International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 41 No. 1 Nov. 2023, pp. 39-47

http://www.ijias.issr-journals.org/

Assessing variation of rabies antibody titers in domestic cats in the context of pet travel from Morocco

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ABSTRACT: Rabies is a deadly disease that can spread between mammals. Morocco is a rabies-endemic country. To maintain rabies-free status, some countries require rabies antibody titration tests. This titration is subject to variation. This retrospective study was conducted to determine the factors associated with humoral response variation in rabies-vaccinated domestic cats in the context of pet movements. Factors such as the animal's sex, its age, the laboratory, the test used (FAVN or RFFIT), the time between vaccination and rabies titration sampling, and the number of vaccinations were taken into account. Information on 959 cats was collected, and structural modeling utilizing logistic multinomial regression was used. The results showed that 57.1% of the cats had a titer exceeding 10 IU/ml, 32.1% had a moderate response (3<t<10) and 10.7% of the cats had a titer between 0.5 and 3 IU/ml. Females had a slightly better antibody response than males. The time between the last vaccination and the sampling for titer measurement had a significant influence on the titration; the longer the delay (more than 60 days), the lower the probability of obtaining a high titer. At the high titration response level, the differences according to the factors laboratories and tests performed (FAVN, RFFIT) were statistically significant. Likewise, the more vaccinations cats receive, the better their humoral response. It comes from this study that cats were well immunized after rabies vaccination, and the number of vaccinations is the most important determinant of titration variation in the feline species. Regular boosters are therefore recommended to protect the feline population from rabies infection.

KEYWORDS: Anti-rabies titration, Cat, Morocco, Rabies, Vaccination.

1 INTRODUCTION

Rabies is caused by neurotropic viruses of the genus Lyssavirus in the family Rhabdoviridae in the order Mononegavirales, and it can affect all mammals [1]. Globally, the rabies virus (RABV) causes an average of 59,000 human deaths each year, with 36.4% in Africa and 59.6% in Asia [2]. Lyssaviruses have a negative-polarity, non-segmented RNA genome that codes for five structural protein genes in the order 3'-N-P-M-G-L-5'. These five structural protein genes are separated by non-transcribed intergenic regions. Glycoprotein (G) represents the major target antigen of the protective immune response [3]. Inactivated RABV particles are highly immunogenic and form the basis of most animal rabies vaccines [4]. The Vaccination Guidelines Group (VGG) recommends that the rabies vaccination protocol for cats should start at 3 months of age, followed by an annual booster. Thereafter, according to the manufacturer's license or local legislation, they could receive either annual or triennial boosters [5]. For international pet travel, some countries require a rabies serological test. To enter European Union countries, tests must be carried out in EU-approved laboratories [6]. The recommended tests for antibody titration are fluorescent antibody virus neutralization (FAVN) and the rapid fluorescent focus inhibition test (RFFIT). A titer of 0.5 IU/ml is considered by the WHO

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(World Health Organization) and WOAH (World Organization for Animal Health) to be the minimum titer level of protective antibodies [1; 7].

The principle of serum neutralization tests (RFFIT and FAVN) is to measure the ability of rabies-specific antibodies to neutralize RABV and prevent the virus from infecting cells in vitro. The presence of fluorescence in the cells correlates with non-neutralized RABV [8]. The ED50 (50% effective dose) neutralization titer is defined as the dilution at which 50% of the observed microscopic field contains one or more infected cells; the ED50 titer is converted to IU/mL if a reference standard is included in the assay [8].

Previous studies have shown that the rabies antibody response of cats vaccinated against rabies depends on certain factors, such as the animals' age and sex, the timing of blood sampling for rabies titer measurement, and the number of vaccinations received [9; 10; 11; 12]. Using information from the archives of national veterinary services about the health certification of cats going abroad, this study aims to find out how immune responses vary depending on things like sex, age, time between vaccination and sample collection, number of vaccinations, laboratory, and test used (FAVN or RFFIT). This kind of study has never been conducted in Morocco.

2 DATA AND METHODS

2.1 DATA AND VARIABLE'S SPECIFICATION

The present study is a retrospective study based on archived data from five Moroccan veterinary services, including those of Rabat, Casablanca, Tanger, Marrakech, and Mohammedia. The data used was taken from the rabies vaccination records and laboratory test reports of 959 cats intended for international travel between 2012 and June 2022. These data included the animals' microchip number, age, sex, name of the vaccine and history of vaccinations, dates of blood samples, the type of tests performed, and the laboratory where the tests were conducted.

The vaccines used were from different national and international manufacturers. The vaccines used were monovalent and multivalent. Two monovalent vaccines from international producers were administered to 63% and 10% of the studied animals, respectively. The remaining 27% of the studied animals were vaccinated with 16 different vaccines.

Titration results ranged from 0.5 IU/ml to values above 13 IU/ml. Some laboratories did not specify the titration level (t) when it was higher than 10 IU/ml or 13 IU/ml; for this reason, the variable "titration level" was adopted as a qualitative variable. Cats were thus classified into three categories: high responders with a high titration level ($t \geq 10$), moderate responders ($3 < t < 10$), and low responders ($0.5 \leq t \leq 3$).

Based on the age at primary vaccination, cats were classified into four groups: <=120 days (d), [120 d, 365 d], [365 d, 2190 d], and +6 years.

Based on laboratory representativeness, they were coded as follows: L1, L2, L3, and others. Over 86% of analysis reports came from L1 (7.82%) with 75 results, L2 (68.71%) with 659 results, and L3 (10.74%) with 103 reports. The remaining 15 laboratories represent 12.72% (122 analysis reports). These laboratories used only one type of test, either FAVN or RFFIT.

According to the number of rabies vaccinations received, the cats were divided into four groups: G1, G2, G3, and G4 and above (+G4). The numbers from 1 to 4 represent the number of vaccinations.

The interval between the vaccination date and the date of blood sampling for titer measurement analysis (TVTM) was divided into five categories: [3d-30d], [31d-60d], [61d-90d], [91d-180d], and more than 180d. The dates of collection for G1 were performed 30 days or more after the vaccination date.

The specifications of the variables (titer groups, sex, age, TVTM, laboratory, test, and number of vaccinations) are shown in Table 1.

Table 1. Variable's specifications

Variables	Type	Levels	Reference level
Titer groups	Target variable, Qualitative with 3 levels	High response= (t≥10) Moderate response = (3<t<10) Low response= (0.5≤t≤3)	Moderate response
Sex	Predictor variable, Qualitative with 2 levels	Male Female	Male
Age at first vaccination	Predictor variable, Qualitative with 4 levels	<=120d] 120d, 365d]] 365d, 2190d] +6 years	<=120d
Time from vaccination to sampling for titer measurement in days (TVTM)	Predictor variable, Qualitative with 5 levels	[3d-30d] [31d-60d] [61d-90d] [91d-180d] + 180 d	[3d-30d]
Laboratory	Predictor variable, Qualitative with 4 levels	L1 L2 L3 Others	L1
Test	Predictor variable, Qualitative with 2 levels	RFFIT FAVN	FAVN
Groups according to the number of vaccinations	Predictor variable, Qualitative with 4 levels	G1= Cats with one vaccination. G2=Cats with two vaccinations G3=Cats with three vaccinations +G4=Cats with four or more vaccinations	G1

2.2 METHODS

The data from this study were analyzed using the R programming language. A structural modelization using multinomial logistic regression has been made to find out how factors including sex, age of the animal, the laboratory, type of test used (FAVN or RFFIT), time between vaccination and collection, and number of vaccinations affect the immune response to rabies vaccination in cats. All codes, outputs, and figures are the author's own work, as are the manipulations under the R programming language [13].

3 RESULTS

3.1 DESCRIPTIVE STATISTICS

Out of 959 studied cats, 57.1% showed a high response to rabies vaccination (t≥10), 32.1% had a moderate response (3<t<10), and only 10.7% had a low response (0.5≤t≤3) (Figure1).

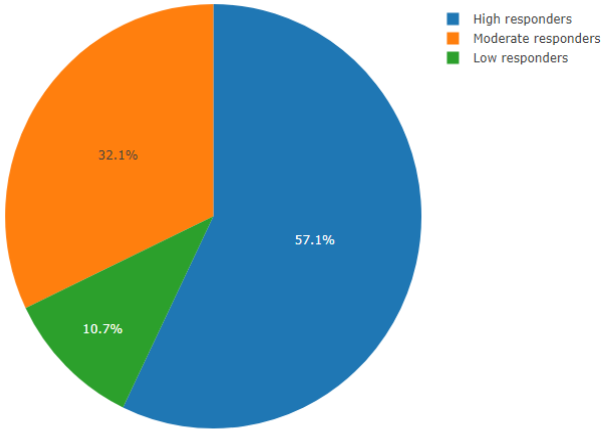

Fig. 1. Distribution of studied cats according to their rabies titration level.

The titration level variation according to animals' sex is shown in Table 2. The sample included 521 females and 438 males. In female cats, 61.04% were high responders, 30.52% were moderate responders, and only 8.45% were low responders. However, in male cats, 52.51% were high responders, 34.02% were moderate responders, and 13.47% were low responders.

Table 2. Number of cat samples by titer range and sex

Sex	Titer groups	Number of animals	Frequency
Female	High responders	318	61.04%
Male	High responders	230	52.51%
Male	Moderate responders	149	34.02%
Female	Moderate responders	159	30.52%
Male	Low responders	59	13.47%
Female	Low responders	44	8.45%

The analysis of the frequencies of the titration level according to the cats' sex shows that females tend to be better represented in the high modality of the titration than males, 61.04% for females against 52.51% for males. In the same logic, females are less represented than males in the low level of titration: 8.45% for females against 13.47% for males (Figure 2).

These results lead to the suggestion that females tend to have better titration levels than males; this funding is only based on observed frequencies, and it remains to be confirmed.

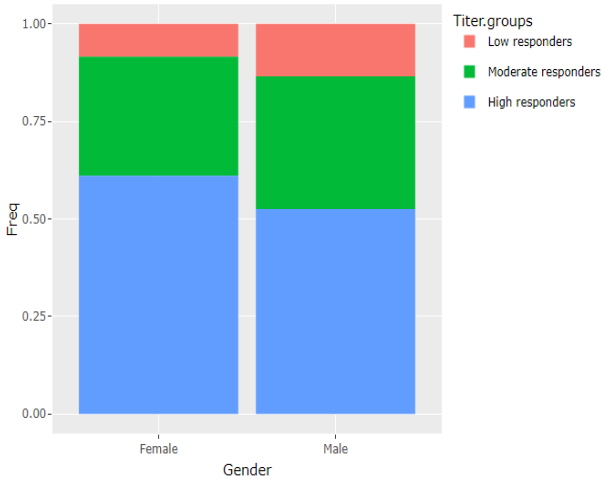

Fig. 2. Variation of rabies antibodies titration level according to cats' sex

The titration level variation according to the number of vaccinations is shown in Table 3. Cats that received one vaccination represent 202 (21.1%) cases; 505 (52.6%) cats received two vaccinations; 132 (13.8%) cats received three vaccinations; and 120 (12.5%) cats received four or more vaccinations.

In the +G4' group, 75.83% were high responders, 16.67% were moderate responders, and only 7.50% were low responders. On the other hand, in the G1' group, 31.68% were high responders, 47.03% were moderate responders, and 21.29% were low responders.

Table 3. Number of cat samples by titer range and by number of vaccination

Groups according to the number of vaccinations	Titer groups	Number of cat	Frequency
+G4	High responders	91	75.83%
G3	High responders	90	68.18%
G2	High responders	303	60.00%
G1	Moderate responders	95	47.03%
G1	High responders	64	31.68%
G2	Moderate responders	158	31.29%
G3	Moderate responders	35	26.52%
G1	Low responders	43	21.29%
+G4	Moderate responders	20	16.67%
G2	Low responders	44	8.71%
+G4	Low responders	9	7.50%
G3	Low responders	7	5.30%

The analysis of the effect of the number of vaccinations on the titration level shows that the frequency of high responders increases with the number of vaccinations received (Figure 3). This suggests that more vaccinated cats tend to have a higher titration. This statement is verified in the subsequent research.

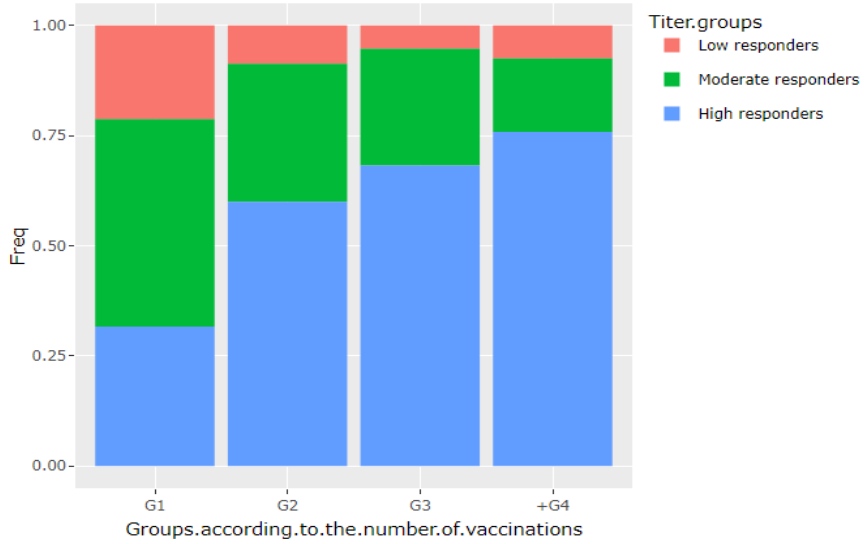

Fig. 3. Variation of rabies antibodies titration level according to the number of vaccination.

Analysis of titration level frequencies according to the test used shows that the RFFIT test tends to be better represented in the high titration modality than the FAVN test (70.50% for RFFIT versus 55.40% for FAVN). The variation of the titration level according to the type of test is presented in Table 4.

Table 4. Number of cat samples by titer range and by type of test

Test performed	Titer groups	Number of animals	Frequency
RFFIT	High responders	79	70.50%
FAVN	High responders	469	55,40%
FAVN	Moderate responders	284	33.50%
RFFIT	Moderate responders	24	21.40%
FAVN	Low responders	94	11.10%
RFFIT	Low responders	9	8%

3.2 RESULTS OF STRUCTURAL MODELLING

The objective of this section is to verify the influence of different factors on the level of rabies titration, namely: sex, age, time between vaccination and collection for testing, type of test, laboratory, and number of vaccinations. We analyze the results of the model built using multinomial logistic regression. In order to do this, we have extracted odds ratios with the help of the tidy library in R. Table 5 and Figure 4 contain and illustrate the odds ratios, organized by the above variation factors.

Table 5. Odd Ratios of Multinomial logistic regression

Factors	Cats categories	y.level	Estimate OR	statistic	P Value
Sex	Low responders	SexFemale	0,73	-1,32	0,19
	High responders	SexFemale	1,69	3,11	**0,00**
Age at vaccination	Low responders	`Age at vaccination`] 120,365]	0,68	-1,22	0,22
		`Age at vaccination`] 365,2190]	0,93	-0,26	0,80
		`Age at vaccination`+6 Years	0,65	-0,82	0,41
	High responders	`Age at vaccination`] 120,365]	1,30	1,24	0,22
		`Age at vaccination`] 365,2190]	1,00	0,02	0,98
		`Age at vaccination`+6 Years	0,60	-1,42	0,15
Time from vaccination to sampling for titer measurement in days (TVTM)	Low responders	TVTM (31d-60d)	1,39	0,61	0,54
		TVTM (61d-90d)	0,74	-0,50	0,62
		TVTM (91d-180d)	1,67	0,88	0,38
		TVTM (More than 180)	2,37	1,50	0,13
	High responders	TVTM (31d-60d)	0,90	-0,33	0,74
		TVTM (61d-90d)	0,46	-2,08	**0,04**
		TVTM (91d-180d)	0,54	-1,66	**0,10**
		TVTM (More than 180d)	0,67	-1,06	0,29
Laboratory	Low responders	`Laboratory name`L2	1,03	0,03	0,97
		`Laboratory name`L3	0,37	-0,95	0,34
		`Laboratory name`Others	0,77	-0,27	0,79
	High responders	`Laboratory name`L2	6,77	3,14	**0,00**
		`Laboratory name`L3	0,03	-3,13	**0,00**
		`Laboratory name`Others	1,70	0,98	0,33
Test	Low responders	`Test performed`RFFIT	0,97	-0,03	0,97
	High responders	`Test performed`RFFIT	6,82	3,63	**0,00**
Groups according to the number of vaccinations	Low responders	G2	0,58	-2,06	**0,04**
		G3	0,35	-2,25	**0,02**
		G4 and more	0,79	-0,50	0,62
	High responders	G2	2,47	4,23	**0,00**
		G3	3,24	4,05	**0,00**
		G4 and more	5,87	5,20	**0,00**

INFLUENCE OF THE CAT'S SEX ON THE TITRATION LEVEL

The analysis of the odds ratios obtained after modeling shows that, at the low titration level, there is no significant difference between the responses of males and females (P=0,19). On the other hand, the females register a better response than the males in the high modality of titration (0, 00***) (Table 5).

INFLUENCE OF AGE AT VACCINATION ON TITRATION LEVEL

In this study, the results showed that the age factor has no significant influence on the level of titration (P>0.1). However, in the high-titration modality, the older the cat, the lower the odds ratios. This observation shows that although the difference between the age groups is not statistically significant, the odds ratios confirm that the older the cat, the more its response to rabies titration is slightly reduced (Table 5).

INFLUENCE OF TIME FROM VACCINATION TO TITER MEASUREMENT (TVTM) ON TITRATION LEVEL

The data in Table 5 show, for high responders, a significant difference (P<0.05) between reference level (3d–30d) and TVTM (61d–90d) (P<0.05) and also between TVTM (91d–180d) with a P equal to 0.10. The odds ratio analysis shows that the longer the time between vaccination and measurement, the lower the response.

However, the P value of TVTM (more than 180 d) is not significant compared to the reference level.

INFLUENCE OF THE LABORATORY ON TITRATION LEVEL

In this section, we investigate the influence of heterogeneity in the source of results (laboratories) on the level of titration.

At the low response level, no significant difference between laboratories was recorded. However, at the high response level, the difference between laboratories becomes statistically significant (0, 00***). (Table 5, Figure 4).

INFLUENCE OF THE TEST TYPE ON TITRATION LEVEL

At the low level of titration, the statistical evidence shows the non-existence of a significant difference between the two tests. On the contrary, at the high level of titration, there is a significant difference between the two tests (0.00***); the use of the RFFIT test greatly increases the probability of the cats' high response (Figure 4).

INFLUENCE OF THE NUMBER OF VACCINATIONS ON TITRATION LEVEL

The analysis of odds ratios and the significance value show that the number of vaccinations is the most influential variable on the level of titration (Table 5, Figure 4). In fact, the higher the number of vaccinations, the lower the probability of a low response to rabies vaccination. Similarly, a cat with more vaccinations has a higher probability of having the highest titration level.

Group 4 cats, on the other hand, contradict this reasoning at the high response level; there is no significant difference between G1 and G4.

4 DISCUSSION

After modeling, the odds ratios show that there is a small, almost insignificant difference between males and females in the level of titration in low responders. Santosh et al. (2017) and Jakel et al. (2008) also discovered this after examining samples of male and female cats and finding no discernible difference between them. However, in high responders to our study, there is a significant difference between females and males (Figure 4), which confirms the finding of Mansfield et al. (2004). It seems that being a female cat significantly favors the probability of a high response to titration. This could be attributed to sex hormones, which can modulate the production of cytokines and contribute to sex-related differences in normal and pathological immune responses [14].

According to the results for age, the study showed that there was no significant difference between the different ages of this sample of cats. However, the odds ratios of high responders indicate that the older the cat, the more its response to rabies titration is slightly reduced. Previous studies have noted that the ability of young cats (<1 year) to develop protective

neutralizing antibody titers is lower than that of adults [9; 10]. Also, cats older than 14 years had a significantly (P=0,04) lower titer [9].

Regarding the time from vaccination to sampling for titer measurement, significant differences were observed in high responders when the duration exceeded 60 days. Similar results were obtained with a significant ($p<0.01$) decrease in neutralizing antibody titer in animals sampled eight to nine weeks after vaccination [9]. Santosh et al. have shown that the best period for serum collection is between 20 and 50 days [10]. However, it should be noted that, in our study, the category "more than 180 days" did not show a significant difference; this is due to the heterogeneity of the population of cats studied. It is very likely that cats in the category TVTM (+180d) have received several vaccinations. In this case, it can be said that the time factor (TVTM) does not greatly affect the rabies titration result if the cat has received several vaccinations.

The multifactorial analysis also allowed us to compare the tests used; a significant difference was observed in the 'high response' category. It can be said that the RFFIT test overestimates the high titration value compared to the FAVN test. Within the same titration category, the statistical analysis showed a significant difference between the laboratories. This result is unexpected. This observed difference between laboratories can be explained by the fact that some laboratories do not provide the exact titration value; for this reason, we initially chose the "anti-rabies titration" variable, a three-level qualitative variable, instead of a quantitative one. In this context, the laboratories are invited to publish the exact values of the titrations, which will allow for further investigation of this fact. On the other hand, Moore mentioned in his review article that interpretation of results can be problematic, not only between methods but also because modifications to the same method can lead to misinterpretations [15].

With regard to the number of vaccinations, it has been found that the number of vaccinations is the factor that most influences the level of rabies titration. The more vaccinations a cat has received, the more likely it is to have the highest level of titration, and the opposite is true. Many studies have reported that a cat with booster vaccinations tends to have better rabies antibody titration than one with fewer vaccinations [9; 12]. However, vaccination breaks (up to more than 3 years in some cats) may contradict this logic, as observed in the +G4 groups in the 'low response' modality (Figure 4).

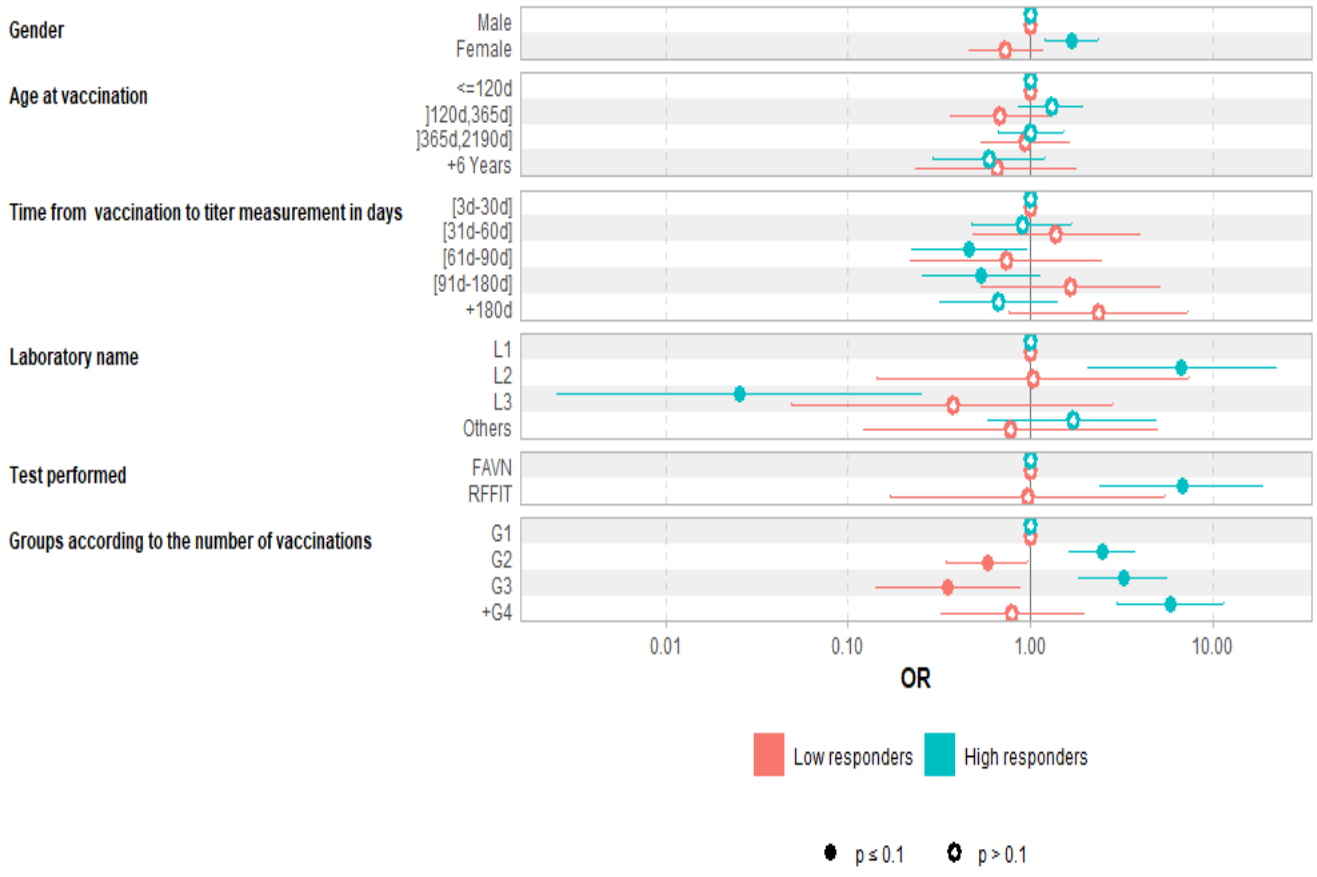

Fig. 4. Graphical representation using the ggcoef_multinom library in R of Odds Ratios.

5 CONCLUSION

Certain factors can influence the level of rabies titers in cats. Indeed, it can be stated that the sex of the cat has a slight influence on the titration level. Females have a slightly better antibody response than males. The time between the last vaccination and the sampling for titer measurement has a significant influence on titration in that the longer this time (more than 60 days), the less chance there is to have a high level of titration. At the high titration response level, the differences according to the factors laboratories and tests performed (FAVN, RFFIT) were statistically significant. Likewise, the number of vaccinations is the most determining variable in the titration; the more the cats have multiple vaccinations, the stronger the chance that they will have the highest level of titration. This observation leads us to recommend regular boosters of the anti-rabies vaccination to protect the feline population and its environment.

ACKNOWLEDGMENT

The authors would like to thank all the veterinarians who facilitated data collection for this study, in particular Hicham Mih, Barkouch Abdessadeq, Fatima Kourbi, Chaib Meryem, Ibtihal Rahmouni Alami, Tarik Abdelmoutalib, Seyagh Mohamed Adil, Taha Abdelhamid Sakr, El Hanchi Abedlhamid and Motassim El Hanafi.

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