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Seroepidemiological survey to cell culture rabies vaccines (CCRV) in Brazil

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A B S T R A C T

Rabies is a contagious viral disease that can be easily transmitted by the saliva and brain/nervous system tissues of the infected animals, causing severe and fatal encephalitis in both animals and humans. Vaccination campaigns are crucial to combat and prevent rabies's spread in dogs and humans. The Modified Fuenzalida & Palicios vaccines have been widely used since the 70s and have proven effective in producing a solid serological response. Since 2008, the Brazilian Ministry of Health has introduced a Cell Culture Rabies Vaccine (CCRV) for all dog mass vaccination campaigns in Brazil. However, to date, there is limited evidence on the immunologic response of dogs to this type of vaccine in field conditions. The present study evaluated the serological response in dogs vaccinated with CCRV from blood samples of 724 dogs using the Simplified Fluorescence Inhibition Microtest – SFIMT. Dogs with a titer equal to 0.5 IU/mL or above were considered seropositive. The results revealed that 59.12% (428/724) of all dogs tested and 48.49% (32/66) of primo-vaccinated animals were seropositive. The percentage of seronegative animals was higher than seropositive for animals that received a single dose during their life ($p < 0.05$). The opposite was observed in animals with five or more doses. The results of this study demonstrated that the CCRV vaccines elicit a satisfactory immunological response in field conditions and can constitute an essential population-level preventive strategy as part of annual canine rabies vaccination campaigns. Although its effectiveness has been studied, there is limited evidence of its immunological response in dogs under field conditions. This paper evaluates the serological response to CCRV in dogs vaccinated during mass vaccination campaigns from 2012 to 2017.

1. Introduction

Rabies is an acute viral disease caused by a *Lyssavirus* from the *Rhabdoviridae* family that can affect all mammals, including humans. It is characterized by acute and progressive encephalitis with approximately 100% mortality [1], causing about 59,000 deaths annually, of which 59.6% occur in Asia and 36.4% in Africa [2]. In 2020, WHO proposed a global plan for rabies control called "Zero By 30" to reduce the transmission from dogs and subsequent human deaths to zero by 2030.

The incidence of reported human and dog rabies cases in Latin America and the Caribbean has decreased considerably through sustainable control [3]. Approximately 40% of suspected rabies exposure incidents occur in children under 15 years [4], with an estimated global economic cost of $8.6 billion [5].

Rabies virus antigenic variant two can be eliminated from the urban environment by adopting efficient prevention measures such as human and animal vaccination, availability of anti-rabies serum, and surveillance actions [6]. Mass animal vaccination campaigns have been shown to reduce the incidence of dog-mediated rabies. In developing countries, 70% of reported rabies cases are caused by dogs and cats, so vaccination campaigns targeting these species are crucial [7].

In Brazil, since its introduction in 1973, the National Rabies Prophylaxis Program (PNPR) has been considered one of the priority programs of the national health policy to promote systematic activities for combatting human rabies and disease control in animals [8]. The canine

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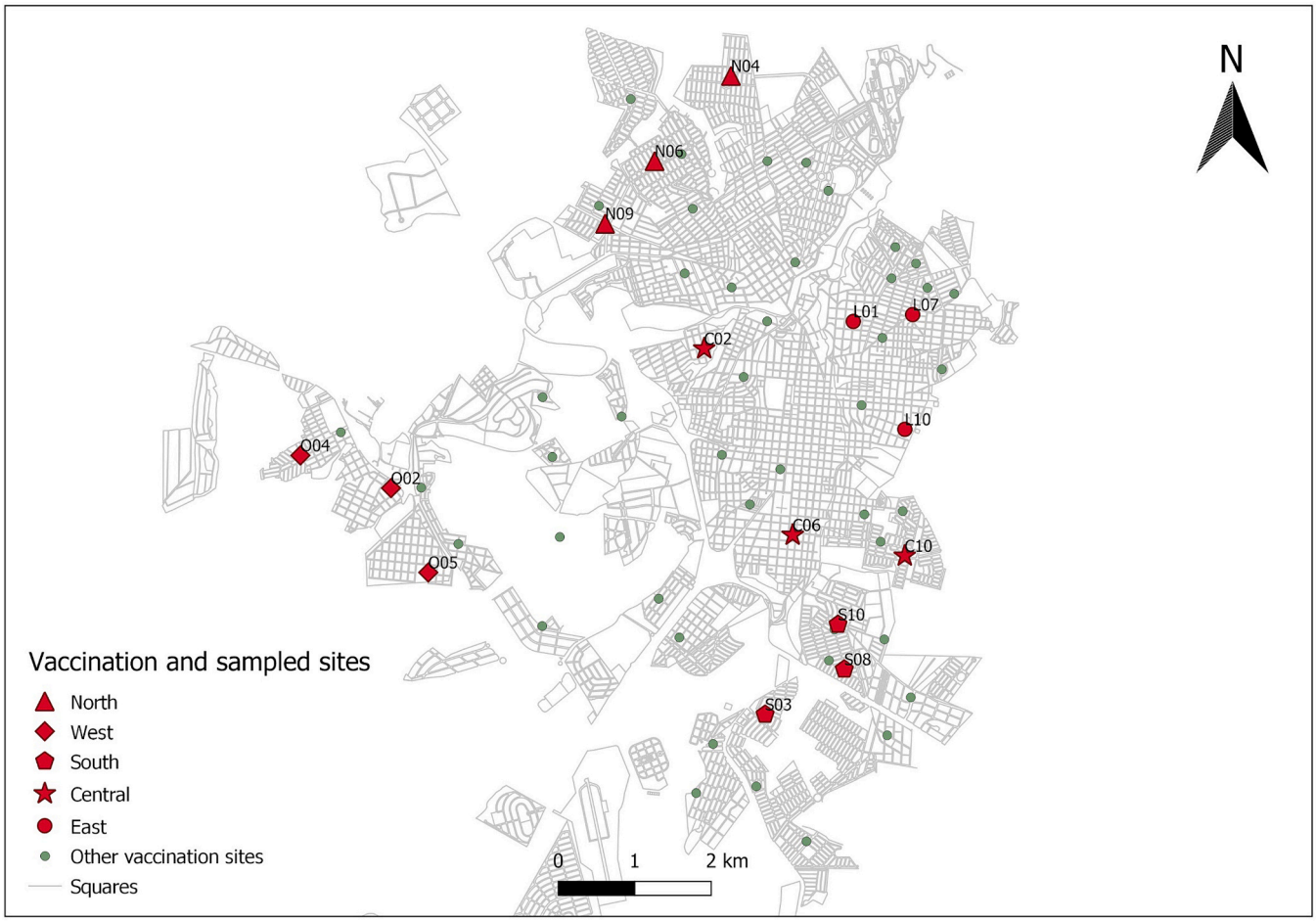

Fig. 1. Vaccination and sampled sites of dog blood collection. Botucatu – Brazil, 2023.

rabies vaccination campaigns in Brazil resulted in a significant decrease in both human and canine rabies notifications from over 1200 canine rabies notifications and a mortality rate of 0.014 human deaths per 100,000 inhabitants in 1999 to 9 canine rabies notifications and no reported human cases in 2018 [1].

In 1973, the São Paulo Government created the Standing Committee on Rabies Control (CPCR), coordinated by the Sao Paulo Pasteur Institute, which coordinates the State's Rabies Control Program [9,10]. However, the first rabies control activity in the Sao Paulo state in Brazil dates back to 1968 in Botucatu, supported by the Faculty of Veterinary Medicine and Animal Science from Sao Paulo State University – UNESP.

Rabies control activities in the municipality of Botucatu have occurred annually for the past 50 years. In 2010, it was awarded "The Best Regional Event in Rabies Prevention and Control in Latin America and the Caribbean" by the Pan American Health Organization (PAHO), the World Health Organization (WHO), and the Global Alliance for Rabies Control [11]. In the municipality of Botucatu, the last case of canine rabies was diagnosed in 1985 [12]. Much of this success can be attributed to the high vaccine coverage achieved by the annual rabies vaccination campaigns. Rabies mortality in dogs is more significant in unvaccinated animals and animals that do not develop detectable antibody levels, as those who produce neutralizing antibodies rarely become sick when exposed to the virus [13].

Public mass animal rabies vaccination campaigns in Brazil were carried out with the modified Fuenzalida & Palacios vaccine, made from infected suckling-mouse brains, before 2009 [14,15]. Since 2008 [16], the Brazilian Ministry of Health has approved using cell culture rabies vaccines (CCRV). The main viral strains used in the manufacture of those vaccines are Pasteur virus (PV), Pitman-Moore (PM), or Flury Low Egg passage (LEP), which effectively protect against Rabies lyssaviruses circulating in Brazil [17].

Developing an adequate immunological response following canine CCRV vaccination is a significant strategy to prevent human rabies, especially when dogs or bats are the main reservoir [18]. Silva et al. [19] evaluated the immune response of 432 healthy dogs in field conditions receiving a single vaccine dose and reported that 21.76% did not present protective titers against the rabies virus. Similarly, Fernandes et al. (2017) [20] investigated the frequency of neutralizing antibodies in dogs with and without a vaccination history, revealing that 30% of vaccinated dogs did not present adequate antibody response.

Previous studies suggest that the humoral response in dogs that received Fuenzalida & Palacios vaccines may be compromised after 12 months, especially in single-dose situations [21]. No further investigations were found in the scientific literature about the humoral response in dogs from Brazil after the vaccine change.

The present study aimed to evaluate the serologic response of dogs who have received CCRV in field conditions.

2. Material and methods

The study received research ethics approval from the Animal Ethics Committee (CEUA) from the Faculty of Veterinary Medicine and Animal Science (FMVZ) of the Sao Paulo State University – UNESP under protocol 0166/2017. All 724 owners of the animals participating in this study signed a consent form.

Botucatu municipality is located 235 km from Sao Paulo State capital, in the central region of the State at 22° 53′ 09" S and 48° 26′ 42″ E and 840 m above sea level with a population of 127,328 inhabitants

Table 1
Composition of vaccines used at the mass vaccination campaigns between 2012 and 2017. Botucatu – Brazil, 2023.

Type	Vaccine 1	Vaccine 2	Vaccine 3	Vaccine 4
2012	X			
2013	X			
2014	X			
2015		No vaccination		
2016	X	X	X	
2017			X	X

Vaccine 1 - High concentrations of fixed rabies virus, grown in cell culture, inactivated by betapropiolactone and added with aluminum hydroxide; Vaccine 2 - Rabies virus suspension, Pasteur Virus (PV) strain, grown in BHK cells and inactivated by Binary Ethyleneimine (BEI); Vaccine 3 - Rabies virus suspension, PV strain, cultured in BHK cells; Vaccine 4 - Fixed rabies virus suspension, PV, of cell culture origin, with aluminum hydroxide gel as adjuvant.

(85.88 inhabitants/km^2), 96% ($n = 106{,}851$) of which live in the urban/metropolitan area. The municipality's area is 1482.874 km^2, of which 154 km^2 corresponds to the urban area and 1329 km^2 to the rural area [22]. 2018, the municipality's total estimated dog population was 27,735 dogs [23].

A random sample of 724 dogs was selected during the 46th Dogs and Cats Rabies Vaccination Campaign, held from September 11th to 15th, 2017, from 15 vaccination sites with the highest vaccination rate in the previous campaign, distributed throughout the urban area (Fig. 1).

Animals participating in the study were randomly selected during vaccination activities at the posts with the highest volume of dogs registered for vaccination in the previous year. Therefore, three stations were chosen for each of the five sectoral regions of the municipality, totaling 15 collection stations. Owners of participating dogs were invited to participate in the investigation, and those who accepted were included in the study. Sampled dogs were chosen from an estimated population of 27,735 animals, considering 75% of participation acceptance with free and informed owner consent, an estimation error of 3.65%, and a confidence level of 95% [24].

Individual animal data, including sex and age of the animal, body condition, clinical signs, treatments, periodical deworming, feeding type, vaccination history and rabies vaccine history number, and contextual factors, including the number of animals housed, place of residence, and vaccination service, was collected from owners via a questionnaire. The same team of trained blood collection officers delivered the questionnaire to all owners.

The vaccines used during the evaluation period are described (Table 1). Four different laboratories produced each type of vaccine, and despite all vaccines being made in cell culture, they presented different compositions. The present study did not evaluate the response of the various types of vaccine compositions.

Blood samples were collected from the cephalic or jugular vein with 5 mL syringes and 25 × 7 mm needles and transferred to test tubes without anticoagulants. After coagulation, the lines were centrifuged at 1600*g* for 10 min. The resulting serum was transferred to microtubes and frozen at −20 °C. Serum determination of neutralizing antibodies to rabies was performed at the CCD-SES Pasteur Institute Rabies Diagnostic Laboratory of Sao Paulo (WHO Collaborating Center on Rabies and National Reference Laboratory for Rabies Diagnosis), by the Simplified Inhibition Microtest. Fluorescence - Simplified Fluorescence Inhibition Microtest – SFIMT [25,26] using the Pasteur Virus (PV) strain. In this study, a neutralizing antibody level of 0.5 IU/mL or higher for RABV was considered an adequate titer (seropositive), as the World Health Organization (WHO) and the World Organization for Animal Health (WOAH) recommended.

Statistical analyses of the association between serum response and animal characteristics were performed in three groups according to the year and the type of vaccine used at the mass campaign (Table 1). The first comparison considered animals with a single dose vaccine nominated as primo vaccinated. The second comparison included animals under five years old who received CCRV only, and the third group included animals over five years old who possibly received Fuenzalida & Palacios vaccine doses. For statistical analysis, animals were classified according to age as "< 12 months, 1 to 5 years, and > five years old"; the number of antirabies vaccine doses as 1,2,3,4 and 5 or more doses; the year of the last antirabies vaccination (classified as 2011, 2012, 2013, 2014, 2015, 2016); the animal's body condition (classified as thin, ideal, overweight and obese).

The association between the presence or absence of adequate titer and intrinsic or extrinsic variables was performed by Goodman's Association Test [27], complemented with multiple comparisons between and within variables. We conducted exploratory analyses using SPSS Statistics, considering the owners' responses. A p-value of <0.05 was considered statistically significant.

3. Results

From sampled dog population (724), 516 (71.27%) presented ideal body conditions, 586 (80.94%) were domiciled, 387 (53.45%) were dewormed, 387 (53.45%) were not neutered, 430 (59.39%) feed on commercial food, 690 (95.31%) were not receiving any disease treatment, and 491 (67.82%) were vaccinated in public vaccinations services (Fig. 2).

Of sampled dogs, 428 (59.12%) were seropositive, and 296 (40.88%) were seronegative.

A statistically significant association was observed between the seropositivity and the number of doses the animals received (Table 2). In animals that received a single dose during their life, the percentage of animals seronegative is higher than the seropositive ($p < 0.05$). The opposite was observed in animals with five or more doses, with a higher seropositivity proportion.

In the group of animals over five years, the proportion of seropositive animals is higher than that of non-seropositive animals ($p < 0.05$). However, the frequency of non-seropositive animals is higher in animals under 12 months of age. In both the seropositive and non-seropositive groups, animals are predominated between 1 and 5 years (Table 3).

Periodic deworming was shown to have a positive association with seropositivity, as the proportion of animals seropositive was significant in the frequently dewormed group ($p < 0.05$) (Table 4).

Regarding primo-vaccinated animals, there was an association between the seropositive group and body scores since the percentage of seropositive animals with ideal body scores was higher than the animals non-seropositive ($p < 0.05$). In contrast, the proportion of non-seropositive animals is higher in overweight animals. It was also observed that dogs who received other vaccines concomitant with rabies presented a better seroconversion ($p < 0.05$). The opposite is true for animals that receive the rabies vaccine only. There was no association between gender, sterilization, age, clinical signs, treatments, type of feeding, place of residence, service used for vaccine administration, and the presence of adequate titer of primo-vaccinated dogs.

4. Discussion

Our results revealed that rabies cell culture vaccines promoted a better humoral response than verified by Rigo and Honer [28] with the modified Fuenzalida & Palacios vaccine in the general dog population, who found that 170 (51.1%) of 333 dogs did not develop adequate titers for the latter vaccine. However, in the same study, researchers found no association between the number of doses and an adequate titer, an association that could be verified in the present study.

There are just a few studies in the scientific literature on the immune response conferred by the cell culture vaccine in domestic animals attending annual rabies vaccination campaigns and are subject to various forms of exposure to the virus in varying clinical, nutritional, environmental, socioeconomic and maintenance, which does not allow

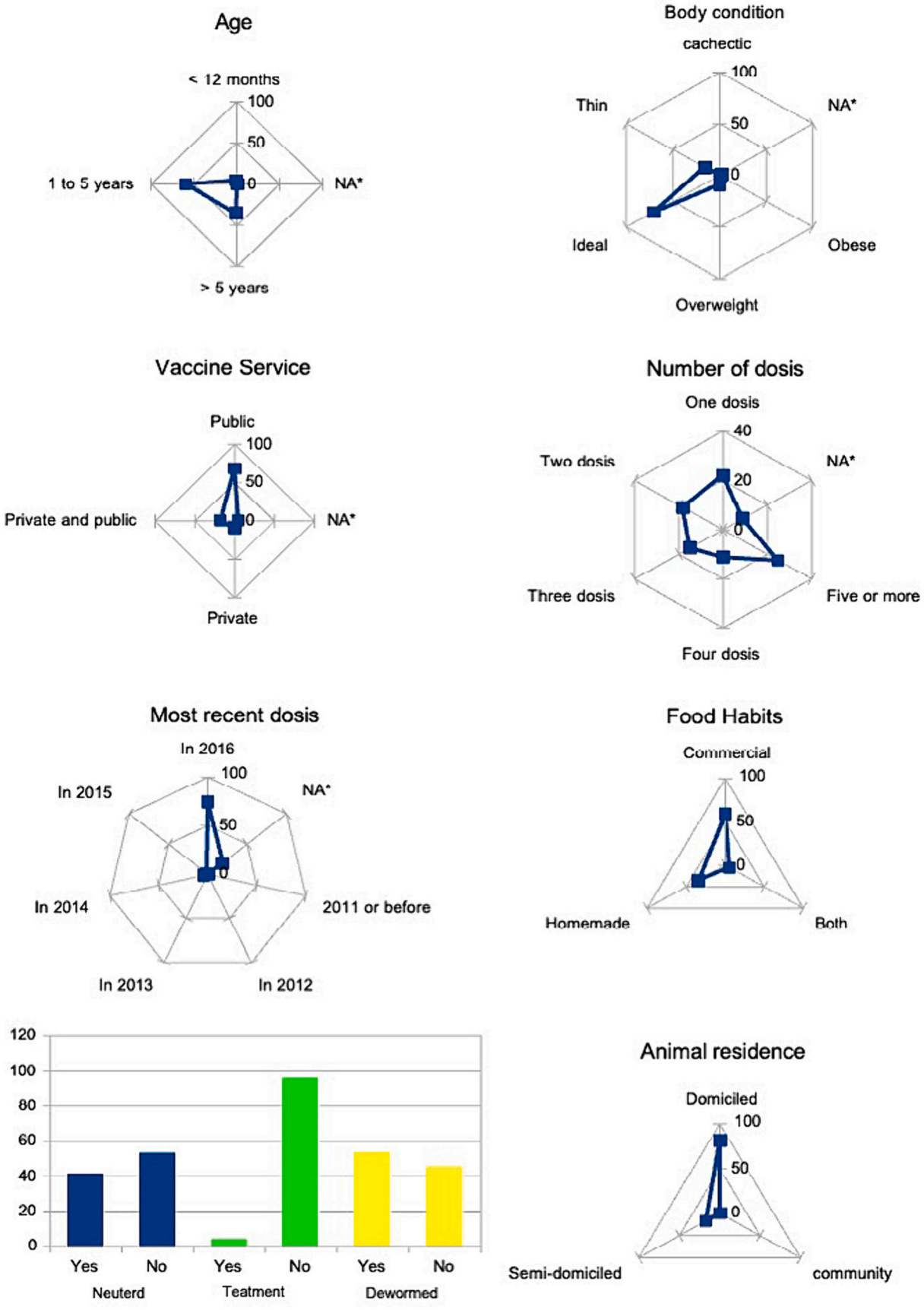

Fig. 2. Frequency of dogs in percentage regarding their intrinsic characteristics. Botucatu – Brazil, 2023.

Table 2
Serum evaluation in the sampled dog population according to the number of doses received. Botucatu – Brazil, 2023.

	Number of doses						
Seropositive	1	2	3	4	+ 5	Total	Not answered
No (<0.5 IU/mL)	107(0.391)bB	53(0.193)aA	43(0.157)aA	23(0.084)aA	48(0.175)aA	274	22
Yes (≥0.5 IU/mL)	56(0.142)aA	78(0,198)aA	68(0.173)aA	59(0.150)bA	133(0.337)bB	394	34
					Total	668	56

Two proportions followed by the same lower case, within a fixed number of doses range, do not differ ($p > 0.05$) from each other in seropositivity; Two proportions followed by the same capital letter, within the same response, do not differ ($p > 0.05$) from each other regarding the number of doses groups.

Table 3
Serum evaluation in the sampled dog population according to age. Botucatu – Brazil, 2023.

	Age				
Seropositive	< 12 months	1 to 5 years	> 5 years	Total	Not answered
No (<0.5 IU/mL)	27(0.092)bA	184(0.626)aC	83(0.282)aB	294	2
Yes (≥0.5 IU/mL)	4 (0.010)aA	247(0.585)aC	171(0.405)bB	422	6
			Total	716	8

Two proportions followed by the same lower case, within a fixed age range, do not differ ($p > 0.05$) from each other in seropositivity; Two proportions followed by the same capital letter, within the same response, do not differ ($p > 0.05$) from each other regarding the age groups.

Table 4
Serum evaluation in the sampled dog population according to the deworming routine. Botucatu – Brazil, 2023.

	Deworming			
Seropositive	No	Yes	Total	Not answered
No (<0.5 IU/mL)	149(0.505)bA	146(0.495)aA	295	1
Yes (≥0.5 IU/mL)	180(0.428)aA	241(0.527)bB	421	7
		Total	716	8

Two proportions followed by the same lower case, within a fixed age range, do not differ ($p > 0.05$) from each other in seropositivity; Two proportions followed by the same capital letter, within the same response, do not differ ($p > 0.05$) from each other regarding the deworming groups.

us to discuss the topic further. However, Silva et al. [19] evaluated the immune response of dogs that were admitted to the European Community, supposedly with adequate nutritional and health status, who received a single dose of the cell culture vaccine and found that out of 432 samples, 21.76% had no adequate titer against the rabies virus, and 67.02% of which were puppies.

When the relationship between immune response and the age of dogs was analyzed, a better immunologic response to the cell culture vaccine in dogs older than five years was observed compared to animals younger than 12 months. This is probably due to the maturation of the immune system and the number of vaccine doses received throughout their life course. This difference was reported by Silva et al. [19], who stated that adult dogs show a better immune response when comparing their average neutralizing antibody levels with younger animals, thus requiring a second dose of vaccine before 12 months, which would increase the possibility of a faster, more significant, and longer-lasting response.

Some authors [21,29,30] observed an abrupt drop in serum anti-rabies antibody concentration for the Fuenzalida & Palacios vaccine, suggesting that a vaccination booster between the annual campaigns is needed. Vaccine boosters were also more efficient in dogs that had received two or more previous vaccinations.

The present study also observed that the number of vaccine doses is associated with the immune response since dogs that received four or more doses of cell culture vaccine responded significantly better than those that received only one dose. This immunologic response can be explained by the immune system's maturation, which becomes more competent over time, and by the consecutive stimulation caused by the vaccine, making the immune response more efficient in antibody production.

It was also possible to verify that dewormed, under treatment, and neutered dogs had a better vaccine response, suggesting a positive relationship with the responsible ownership as owners who are worried about their dog's health are more likely to maintain a regular vaccination schedule, deworming, and feeding care. Additionally, animals free from other infections and in good health have the most competent and responsive immune systems, whether by natural or artificial active immunization.

The World Health Organization posits that 70% canine vaccination coverage is necessary to eliminate canine-mediated transmission. However, vaccination may not guarantee that the animals retain adequate seroprotection [4]. Although previous studies have shown that most states in Brazil surpass 80% canine vaccination coverage, our results found that <60% of sampled dogs had protective rabies-neutralizing antibody titer. In particular, primo-vaccinated dogs had lower seropositivity than other groups, suggesting additional boosters may be necessary to maintain herd immunity between annual campaigns [31].

In the primo-vaccinated dog group, it was found that the characteristics related to adequate titer differ concerning the general canine population. In this group, body score proved an important attribute linked to the animal response. Dogs classified as having an ideal body score stood out considerably compared with the other scores in the adequate titer primo-vaccinated animal category. This finding corroborates that well-nourished animals become more competent in developing antibodies against natural infections and more responsive when subjected to vaccination protocols. This is also true for primo-vaccinated dogs receiving doses of vaccines not only against rabies but also other diseases.

5. Conclusions

The immunologic response of dogs to rabies culture cell vaccine is satisfactory in the context of mass dog vaccination campaigns. The immunologic response is likely to be improved by multiple dog vaccinations and adopting responsible ownership practices such as caution

with animal welfare, neuter-ing, deworming, and maintaining vaccination against other diseases.

The present study's findings suggest that the seroconversion after the primo vaccination is unsatisfactory, indicating that a vaccine booster may be needed after one month.

It was also possible to conclude that the serum titer of neutralizing antibodies against rabies decays within a year, especially revealing the need for an annual vaccine booster.

AI statement

While preparing this paper, the author(s) used Grammarly® to review the translation from Portuguese to English. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the publication's content.

CRediT authorship contribution statement

Rodrigo Iais da Silva: Validation, Resources, Investigation, Formal analysis. **Luciana Botelho Chaves:** Validation, Methodology, Formal analysis. **Sandriana dos Ramos Silva:** Validation, Methodology, Formal analysis. **Iana Suly Santos Katz:** Validation, Methodology, Formal analysis. **Elaine Raniero Fernandes:** Validation, Methodology, Formal analysis. **Rene Cunha Neto:** Validation, Methodology, Formal analysis. **Carlos Roberto Padovani:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Jose Rafael Modolo:** Writing – review & editing, Validation, Methodology. **Ricardo J. Soares Magalhaes:** Writing – review & editing, Validation, Methodology. **Holly Crompton:** Writing – review & editing, Validation, Methodology. **Cassiano Victoria:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Data curation, Conceptualization.

Declaration of competing interest

The author(s) declares no conflict of interest in this work.

Data availability

Data will be made available on request.

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