

## Evaluation of maternal antibodies against rabies in puppies and kittens in Mazandaran Province, Iran

[Avaliação de anticorpos maternos contra a raiva em filhotes de cachorro e gatos na província de Mazandaran, Irã]

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### ABSTRACT

Rabies is a critical zoonotic high-risk disease, which has emerged as an important public-health problem. There are limited investigations that explore maternally derived antibody (MDA) in puppies and kittens from around the world. Furthermore, there is no such research in any province of Iran. This study measured the serum level of MDA in 10 puppies and their vaccinated bitches (n=20), 10 puppies and their unvaccinated bitches (n=20), 10 kittens with vaccinated queens and 10 kittens with unvaccinated queens. Antibodies against rabies were measured using quantitative, enzyme-linked immunosorbent assay (ELISA). Our results showed that 90% (9 out of 10) of puppies from vaccinated bitches were positive for anti-rabies antibodies, while test results for all puppies from non-vaccinated bitches were negative (P=0.001). Moreover, puppies' titers were significantly higher in the vaccinated group than the non-vaccinated group (P=0.003). There was a similar condition between vaccinated and non-vaccinated bitches (P=0.008). Although kittens' titers were significantly higher in the vaccinated group than the non-vaccinated group (P=0.03), only 20 percent of the kittens with vaccinated queens showed positive results, while all kittens from non-vaccinated queens were negative for anti-rabies antibodies (P=0.137).

Keywords: Puppies, Kittens, Anti-rabies antibodies, MDA, Mazandaran Province

### RESUMO

A raiva é uma doença zoonótica crítica de alto risco, que surgiu como um importante problema de saúde pública. Há investigações limitadas que exploram anticorpos derivados da maternidade (MDA) em filhotes e gatos de todo o mundo. Além disso, não existe tal pesquisa em nenhuma província do Irã. Este estudo mediu o nível sérico do MDA em 10 filhotes e suas cadelas vacinadas (n=20), 10 filhotes e suas cadelas não vacinadas (n=20), 10 filhotes com rainhas vacinadas e 10 filhotes com rainhas não vacinadas. Os anticorpos contra a raiva foram medidos usando o ensaio quantitativo de imunoabsorção enzimática (ELISA). Nossos resultados mostraram que 90% (9 em cada 10) dos filhotes de cadelas vacinadas foram positivos para anticorpos anti-rábicos, enquanto os resultados dos testes para todos os filhotes de cadelas não vacinadas foram negativos (P=0,001). Além disso, os títulos dos filhotes foram significativamente mais altos no grupo vacinado do que no grupo não vacinado (P=0,003). Havia uma condição similar entre cadelas vacinadas e não vacinadas (P=0,008). Embora os títulos dos filhotes fossem significativamente mais altos no grupo vacinado do que no grupo não vacinado (P=0,03), apenas 20% dos filhotes com rainhas vacinadas apresentaram resultados positivos, enquanto todos os filhotes de rainhas não vacinadas foram negativos para anticorpos anti-rábicos (P=0,137).

Palavras-chave: filhotes, anticorpos anti-rábicos, MDA, província de Mazandaran

### INTRODUCTION

Rabies is a high-risk zoonotic disease, which could have 100% mortality rate. According to

global statistics, this disease is a problem caused by transmission between domestic dogs, mainly in Asia and Africa. For decades, the direct threat of human and dog with rabies exposure has been significantly reduced through continuous

vaccination of dogs, as well as the control of stray dogs and the elimination of dog-to-dog strains in Europe and North America (Greene, 2012).

Because of the endotheliochorial placentas in dogs and cats, only 2-18% of maternally derived antibody (MDA) can be transferred to the fetus, which protects puppies and kittens for a short time after birth. In contrast, a high concentration of MDA in colostrum can protect puppies/kittens for a longer period, which decreases gradually during lactation. MDA has an inhibitory effect on the production of antibodies in puppies/kittens, so that immunoglobulin production begins when MDA falls below a certain level (Greene, 2012). Various studies investigated the prevalence of rabies antibodies in dogs in different countries (Wosu and Anyanwu, 1990; Seghaier *et al.*, 1999; Kasempimolporn *et al.*, 2007; Suzuki *et al.*, 2008; Berndtsson *et al.*, 2011; Olugasa *et al.*, 2011). Furthermore, a rare number of studies detected MDA for rabies in dogs, raccoon dogs, and fox. For example, using the ELISA method, a study showed that 15.6 percent of 32 puppies had positive rabies antibodies before primary vaccination (Kasempimolporn *et al.*, 1996). In another study, the results indicated that there was maternal antibody  $\geq 0.5$  IU/ml up to 36 days postpartum in ten cubs born from a rabies-immune animal (Vos *et al.*, 2001). As far as we know, no study has explored the situation of maternal antibodies in Iran. Hence, we conducted this investigation to assess maternal antibodies against rabies in puppies and kittens from vaccinated and none vaccinated mothers in Mazandaran province, Iran.

## MATERIALS AND METHODS

This study was performed during the period from August 2019 to August 2020.

The current research has been done in the Mazandaran province located along the southern coast of the Caspian Sea and in the adjacent Central Alborz Mountain range, in central-northern Iran. The total area, total population, and density of this province are 23,833 km<sup>2</sup>, 3,073,943, and 130/km<sup>2</sup>, respectively. The climate of Mazandaran is divided into three types: moderate Caspian weather, moderate mountainous weather, and cold mountainous

weather. Mazandaran is divided into 20 counties ([https://en.wikipedia.org/wiki/Mazandaran\\_Province](https://en.wikipedia.org/wiki/Mazandaran_Province)).

Blood samples were taken from 10 puppies and their vaccinated bitches (n=20), 10 puppies and their unvaccinated bitches (n=20), 10 kittens from vaccinated queens and 10 kittens from unvaccinated queens in the first 9 days of birth and randomly from both sexes. All mothers were under two years old. Blood was collected for serology by cephalic or jugular venipuncture in the amount of 2-3 ml using a sterile syringe and a 21-gauge needle. Blood samples were left at room temperature (30 minutes to 2 hours) for clotting and centrifuged at 3000 rpm for 10 minutes to separate the sera. Serum samples were labeled and kept frozen at minus 20°C throughout the study period. All characteristics of puppies and kittens tested, including breed, age, sex, owner name, address, sampling date were recorded in numbered sheets.

Anti-rabies antibody titer for kittens was measured with a quantitative enzyme-linked immunosorbent assay (ELISA): (SERELISA Rabies Ab Mono Indirect, Synbiotics, France). The kit used an indirect immunoenzymatic technique and contained both negative and positive controls, and the procedure was followed as the manufacturer recommended. The results (optical density-OD) were read using an ELISA reader (Hiperion-Germany) at wavelength 450-620 nm.

Anti-rabies antibody titer for bitches and puppies was measured with a quantitative ELISA: (Rabies Virus IgG Ab (Dog) ELISA, Demeditec (REF, DE 2486), Germany). The kit used contained both negative and positive controls, and the procedure was followed as the manufacturer recommended. The results (optical density-OD) were read using an ELISA reader (Lab system-Finland-series: 3520900102) at wavelength 450-620nm.

A minimum titer of 0.5 IU/ml Rabies antibodies is required to protect against Rabies infection, according to the World Health Organization recommendations (Guide..., 2002).

Antibody titer between cases with vaccinated and unvaccinated mothers was compared using Student's t-test in both kittens and puppies. An antibody titer of 0.5 IU/ml was considered as the

protective level (Pimburage *et al.*, 2017) and the percentage of immunized dogs in each group was calculated and compared using Fisher's exact test. All statistical analysis was carried out using SPSS.16 statistical package (Chicago, USA). A *P* value lesser than 0.05 was considered significant.

## RESULTS

Our cases included 20 puppies and 20 kittens. Puppies were selected from different breeds, and the breed of all kittens was merely Domestic Short Hair (DSH). Puppies included 14 (70%) females and 6 (30%) males, and kittens included 10 (50%) females and 10 (50%) males (Table 1, and 2).

Table 1. Categorical information on 10 puppies in both vaccinated and non-vaccinated groups

		Vaccinated	Non-vaccinated	Total
Breed	Golden Retriever	1	0	1
	German Shepherd	4	7	11
	Husky	2	1	3
	Pitbull	0	1	1
	Pointer	2	0	2
	Rottweiler	1	0	1
	Terrier	0	1	1
Sex	Female	7	7	14
	Male	3	3	6
Test result	Negative	1	10	11
	Positive	9	0	9

Table 2. Categorical information on 10 kittens both vaccinated and non-vaccinated groups

		Vaccinated	Non-vaccinated	Total
Breed	DSH	10	10	20
Sex	Female	6	4	10
	Male	4	6	10
Test result	Negative	8	10	18
	Positive	2	0	2

The mean age of puppies and kittens were  $8.55 \pm 0.76$  days and  $8.1 \pm 0.72$  days, respectively, which was not significantly different between vaccinated and non-vaccinated groups in both species ( $P=0.777$ ,  $P=0.990$ , respectively). Furthermore, the mean age of bitches and queens was  $20.70 \pm 3.25$  and  $17.9 \pm 5.09$  months, which was not statistically different between vaccinated and non-vaccinated groups ( $P=0.791$ ,  $P=0.800$ , respectively) (Table 3, and 4).

Our findings revealed that 90% (9 out of 10) of puppies from vaccinated bitches were positive for anti-rabies antibody, while test results for all

puppies from non-vaccinated bitches (100%) were negative ( $P=0.001$ ). Furthermore, puppies' titer was significantly higher in the vaccinated group than the non-vaccinated group ( $P=0.003$ ). There was a similar condition between vaccinated and non-vaccinated bitches ( $P=0.008$ ). Although kittens' titer was significantly higher in the vaccinated group than non-vaccinated group ( $P=0.03$ ), only 20 percent of kittens from vaccinated queens showed positive results, while all kittens from non-vaccinated queens were negative for anti-rabies antibody ( $P=0.137$ ) (Table 3, and 4).

Table 3. Information of continuous variables on 10 puppies and their corresponding bitches in both vaccinated and non-vaccinated groups

		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum	P value
Bitch age (months)	Vaccinated	10	20.90	2.77	0.87	18	24	0.791
	Non-vaccinated	10	20.50	3.81	1.20	14	24	
	Total	20	20.70	3.25	0.73	14	24	
Puppy age (days)	Vaccinated	10	8.60	0.70	0.22	7	9	0.777
	Non-vaccinated	10	8.50	0.85	0.27	7	9	
	Total	20	8.55	0.76	0.17	7	9	
Bitch titer (IU/ml)	Vaccinated	10	2.04	2.04	0.64	0.5	6.77	0.008
	Non-vaccinated	10	0.12	0.03	0.01	0.07	0.15	
	Total	20	1.08	1.71	0.38	0.07	6.77	
Puppies' titer (IU/ml)	Vaccinated	10	0.69	0.38	0.12	0.13	1.46	0.003
	Non-vaccinated	10	0.07	0.02	0.01	0.04	0.11	
	Total	20	0.38	0.41	0.09	0.04	1.46	

Table 4. Information of continuous variables on 10 kittens and their corresponding queens in both vaccinated and non-vaccinated groups

		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum	P value
Queen age (months)	Vaccinated	10	18.2	3.22	1.02	12	22	0.800
	Non-vaccinated	10	17.6	6.64	2.10	1	24	
	Total	20	17.9	5.09	1.14	1	24	
Kitten age (days)	Vaccinated	10	8.1	0.74	0.23	7	9	0.990
	Non-vaccinated	10	8.1	0.74	0.23	7	9	
	Total	20	8.1	0.72	0.16	7	9	
Kitten titer (IU/ml)	Vaccinated	10	0.295	0.37	0.12	0.03	1.04	0.030
	Non-vaccinated	10	0.018	0.02	0.00	0	0.04	
	Total	20	0.1565	0.29	0.07	0	1.04	

## DISCUSSION

Different studies investigated the prevalence of rabies antibodies in dogs. A study by Kasempimolporn *et al.* (2007) on 3314 stray dogs in Bangkok, Thailand, indicated that overall antibody seroprevalence assay was 62%, while dogs captured within central Bangkok had a higher prevalence (86%) (Kasempimolporn *et al.*, 2007). Olugasa *et al.* (2011) evaluated anti-glycoprotein antibodies against rabies virus in a transit city of Nigeria on 190 dogs, including 116 confined, 61 free-roaming, and 13 stray dogs. Their findings showed that 81 out of 190 (42.6%), including 57 confined (49.1%), 23 free roaming (37.7%), and 1 stray (7.7%), were positive for rabies antibody (Olugasa *et al.*, 2011). In another study in Nsukka environs, Nigeria, using the haemagglutination-inhibition technique, antibodies to rabies virus were detected in 254 non-vaccinated and apparently healthy dogs. This study reported an overall 16.1% prevalence, while 22.8% of cases older than 6 months, 17.5% cases younger than 3

months, and 7.3% of cases between 3-6 months had positive titer (Wosu and Anyanwu, 1990). In Bolivia, Suzuki *et al.* (2008) reported a prevalence of 58% of positive antibody titer in 236 dogs vaccinated using an inactivated suckling mouse brain vaccine against canine rabies (Suzuki *et al.*, 2008). A study on 6,789 vaccinated dogs in Sweden revealed that 6,241 (91.9%) were positive for antibodies against the rabies virus. Furthermore, analyses revealed that larger breed size, age younger than 6 months, and age older than 5 years were potential risk factors for having antibody titers of <0.5 IU/ml (Berndtsson *et al.*, 2011). Seghaier *et al.* (1999) showed that one month after vaccination on 301 dogs in Tunisia, 76-84% of the older than 1-year dogs and 65% of the dogs less than 1 year of age had positive anti-rabies antibody (Seghaier *et al.*, 1999).

Few studies were also explored maternal antibodies against rabies in the puppies. Kasempimolporn *et al.* (1996) indicated that 5 out of 32 puppies before primary vaccination had

positive rabies antibodies by ELISA testing (Kasempimolporn *et al.*, 1996). In another study, findings showed there was maternal antibody  $\geq 0.5$  IU/ml up to 36 days post-partum in ten cubs born from a rabies-immune animal. At 56 days post-partum, the level of maternal antibody was drastically decreased below 0.5 IU/ml (Vos *et al.*, 2001). In a similar report, serum titer of vaccinated (maternally vaccinated [MV+]) cubs (0.23 IU/ml) was significantly higher than that of non-vaccinated (MV-) cubs (0.15 IU/ml) (Müller *et al.*, 2001). We could not find any study that explored the MDA against rabies in Iran. Morshedi and Aslani (2003) compared the antibody titers between three groups –namely, vaccinated pet dogs (n=60), non-vaccinated pet dogs (n=25), and stray dogs (n=15) in Urmia, Iran. They found that 49 out of 60 vaccinated dogs (81.6%) had protective antibody titers (between 0.5-2.25 IU/ml), while not all non-vaccinated domestic dogs and the stray dogs had significant antibody titers (Morshedi and Aslani, 2003).

Our study can be considered a starting point to run a further study to explore MDA against rabies in more detail. Investigation on a larger population of puppies and kittens and the corresponding parents and measure of MDA at different intervals (such as different ages and various vaccination steps) could result in more accurate and reliable findings.

### CONCLUSIONS

According to the results of this study, it can be concluded that although vaccination of the bitches and queens against rabies virus causes higher serum IgG levels in the blood of puppies and kittens than non-vaccinated mothers, this level of IgG cannot protect them from the rabies virus. Therefore, caring of puppies and kittens until the first period of vaccination is necessary and inevitable.

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