

Assessment of anti Rabies post-vaccinal immunity in domestic carnivores pets in low resources setting

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Research note

Keywords: Ivory Coast, Rabies, ELISA, post-vaccinal immunity, dogs, cats

Posted Date: January 28th, 2020

DOI: <https://doi.org/10.21203/rs.2.21907/v1>

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Abstract

Objective

Rabies is a zoonotic disease transmissible to humans mainly by dogs. It is a vaccine preventable disease. In Ivory Coast, canine rabies is a priority and notifiable animal disease since 1962. Evaluation of post-vaccination immunity against rabies was undertaken between 2003 and 2009 by an immunoenzymatic test Platelia Rabies II ELISA Kits (Bio-Rad). We aim to investigate anti Rabies vaccination efficacy in Ivory Coast, using data obtained purposely for antibody quantification prior to international movement of pets.

Results

A total of 527 dogs and cats sera were analyzed. Generally cats had developed higher level of post vaccinal immunity than dogs. We have found that more than 95.80% (114/119) of cats had a serum with antibodies titer above 0.5 EU /ml so like 74.75% (n = 305) of dogs. Undetectable or insufficient level of seroconversion was noted either in the canine specie (25.25%) or in cats (4.2%). Multivaccinated Dogs, older than 4 years, represented the most important group (62.25%) of seronegative dog population. Associated with vaccination, serological results were used as data based-evidence in compliance with official requirements governing free movements of pets from rabies-infected to rabies-free european countries.

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Introduction

Rabies is a fatal zoonosis of global distribution. The disease could be controlled by vaccination, but still a neglected disease in most infected countries. Rabies is enzootic in Ivory Coast [1]. To regulate the international movement of domestic carnivores from infected countries to free territories from rabies, a system combining the anti-rabies vaccination of the animal and serological testing was launched. Preventive vaccination is considered successful by World Health Organisation (WHO) and World Organisation for Animal Health (OIE) when the neutralising antibody titre is at least 0.5 IU/ml in serum from both vaccinated humans and animals [2]. Neutralizing antibodies are good correlates of protection [2]. They were titrated by Fluorescent antibody virus neutralisation test (FAVN) [2] and the Rapid Fluorescent Focus inhibition test (RFFIT) [2] which are the reference methods [2]. Those reference methods needed high level biosafety laboratory and well skilled staff to be implemented [2]. The enzyme-linked immunosorbent assay (ELISA) technique could easily be performed in resources constraint setting. The use of Platelia Rabies II ELISA kit as an alternative method to quantify antibody to Rabies was certified by OIE [2]. It was compulsory for pets owners willing to travel to Europe with their animals, to strictly follow those above mentioned requirements. In that context, from 2003 to 2009, 119 cats sera and 408 dogs sera were tested in our referral laboratory in Ivory Coast. In this study, we aim to initiate a

preliminary investigation of vaccination efficacy in Ivory Coast using results obtained when were quantified anti rabies antibody titer in dog and cat serum samples.

Material And Methods

A total of 408 dog serum samples and 119 cat serums samples were collected by private veterinary clinics from previously vaccinated. Samples were packed and brought to the virology laboratory for testing under local regulations. The Virology laboratory is located in Bingerville, close to Abidjan, the capital City of Ivory Coast. It is a unit of the National Laboratory for the Development of Agriculture (LANADA). Antibodies to the glycoprotein of Rabies virus present in those sera have been titrated using the Platelia ELISA Kit, an indirect ELISA developed and manufactured by BIO-RAD (Marne-la Coquette-France). The test is performed including controls and standards following the instructions of the manufacturer. Test Validation and Calculation of serum titer were carried as previously described [3, 4]. Simultaneously, Sera titres were obtained after plotting corrected optical density of unknown serum sample against a standard curve. Titre is expressed as Equivalent Units per ml (EU/ml), unit equivalent to the international units defined by seroneutralization test.

Results

Serological efficacy of the vaccine

Antibodies titers covered a wide range of values. In canine specie, 103 out of 408 Sera from vaccinated dogs (25.50%) had a titer lower than the threshold value (0.5 EU / ml), and 74.75% (n = 305) have developed a detectable post-vaccinal immunity. More than 95.80% (114/119) of cats had a serum with antibodies titer above 0.5EU / ml). High levels of antibodies titres (Titre \geq 4EU/ml) were obtained for 84.87% (96/119) of vaccinated cats. Only, 4.20% of cats did not seroconvert. Cats have generally high antibodies titers. They presented a better immunization (Table 1). The rate of immunized Cats is significantly higher than dogs ($\chi^2 = 25.04 > 3.84$; $p < 0.0001$) (Table 2).

Table 1
Sera titers range obtained by ELISA PLatelia

	Titers (EU/ml)			Total
Species	< 0.5	[0.5 ;4 [\geq 4	
Dogs	103	240	65	408
Cats	05	13	101	119

Table 2
Seroconversion of dogs and cats

Serological status	Species	
	Cats	Dogs
Non Immunised (< 0.5EU/ml)	05(4.20%)	103(25.25%)
Immunised (\geq 0.5EU/ml)	114(95.80%)	305(74.75%)

Emphasis on negative canine sera

The percentage of non immunized dogs after vaccination is ranging from 21.80% to 28,20% (95% confidence interval). 79 dogs serums having undetectable or insufficient seroconversion level were considered along with vaccinal status and age of concerned pets. 62% (n = 47) of those sera were for dogs aged above 4 years and boosted several times. Whereas 15%(n = 17) of seroconversion failure was recorded during prime vaccination of dogs (Table 3).

Table 3
Characteristics of negative canine sera

Age	vaccinal status	
	Prime vaccination	Boosting
Puppies] 0–5 months]	07(6.41%)	
Young] 5-11months]	10(8.97%)	
Adults] 12–22 months]		02(01.28%)
] 22–48 months]		13(16.67%)
Adults] 4–6 years]		14(17.95%)
] 6–8 years]		21(26.92%)
] 8–10 years]		08(10.26%)
> 10 years		04(05.13%)

Discussion

During last 2000 decade, an evaluation of anti Rabies post-vaccinal immunity in domestic carnivores (cats/dogs) showed a satisfactory rate of immunization of 74.75% for dogs and 95.8% for cats (titre \geq 0.5 EU/ml). In addition, 84.870% of cats had antibodies titer greater than 4 EU/ml (\geq 4 EU/ml). Only 14.46% of dogs have reached such titers. Anamnestic immune response observed during this study for

cat (84.870%) and for some multivaccinated dogs (14.46%) are consistent with results obtained by Derbyshire and co-workers [5] and Cliquet and collaborators [6]. This hyperimmunisation of cats was probably linked to the ratio of the antigen to the animal weight [7]. Our study had revealed that 25.25% and 4.2% respectively of dogs and cats were seronegative. In France, a rate of seroconversion failure estimated to 7.4% and 1.9% has been reported in dog and cat, respectively [6]. Also, we had found especially adults dogs, which did not seroconvert. Mainly, dogs older than 4 years and multivaccinated represented the most important (62.25%) seronegative dog population [8]. This result probably could be explained by a deficiency of the immune system of old dogs. We also observed that 21.52% of seroconversion failure occurred during prime vaccination of puppies and juvenile dogs. It was likely due to maternal antibodies interference leading to inhibition of post vaccinal immunity [9–11]. Due to the increasing number of puppies affected by rabies, vaccination is promoted for puppies aged above 8 weeks with an attenuated vaccine [12]. Therefore, many reasons had to be considered in case of vaccination failure such as the health status, immunization protocol and the race of the animal. But also, poor quality of sera due to collection and transportation conditions to the laboratory could affect the ELISA result. Sometimes, dogs are bled just after vaccination. Normally, animals should be sampled after thirty post vaccination days at least to enable the immune response to reach a detectable level [2, 6].

In Ivory Coast, regards to the failure rate of seroconversion, exposure to dog though vaccinated might be harmful. Therefore, the role of vaccinated dogs in transmission of Rabies should widely be investigated to assess the real risk they represent. In addition, this may help to estimate optimal vaccination coverage for the control of rabies in the country.

In conclusion, it was estimated a rate of seroconversion failure around 28% by Platelia ELISA for dog. This result suggested to pay attention to dog vaccination practice. Findings may suggest reschedule of immunisation protocol. Subsequently, sustainable management of old dogs population should be implemented. ELISA is a versatile tool for the titration of antibody to Rabies virus in low resources countries such as in Ivory Coast. It is easier to be performed than gold standard FAVN or RFFIT. Recent developments had prompted out others ELISA kits with satisfactory performance [13].

Limitations

Our study may present limitations. Indeed, it has been also reported the Platelia ELISA has a weak sensitivity in quantifying antibody in sera which had low titer [2]. Titers obtained by Platelia Elisa were regarded as underestimated [4]. The use of this kit was abandoned due to controversies on its performance and validation process [2]. Today, OIE stated that Virus neutralisation and enzyme-linked immunosorbent (ELISA) assays are suitable tests for monitoring the antibody response of vaccinated animals in sight of rabies control. For the purposes of measuring antibody responses to vaccination prior to international animal movement or trade, only viral seroneutralisation methods (FAVN test and RFFIT) are acceptable. ELISA does not fit for this purpose anymore.

Abbreviations

WHO

World Health Organisation

LANADA

Laboratoire National d'Appui au Développement Agricole

OIE

World Organisation for Animal Health

FAVN

Fluorescent antibody virus neutralisation test

RFFIT

Rapid Fluorescent Focus inhibition test

ELISA

Enzyme linked immunosorbent assay

Declarations

Ethics approval and consent to participate

Our study is a non-experimental research on animals. It has been carried according to the official mandate given to LANADA by the Government of Ivory Coast. Therefore, all applicable international, national, and institutional guidelines for the use and care of animals were followed.

Consent for publication

Not Applicable

Availability of data and material

Not Applicable

Competing interests

The authors stated that they have no conflict of interests to declare.

Funding

This study was supported entirely by the budget of the Virology Laboratory which a unit of the LANADA.

Author's contributions

YMK, coordinated laboratory testing, performed the analysis and wrote the original draft of manuscript. CKK participated to testing and data interpretation. VKK, LK, HGP, KHA, LSAK were involved in preparation of the manuscript. CHE designed the study and supervised laboratory investigation, approved the final version of the manuscript like all co-authors.

Acknowledgements

We would like to thank some colleagues, Mr Adje Kouassi, Mr Konan Kouadio for their great contribution to the diagnosis of rabies. We are also grateful to private veterinarians for their huge contribution to samples collection and submission to the Virology Laboratory.

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