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A Serosurvey of Rabies Antibodies in Dogs in Gaborone, Botswana

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Abstract: During a serosurvey of domestic dogs in Botswana, an immuno-enzyme technique (ELISA) was used to measure rabies antibodies in dogs. Serum samples were collected from the dogs at their visits to three small animal clinics, in and around Gaborone. Overall, 148/276 (54%) of the sera samples were positive with antibody levels ≥0.5 IU mL^{-1} indicative of effectiveness of protection against rabies challenge. Where vaccination history was available 90 dogs, 47/90 (52%) serum samples demonstrated ≥0.5 IU mL^{-1} with 43/90 (48%) having antirabies antibodies <0.5 IU mL^{-1}. Dogs that had history of two, three and four successive rabies vaccinations showed better responses with 63, 66 and 100%, showing levels ≥0.5 IU mL^{-1}, respectively, as compared to only 46% of dogs with a history of only one vaccination being positive. It is recommended that the practice of vaccinating of dogs against rabies annually be continued since multiple vaccinations appear to give effective protection against rabies in dogs.

Key words: Rabies, canine, ELISA, neutralizing antibodies

INTRODUCTION

Rabies is a serious global disease which invariably results in death of the affected individual which includes man, his domestic as well as wild animals. In Botswana as well as elsewhere, the domestic dog is the principal host and major vector of rabies (Crick, 1981; Wandeler *et al*., 1993; Mitoonpitak *et al*., 1998). Rabies remains endemic in animals in Botswana with rabid cases occurring consistently in considerable numbers throughout the country in both domestic and wild animals. The number of confirmed rabies cases in animals during a 15 year period (1977-1992) was 1651 of which 1413 (86%) cases occurred in domestic animals (25% were dog cases), while the remainder 238 (14%) were of wildlife origin. During the same period (1978-1991), 23 human rabies cases were confirmed in various districts (Tremlet, 1993). The persistent occurrence of rabies in large numbers in animals contributes to economic losses through deaths of animals and the resources needed in its control. Because of rabies's zoonotic potential, the disease constitutes a constant public concern generating steady interest in rabies virus over the years in Botswana and worldwide (Kuwert *et al*., 1985; Fekadu, 1991; Tremlet *et al*., 1994; Clique *et al*., 2004; Fachin *et al*., 2005; Ohore *et al*., 2007.

Vaccination has been found to be an effective protection against rabies virus infection in both man and animals (Kuwert *et al*., 1985; Hooper *et al*., 1998; Mattos *et al*., 2001). Various rabies vaccines have proven their safety and ability to efficiently induce high titres of protective antibody in man and animals elsewhere (Cleaveland *et al*., 1999; Mattos *et al*., 2001) however, there is lack of information regarding serological responses following vaccination against rabies in animals in Botswana. Serological tests for rabies virus neutralizing antibody vary from those employing infectious virus which necessitates the use of special containment facilities and are highly risk to the worker (Kuwert *et al*., 1985; Mattos *et al*., 2001).

Immuno-enzyme techniques (ELISA) for the detection of rabies virus antibodies in serum or plasma have proven more practical for detecting serological responses following vaccination and to quantify antirabies antibodies in serums or plasma of animals such as dog and cat (Atanasius *et al*., 1980; Sugiyama *et al*., 1997; Clique *et al*., 2004; Fachin *et al*., 2005). When assessing the efficacy of rabies vaccination, the World Health Organisation (WHO), Recommended the Mouse Neutralization Test (MNT) and the Fluorescent Serum Antibody Neutralizing Test (FSANT) (WHO, 1973). An antibody titre of 0.5 IU mL^{-1} and above after vaccination indicates protection against rabies according to the guidelines of the (WHO, 1992).

It was of interest to the present study to investigate the serological responses against rabies virus in dogs in Gaborone, Botswana.

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MATERIALS AND METHODS

Research design: The study area consisted of the urban and adjoining localities of Gaborone city. Serum samples were obtained from dogs from three veterinary clinics, during their normal scheduled visits.

ELISA procedure for detection of rabies antibodies: The serum samples were titrated using an ELISA technique in order to detect antiglycoprotein rabies antibodies in dogs (Kuwert *et al*., 1985). The test procedure used was as recommended by the supplier of the ELISA kits (Plateria Rage Rabies kits; (Bio-rad, France). The ELISA involved calibration of a standard curve using OD determinations obtained from reference positive and negative samples supplied in the kit. The titer of the unknown sample was determined with reference to the standard curve. The 96 standard microtiter plates supplied by the manufacturer were coated with the rabies glycoprotein antigen. The positive and negative control serums were diluted to 1/100th; followed by doubling dilutions in order to obtain the 1/200th, 1/400th, 1/800th, 1/1600, 1/3200, 1/6400 and 1/12800. The unknown serums were diluted to 1/100.

Calculations and interpretation of the results

Quantitative determinations: The means (OD) for each unknown serum and for each dilution of the positive and negative reference serums were calculated. The corrected values of the OD for each unknown serum: OD = OD of the 1/100th unknown serum minus (-) OD of the 1/100th negative control serum. For each dilution of the reference serums: OD = OD of the 1/×positive serum minus (-) OD of the 1/×negative serum. A graph plot was made of the OD reference curve (Y axis) as a function of the reciprocal of the reference serum dilutions (X-axis). The titre of the unknown serum was determined in International Units (IU) per mL (IU mL^{-1}), by finding the OD calculated for the serum on the Y-axis and then finding the corresponding point on the X-axis of the reference graph (curve).

Fluorescent serum antibody neutralization test: In order to validate the ELISA findings, 10 dog sera samples were randomly selected and sent for the fluorescent serum neutralisation test at OIE (Office international des epizooties/Organisation mondiale de la sante animale) reference laboratory at the Onderstepoort Veterinary laboratory, South Africa (OIE Manual, 2004).

RESULTS AND DISCUSSION

A total of 276 serum samples were obtained from three small animal clinics in and around Gaborone and analysed for antirabies antibodies using an indirect ELISA test. Some of the samples could not be analysed because they were dark or haemolysed.

Antirabies antibodies are measured in specialised laboratories in order to determine the degree of immunity of vaccinated subjects. WHO experts consider that a level of antibodies equal to or superior to 0.5 IU mL^{-1} adequately protects subjects exposed to the risk of contamination. In fact, animals with this level of antibodies have been shown to resist experimental infection induced by injection of the wild rabies virus (WHO, 1973, 1992; Cox *et al*., 1997).

Overall, 148/276 (54%) of the samples in this study were positive with rabies antibody levels ≥0.5 IU mL^{-1}. The level of immunity in the dog population sampled indicates that about half of the dog population sampled had levels of antirabies antibodies considered protective against rabies virus challenge. A considerable proportion of the dogs had antibody levels not considered protective against rabies in the wild. Indeed rabies in dogs and other species remains endemic in Botswana (Report, 2002). A higher prevalence of protective antirabies antibodies in vaccinated dogs may help reduce the incidence of rabies in dogs and other species. To prevent rabies outbreaks, WHO recommended that at least 70% of the dogs in a population be immunised (Coleman and Dye, 1996).

Dogs that had received two, three and four vaccinations against rabies showed better responses to rabies vaccine with of 63% (12/19); 66% (4/6) and 100% (2/2), respectively of them compared to only 46% (29/63, of dogs that had received one vaccination having rabies antibody levels of ≥0.5 IU mL^{-1} (Fig. 1). Dogs vaccinated twice or more in Japan remained seropositive for over one year (Sigiyama *et al*., 1997). Repeat vaccinated dogs had rabies antibody titers above 0.5 IU mL^{-1}, considered protective against rabies challenge (Shimazaki *et al*., 2003).

Table 1: A comparison of ELISA and fluorescent serum neutralization for detection of antibodies to rabies virus in dog sera

Sample number	ELISA results	FSNAT
D 9	Positive	0.9 IU mL^{-1}
D 64	Positive	0.7 IU mL^{-1}
D 148	Positive	0.7 IU mL^{-1}
D 149	Positive	7.9 IU mL^{-1}
D 173	Positive	2.0 IU mL^{-1}
D 228	Positive	0.7 IU mL^{-1}
D 5	Negative	0.1 IU mL^{-1}
D 141	Negative	0.0 IU mL^{-1}
D 151	Negative	0.2 IU mL^{-1}
D 232	Negative	0.01IU mL^{-1}

FSNAT = Fluorescent Serum Neutralisation Test, ELISA: Positive: ≥0.5 IU mL^{-1}; negative<0.5 IU mL^{-1}

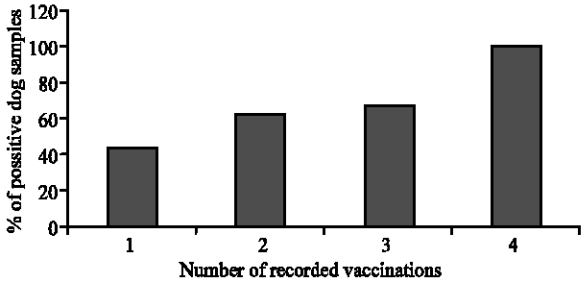

Fig. 1: Percentage of positive dog samples with = 0.5 IU mL^{-1} rabies antibodies following vaccination

The results of present ELISA study correlated with the results of the assays carried out by the OIE fluorescent antibody virus neutralisation test (Table 1). Based on the fact that virus glycoproteins induce neutralizing antibodies, serological tests that measure the vaccination response to glycoproteins should have a good correlation with OIE fluorescent antibody virus neutralization test, the latter test being one of the standard tests recommended by WHO/OIE (Habel, 1996). Antibodies induced by vaccination, particularly those with neutralising activity, play a prominent role in immune defence against infection (Hooper *et al*., 1998). Glycoprotein G-protein) is the primary surface antigen capable of inducing and reacting with the rabies Virus- Neutralising Antibodies (VNAs) (Cox *et al*., 1997).

Almost all major human and veterinary vaccines are based on the functional aspects of the G protein of rabies virus (Crick, 1981; Mattos *et al*., 2001). VNA exert their protective effect by neutralisation of extracellular virus, by complement-mediated lysis of virus- infected cells and by antibody-dependent cytotoxicity (Mattos *et al*., 2001). VNA can mediate viral clearance from the Central Nervous System (CNS) without other immune effectors (Dietzschold *et al*., 1992).

A number of serum samples taken from dogs with unavailable vaccination history had rabies antibody levels of ≥0.5 IU mL^{-1} indicating possible prior vaccination exposure since in Botswana, it is recommended that dogs be vaccinated annually, as this would boost their immune response to the rabies vaccine. The limited observation made in this study support the view that continued annual vaccination of dogs can generate adequate protection against rabies exposure in dogs and contribute towards control of rabies as a whole in Botswana.

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