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The Seroprofile of Rabies Antibodies in Companion Urban Dogs in Ibadan, Nigeria

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Abstract: This study evaluates the immune status of companion dogs in an urban setting in Nigeria using ELISA technique. A total of 630 companion dogs made up of six major breeds were sampled for rabies virus antibodies by the indirect ELISA method. An overall prevalence of 71.4% (52.9±39.5 EU) was observed, with the males having a prevalence of 69.1% compared to 74.0% for females. The mongrels were observed to have the highest prevalence during the Post-Vaccination (PV) periods while the highest antibody titre was obtained 6-9 months PV and the lowest between 9-12 months PV. The comparative prevalence and antibody titres of dogs vaccinated with different commercial vaccines showed no remarkable difference. There was no significance in the mean SP ratio of dogs of different breeds, sexes and commercial antirabies vaccines also in the PV periods. The prevalence indicates a relatively good protection against rabies outbreak and uniform potency of the common antirabies vaccines in use in Nigeria. It also showed that annual booster vaccination should further be enforced as it enhances immune response in the dogs.

Key words: Seroprofile, rabies, antibodies, enzyme-linked immunosorbent assay, dogs

INTRODUCTION

Rabies is a highly fatal viral zoonosis that affects the central nervous system of warm-blooded animals worldwide (Radostits *et al*., 1995). Rabies has been well reported in humans (Ali, 2002; Ogunkoya *et al*., 2003), companion animals (Taiwo *et al*., 1998; Awah-Noukum *et al*., 2002), farm animals (Muriuki *et al*., 1994), wild carnivores (Binepal *et al*., 1991) and experimentally in local chicken (Nottidge and Oyejide, 1992). Apart from skunk and vampire bats, dogs and, to a lesser extent, cats are the major transmitters of the virus in the urban areas (Ali, 2002; Binepal *et al*., 1991). The close relationship between dogs and humans makes human rabies to be more urban in nature (Awah-Noukum *et al*., 2002).

Despite reports of rabies in young puppies of two months of age (Adeyanju and Addo, 1977), vaccinated dogs (Okoh, 1983) and unvaccinated dogs (Aghomo *et al*., 1986; 1989), there has been a decline in dog owners' response to routine vaccination (Adeyemi and Zessin, 2000), which makes the dog population to be susceptible to rabies. The diagnosis of rabies had been by examination of the brain at necropsy for Negri bodies, the use of fluorescent antibody test, immunoperoxidase and mouse inoculation (Muriuki *et al*., 1994; Binepal *et al*., 1991; Anjaria and Ihala, 1985). In Nigeria however, serodiagnosis of rabies was mainly based on fluorescent antibody test (Adeyanju and Addo, 1977) while there are few reports on the use of enzyme-linked immunosorbent assay especially in the evaluation of vaccination programmes (Aghomo *et al*., 1986).

Control of rabies in humans and animals is mainly achieved by vaccination, control of stray dogs and wildlife carriers of the virus (Binepal *et al*., 1991; Lee *et al*., 2001). Although compulsory vaccination programmes in other parts of the world have been observed to reduce the incidence of rabies in dogs (Coleman and Dye, 1995), the duration of the induced immunity has been inconsistent (Coryne *et al*., 2001). The situation in Africa is that of inconsistencies in vaccination programmes and vaccine failures (Taiwo *et al*., 1998) and the immune status of urban dogs is relatively unknown. This study was therefore aimed at evaluating the humoral immune status of companion dogs in an urban setting with a view to determining the seroprofile of antibodies among these companion pets. It also revealed the effect of sex, vaccine type and post-vaccination period on the rabies virus antibody titres of dogs.

MATERIALS AND METHODS

Study area: The study focused on Ibadan, the largest city in Nigeria, with influx of people and dogs from all regions in Nigeria since trade in dogs across the country is common and unregulated (Adeyemi and Zessin, 2000).

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The three veterinary clinics used were the major ones in Ibadan that attend to both exotic and mixed breeds of dogs. The government-owned hospitals serve as referral centers to these clinics.

Survey animals: A total of 630 companion dogs made up of five major breeds of both sexes and various ages were sampled from three selected veterinary clinics in Ibadan metropolis. The sample characteristics which consisted of age, sex, breed and previous antirabies vaccination record, including the vaccine type were obtained from the clinical records. The dogs sampled were among the animals presented to the clinics for various routine clinical examinations. Care was taken to note records to avoid repeated sampling. 492 (78.1%) of the 630 dogs had record of previous antirabies vaccination while 138 (21.9%) had no known record of vaccination. All dogs were aged 3 months to 6 years. The survey animals consisted of 252 Alsatians, 84 Rottweilers, 78 Pit-bull terriers, 84 mongrels, 42 Ridgebacks, 36 Doberman and 52 other uncommon exotic breeds, whose sample sizes were too small to be grouped separately.

Blood collection: The blood samples were obtained via the cephalic vein and allowed to clot at room temperature for about 5-6 h. Separated sera were stored at -20°C until tested.

Antirabies vaccines: The four types of imported commercial antirabies vaccines used among the dog population sampled were designated as type I (the commonest), II, III and IV. 240 dogs were vaccinated with type I while 60, 54 and 60 dogs were vaccinated with types II, III and IV, respectively. The vaccine types used for the remaining 204 vaccinated dogs were not specified. Each vaccine was first used at 3 months or 6 months of age as specified by the manufacturer. The dates of administration of subsequent booster doses were obtained from the clinical records of the dogs. For each dog, the period between the last antirabies vaccination and sample collection was noted as the Post-Vaccination (PV) period.

ELISA technique: The ELISA technique used was as described (Aghomo *et al*., 1986). Optimal working dilutions obtained following chequerboard titration were antigen 1:500, sera 1:100 and conjugate 1:1000. The cut-off SP ratio was 0.25, which corresponded to twice the O.D. value of the negative control serum. Results were considered valid only when the difference between the mean O.D. of the positive and negative controls was greater than 0.2 and the mean O.D. of the negative control was less than or equal to 0.25. ELISA Unit = 100 (SP ratio).

Statistical analysis: Data obtained was subjected to Student's *t*-test and Chi-squared analysis to determine the significance of differences between breed, age, sex, vaccine types, seroconversion, booster vaccination and post-vaccination time.

RESULTS AND DISCUSSION

The prevalence of rabies virus antibodies among 630 dogs, consisting of six breeds of both sexes and varying ages, as well as the mean SP ratio of the samples are shown in (Table 1). An overall prevalence of 71.4% was observed in the dogs sampled. Among the six sampled breeds, the prevalence of rabies antibodies ranged from 66.7% in Alsatians to 88.6% in mongrels. It was observed that 74.0% of the female dogs had antibodies compared to 69.1% in the males. There was no significant difference ($p>0.05$) in the mean SP ratio between the sexes. The comparative prevalence of rabies virus antibodies and the mean SP ratio of positive samples at various periods PV are shown in (Table 2). The highest prevalence of 87.5% was obtained at 3-6 months PV while the lowest was obtained after 12 months PV. The highest mean positive SP ratio of 0.665 was obtained at 6-9 months PV while the lowest 0.387 was observed at 9-12 months PV. (Table 3) shows a comparison of the prevalence of rabies virus antibodies and the mean SP ratio of dogs given different commercial antirabies vaccines. While the prevalence of antibodies varied from 61.0% with vaccine type I to 90.0% with vaccine type IV, the mean positive SP ratio was not significantly different between the vaccine types ($p>0.05$).

Table 1: Prevalence of rabies virus antibodies among 630 companion dogs in Ibadan

				Male			Female		
Breed of dog	No.	No. positive	Mean±SD	No.	No. positive	Mean±SD	No.	No. positive	Mean±SD
Alsatian	252	168 (66.7%)	0.503±0.254	150	102 (68%)	0.440±0.254	102	66 (64.7%)	0.587±0.280
Rottweiler	84	66 (78.6%)	0.628±0.280	30	24 (80%)	0.688±0.288	54	42 (77.8%)	0.593±0.410
Mongrel	84	72 (85.6%)	0.747±0.290	42	30 (71.4%)	0.851±0.290	42	42 (100%)	0.673±0.310
Ridgeback	48	36 (75.0%)	0.551±0.261	24	18 (75.0%)	0.53±0.3130	24	18 (75.0%)	0.549±0.261
Doberman	42	30 (71.4%)	0.589±0.294	36	24 (66.7%)	0.566±0.294	6	6 (100%)	0.683±6.250
Pit-bull terrier	78	60 (76.9%)	0.419±0.316	30	18 (60%)	0.378±0.318	48	42 (87.5%)	0.436±0.316
Others	42	18 (42.9%)	0.438±0.318	18	12 (66.7%)	0.462±0.269	24	6 (25.0%)	0.389±0.340
Total	630	450 (71.4%)	0.572±0.284	330	228 (69.1%)	0.564±0.284	300	222 (74.0%)	0.559±0.281

Table 2: Prevalence of rabies antibodies in dogs at various periods post-vaccination

Post-vaccination period	No. of dogs	No. positive (%)	Mean ELISA Units±SD
0-3 months	114	84 (73.7%)	64.7±56.5
3-6 months	96	84 (87.5%)	40.9±12.0
6-9months	66	48 (72.7%)	66.5±62.2
9-12months	90	66 (73.3%)	38.7±19.5
>12 months	126	66 (52.4%)	58.0±40.0
No. record	138	102 (73.9%)	52.0±35.0
Total	630	450 (71.4%)	52.9±39.5

Table 3: Relative prevalence of rabies virus antibodies in dogs vaccinated with different antirabies vaccines

Type of Vaccine	No. of dogs vaccinated	No. positive (%)	Mean ELISA Units±SD
I	246	150 (61.0%)	53.1±43.3
II	66	48 (72.7%)	52.9±48.5
III	54	42 (77.8%)	49.6±36.6
IV	60	54 (90.0%)	54.2±15.7
Unspecified	204	156 (76.5%)	54.5±45.0
Total	630	450 (71.4%)	52.9±39.5

In this study 71.4% of the companion dogs sampled had rabies virus antibodies, indicating a high level of vaccination awareness among the owners of imported dogs in Ibadan metropolis. This observation indicates a relatively good protection against rabies outbreak in this locality as it has been reported that transmission effectively stops when over 70% of the canine population is immune (Coleman and Dye, 1995; Beran, 1981). The reports of continued outbreak of this disease (Taiwo *et al*., 1998) and low vaccination coverage especially in local dogs (Adeyemi and Zessin, 2000), support the fact that mass vaccination does not cover stray or ownerless dogs which remain a potential source of transmission of rabies (Binepal *et al*., 1991). The dogs considered in this study were mainly imported, highly expensive and mostly kept in fenced houses where contact with stray dogs is remote. There was no significance in the sex predisposition to rabies vaccination but a slightly higher prevalence of antibodies (74.0%) was observed in females than in males (69.1%). This slight difference could be due to the better care of bitches for breeding purposes than the males and the tendency to vaccinate bitches to facilitate maternally-derived immunity in the puppies, which has been reported to interfere with immune response (Tizard and Yawei, 1998).

There was no significant difference in the response of the defined breeds to the different rabies vaccines despite the variance in the prevalence of antibodies from 66.7% in Alsatians to 85.6% in mongrels. This indicates higher vaccination coverage among the companion mongrels routinely brought to the private veterinary clinics. The relatively higher vaccination coverage among these mongrels as compared to reports from government or university clinics (Adeyemi and Zessin, 2000; Adeyemi *et al*., 2000) could be due to the perceived high risk of exposure of these less priced breeds to rabies virus by the owners who are also well enlightened and economically stable to pay for antirabies vaccination, even in a private clinic. This observation seems to indicate that dog owners vaccinate their pets when there is perceived risk of rabies exposure and for breeding purposes rather than as a rule.

The comparative prevalence of rabies virus antibodies in dogs at various periods PV showed the highest prevalence of 87.5% at 3-6 months PV while the lowest prevalence of 52.4% was obtained in dogs over 12 months PV. This observation supports the adoption of first vaccination at 3 months of age in enzootic areas (Aghomo *et al*., 1986). The low prevalence and marked decline in antibody titres at 12 months PV could be a strong reason in support of the annual booster vaccination suggested by some workers (Taiwo *et al*., 1998; Aghomo *et al*., 1986). It should be noted also that there was no significant difference in the response of the different age groups to the various antirabies vaccines. Of particular interest was the mean titres and SP ratio for dogs over 12 months PV. There were relatively high and variable antibody levels among this group of dogs. This may be attributed to some field exposure to rabies or rabies-related viruses since antibodies obtained from vaccinations would have declined considerably by 12 months PV. This group of dogs could constitute a canid climax host which harbor inapparent infection and shed the saliva in the absence of clinical disease (Aghomo *et al*., 1986). A comparative prevalence and SP ratio of antibodies among dogs given different vaccines showed no significant difference ($p>0.05$). This may indicate a relatively uniform potency among the commonly available rabies vaccines in use in Nigeria. There should then be a periodic evaluation of these vaccines as the disease is an important zoonotic disease and poorly immunogenic vaccines would be a big public health risk. There is a significant relationship in the SP ratio and the booster vaccination. The percentage of dogs that were given booster vaccination in private clinics is higher than that reported for government-owned clinics (Adeyemi and Zessin, 2000). This may be related to the economic class of those that patronize the clinics and types of dogs involved. In this study, imported and expensive dogs were involved and the cost of antirabies vaccination cannot be a burden as compared to the low economic class that patronize the government-owned clinics where cheaper, locally produced but also effective vaccines are used (Adeyemi and Zessin, 2000; Adeyemi *et al*., 2000).

In adequately controlling this dreadful disease, the culling of stray and unvaccinated dogs should be considered a routine policy and intensive public health education programmes should be adopted. Moreover, the link between the veterinary and human health authorities should be strengthened and sharing of data on rabies and other zoonotic diseases should be encouraged. The supply of modern diagnostic tools to laboratories in order to aid quick detection of the disease is highly recommended.

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