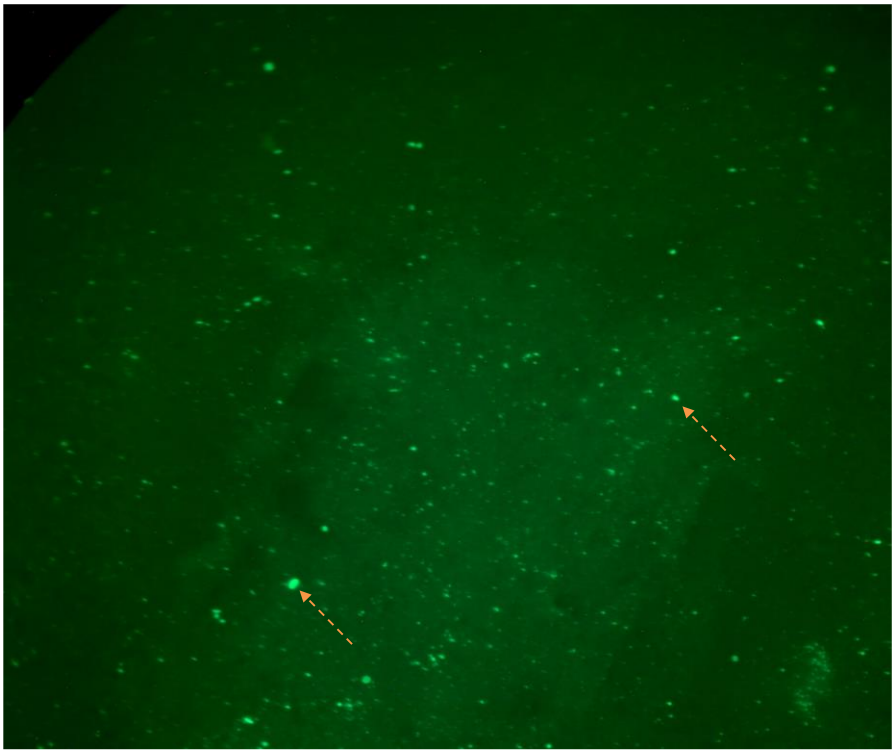

Figure 2. Brain tissue of a dog positive to rabies antigen detection using a direct fluorescent antibody test (DFAT) showing the apple green fluorescence (arrows).

Table 1. Prevalence of canine rabies antigen in dog brains detected by Fluorescent Antibody Test (FAT) in dogs slaughtered at the abattoirs in Southern Gombe, Nigeria.

Variables	Number of Dogs sampled	Fluorescent antibody Test (FAT) (Negative %)	Fluorescent antibody Test (FAT) (Positive %)	Chi-Square (x^2)	p-value
Location of abattoir				1.509	0.470
Billiri	55	47	8 (14.55)		
Kaltungo	35	32	3 (8.57)		
Shongom	30	28	2 (6.67)		
Sex				0.282	0.596
Male	75	66	9 (12)		
Female	45	41	4 (8.89)		
Breed				1.041	0.307
Indigenous breed	112	99	13 (11.61)		
Mixed breed	8	8	0 (0)		

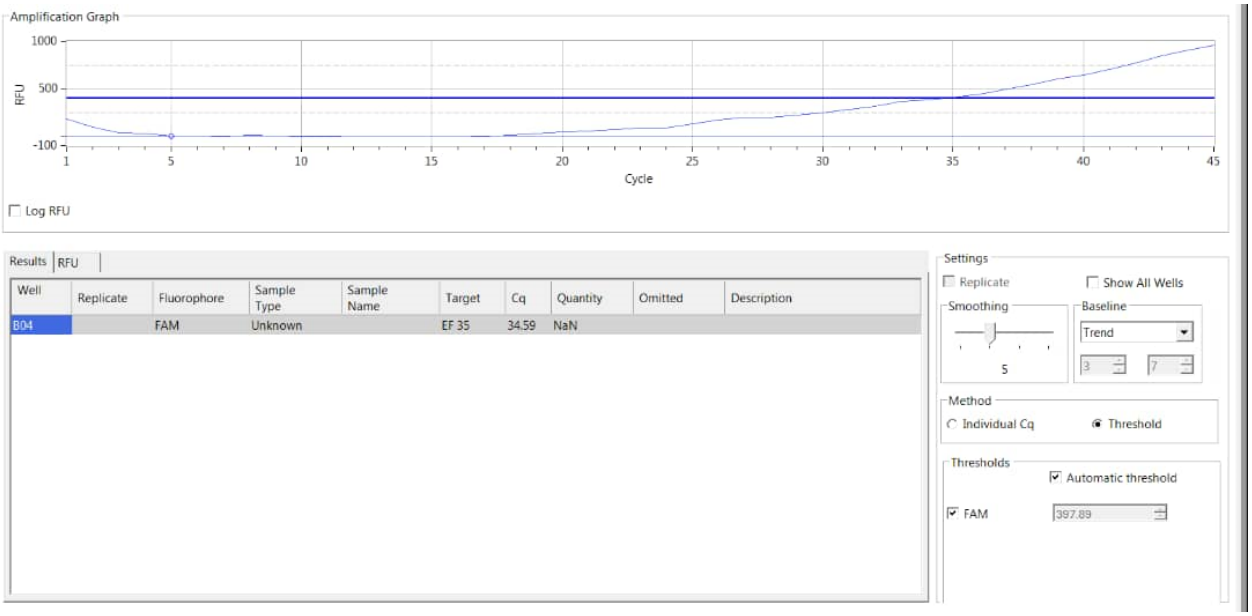

Figure 3. Rabies-positive dog brain samples detected with Real-Time PCR.

possibly caused by a four-day power outage at the institution due to storm damage.

The real-time PCR results demonstrated positive amplification curves for rabies virus. This is evident in the exponential increase in relative fluorescence units (RFU), surpassing the threshold line after cycle 30, crossing the threshold line (Figure 3). The upward curve indicates the amplification of the rabies virus genetic material over successive PCR cycles. The point where the curve crosses the threshold is known as the quantification cycle (Cq) (Figure 3). The observed Cq value was 34.59, indicating low viral RNA concentration in the sample. A lower Cq value generally indicates a higher amount of starting viral genetic material in the sample (Figure 3).

DISCUSSION

The prevalence of canine rabies in Southern Gombe, Nigeria, as revealed by DFAT, was 10.83%. The direct fluorescent antibody test (DFAT) is considered the gold standard recommended by World Health Organisation and office of international des epizooties (OIE) for diagnosing rabies in animals and humans because is it a rapid, highly sensitive, and specific method (between 96-99%) (Robardet *et al*., 2011). The study's results indicated varying prevalence rates of rabies across different locations in the Southern Gombe, with Billiri recording the highest prevalence (14.55%) among the surveyed locations, with 8 out of 55 samples testing positive. In

contrast, Kaltungo and Shongom exhibited lower prevalence rates of 8.57% and 6.67%, respectively. The chi-square analysis ($\chi^2 = 1.509$, $p = 0.470$) revealed no statistically significant difference in rabies prevalence among the locations, suggesting a relatively uniform distribution of the disease across the region. These findings align with previous studies highlighting regional variations in rabies prevalence. The research conducted in other parts of Nigeria, such as Lagos and Enugu, also reported substantial rabies incidence in urban and rural settings, emphasising the widespread nature of the disease across the country (Ojo *et al*., 2016; Mshelbwala *et al*., 2021). The consistent prevalence across study areas supports the need for comprehensive rabies control programmes that address both urban and rural areas. A study conducted in Abuja, the Federal Capital Territory (FCT), found multiple outbreaks of rabies with associated human deaths. The study revealed a low dog vaccination rate of 38% compared to the recommended 70%, a high number of free-ranging dogs, and the presence of the rabies virus in dogs slaughtered for human consumption. The findings emphasise the need for a robust One Health approach that integrates human, animal, and environmental health strategies to control rabies effectively (Mshelbwala *et al*., 2023).

Interestingly, the study included only adult dogs, with no samples collected from younger dogs, which had a prevalence rate of 10.83%. This finding may be attributed to the higher risk of exposure in adult dogs, which are more likely to roam and interact with other animals compared to younger dogs. The absence of young dog samples may reflect the demographic composition of dogs typically slaughtered in the study areas. Previous studies have documented varying rabies prevalence among different age groups of dogs. In a study conducted in southwestern Nigeria, higher prevalence rates were observed among adult dogs compared to puppies, likely due to increased exposure risk over time (Eze *et al*., 2020). These patterns emphasise the importance of targeting adult dogs in rabies vaccination and control efforts to effectively reduce the disease burden.

The study revealed a slightly higher prevalence rate among male dogs (12%) compared to females (8.89%). However, chi-square analysis ($\chi^2 = 0.282$, $p = 0.596$) indicated no statistically significant difference in prevalence based on sex. This finding is consistent with the general understanding that both male and female dogs are equally susceptible to rabies infection. Sex-based differences in rabies prevalence have been the subject of research in various studies. While some studies report higher prevalence in male dogs due to their more aggressive and roaming behaviour, others find no significant difference between sexes (Atuman *et al*., 2014). The results from Gombe align with the latter, suggesting that rabies control efforts should target both sexes equally.

The study also examined rabies prevalence by breed, finding that indigenous breeds had a prevalence rate of 11.61%, while no rabies antigen was detected in mixed breeds. The chi-square analysis ($\chi^2 = 1.041$, $p = 0.307$) showed no statistically significant difference between the two groups. The higher prevalence observed in indigenous breeds may be attributed to their larger population and more frequent interaction with wildlife and other stray dogs. Indigenous breeds often have greater freedom to roam, increasing their risk of exposure to rabid animals. In contrast, mixed breeds, which are often kept as pets with restricted movement, might have a lower exposure risk. These findings are consistent with previous research indicating that indigenous breeds are more at risk due to their roaming behaviour and lower confinement (Oluwayelu *et al*., 2015). Effective rabies control programmes should therefore prioritise indigenous breeds without neglecting mixed-breed populations to ensure broad coverage.

The findings from this study have significant implications for rabies control and prevention in Gombe and other regions of Nigeria. The relatively high prevalence rates and uniform distribution across different locations highlight the need for widespread vaccination and control programs. The higher prevalence in adult and indigenous breeds suggests that these groups should be prioritised in vaccination campaigns.

The Nigerian government has set a target to eliminate dog-mediated human rabies by 2030. The government has been procuring anti-rabies vaccines for animals and pre- and post-exposure vaccines for humans at high risk of rabies, such as animal health workers and hunters. Despite ongoing efforts, several challenges persist, including limited access to post-exposure prophylaxis (PEP) for dog bite victims. Many victims are unable to afford PEP or access it in time, leading to preventable deaths (The Guardian Newspaper, 2023).

Nigeria faces significant challenges in controlling rabies; ongoing efforts and research provide a roadmap for eliminating the disease by 2030. Collaboration across sectors, improved healthcare accessibility, and public education are critical components of this strategy. The fluorescence microscopy results from Gombe highlight the continued prevalence of rabies and underscore the need for sustained and coordinated efforts to achieve rabies elimination.

Conclusion

The study's findings highlight increased rabies prevalence among indigenous dog breeds, which should guide the design of targeted vaccination campaigns. Targeted vaccination of indigenous breeds could significantly reduce the rabies burden. The data further confirm that sex is not a significant risk factor, and rabies control strategies should be uniformly applied to all dogs. Future research

should include molecular characterisation of rabies virus strains to better understand transmission patterns.

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CONFLICTS OF INTEREST

The authors have stated that there are no competing interests.

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