Factors Associated With Rabies Antibody Titers in Dogs in Mapanget District, Manado City

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Abstract.

Rabies remains a highly fatal zoonotic disease and continues to pose a substantial public health challenge in Indonesia, including in Manado City. Mapanget District hosts a considerable dog population, yet empirical data on herd immunity based on rabies antibody titers are scarce. This study aimed to evaluate the determinants associated with rabies antibody titers among dogs in Mapanget District, Manado City. A cross-sectional study was conducted involving 97 dogs selected through purposive sampling. Serum samples were analyzed using the Enzyme-Linked Immunosorbent Assay (ELISA) to classify antibody titers as protective (≥0.5 IU/mL) or non-protective (<0.5 IU/mL). Statistical analyzes included Fisher's Exact Test and multivariable logistic regression to assess associations between host factors and antibody titer status. Only 8.2% of dogs exhibited protective antibody titers, whereas 91.8% demonstrated non-protective levels. Vaccination status showed a highly significant association with antibody titers (p < 0.001). Vaccinated dogs were markedly more likely to achieve protective titers compared to unvaccinated dogs (OR = 35.71; 95% CI: 3.888–328.026). No significant associations were observed for dog type, age, or sex (p > 0.05). The findings underscore a critically low level of immunity within the dog population in Mapanget District, primarily attributable to insufficient vaccination coverage. Vaccination emerged as the predominant factor influencing the development of protective antibody titers. Strengthening routines and mass vaccination programs, coupled with systematic serological monitoring, are imperative to achieve adequate herd immunity and to advance rabies elimination strategies in the region.

Keywords: *Antibody Titer; Canine Immunity; Mapanget District and Rabies.*

I. INTRODUCTION

Rabies is an acute infectious disease that attacks the central nervous system, caused by the rabies virus, and can be spread through saliva and bites from animals that transmit rabies (GHPR).(Ministry of Health of the Republic of Indonesia, 2023)The disease has spread to almost every continent except Antarctica, and more than 150 countries have reported cases of rabies. Each year, more than 55,000 people die from rabies, while more than 15 million people worldwide receive prophylactic vaccination to prevent transmission of the disease (WHO, 2018).Indonesia still faces a serious rabies problem, with 26 provinces declared rabies endemic and 12 provinces free. These provinces include Jakarta, Central Java, East Java, Yogyakarta, Riau Islands, Bangka Belitung Islands, Papua, West Papua, Southwest Papua, Central Papua, Highland Papua, and South Papua. Rabies remains a zoonotic health problem that must be addressed. According to the 2024 monthly zoonotic report, there were 185,359 cases of GHPR and 122 deaths due to rabies in humans. From January to March 7, 2025, there were 13,453 reported cases of GHPR, with 25 deaths related to rabies in humans (Ministry of Health of the Republic of Indonesia, 2025).The spread of rabies in North Sulawesi has occurred since 2014. Between 2020 and 2024, 32,059 bite cases were reported in North Sulawesi, with the highest number of bites occurring in 2023 with 9,153 cases (North Sulawesi Provincial Health Office, 2025). Data on GHPR cases in Manado City between 2020 and 2024 showed 5,099 cases and 7 deaths. The peak of GHPR cases occurred in 2023 with 1,250 cases. In 2024, 1,086 GHPR cases were recorded with 2 deaths.

Laboratory analysis of animal specimens in 2023 showed that 34 specimens were examined, with 19 positive for rabies. Meanwhile, in 2024, of the 68 specimens examined, 51 were detected positive for rabies (North Sulawesi Provincial Health Office, 2025).The key to controlling rabies in endemic areas is animal vaccination. Several health organizations, including the World Health Organization (WHO) and the World Organization for Animal Health (WOAH), state that the best way to treat rabies is to prevent it at its source, through vaccination. Rabies vaccination aims to stimulate the development of immunity or antibodies against rabies virus infection. The level of the body's humoral immune response, or rabies antibody titer, represents the level of immunity against rabies virus infection. Measuring rabies antibody titers in dogs is important because the resulting antibody titer will serve as a benchmark for determining whether the dog has immunity to the rabies virus.Based on WHO (2018) and WOAH (2023) recommendations, the antibody titer that can provide protection against rabies virus infection is equal to or higher than 0.5 IU/ml. A rabies antibody titer ≥ 0.5 IU/ml is seropositive or protective against the rabies virus. A rabies antibody titer less than 0.5 IU/ml is seronegative or not protective against the rabies virus.

Laboratory examination data from the Maros Veterinary Center for rabies antibody titers in dogs in the North Sulawesi region in January - June 2025 from 15 samples tested there were 12 samples with antibody titers ≥ 0.5 IU / ml and 3 samples with antibody titers ≤ 0.5 IU / ml. Data on rabies antibody titers in dogs in Manado City from 11 samples tested there were 9 samples with antibody titers ≥ 0.5 IU / ml and 2 samples with antibody titers ≤ 0.5 IU / ml (BBVet Maros, 2025). From the rabies antibody titer data, it can be seen that the rabies vaccination program in North Sulawesi has been carried out but is still not optimal with the discovery of low rabies antibody titers.The continued low rabies antibody titers may be due to a lack of public awareness about rabies vaccination for pet dogs. To eliminate or prevent rabies outbreaks, dog vaccination coverage must reach at least 70% of the total dog population to achieve herd immunity. Low or uneven vaccination coverage can worsen the situation and threaten rabies elimination programs. Rabies vaccination must be continuously implemented to prevent the spread of rabies. Rabies vaccination in dogs will stimulate the formation of an immune response or antibodies. Monitoring antibody titers in dogs is important, as the resulting antibody titer will determine whether the dog is immune to the rabies virus. It also serves as an indicator of vaccination success. Variations in antibody titers in dogs can be influenced by many factors, such as age, genetics, the presence of infection, maintenance methods, and nutritional adequacy.

Rabies antibody titer testing in dogs uses the Enzyme-Linked Immunosorbent Assay (ELISA) method. Antibody responses can also be influenced by various factors such as vaccination status, age, breed type, and the time between vaccination and sampling (Kaila et al, 2019).Measuring the humoral immune response, or antibody titer, in dogs is crucial for evaluating their ability to protect against rabies virus infection. Furthermore, understanding the factors associated with the development of rabies antibody titers is crucial for more effective control and prevention interventions.Mapanget District, Manado City, is an urban area with high levels of human-dog interaction. Dogs are often kept as pets and as guard animals. According to the Manado City Agriculture Service data for 2024, the dog population in Mapanget District was 3,480 dogs. This high level of human-dog interaction, coupled with high dog mobility, can increase the risk of rabies infection through bites from infected dogs. Data on rabies antibody titers in dogs in Mapanget District is very limited, making it impossible to determine the prevalence of rabies antibody titers. Therefore, research on rabies antibody titers in dogs in Mapanget District is necessary. Rabies antibody titer research is needed to measure rabies antibody titers, and it also replaces the calculation of vaccination coverage, which cannot be done accurately. Measuring antibody titers can provide direct evidence of vaccination success and determine whether revaccination is necessary. Serological data on rabies antibody titers will be a useful source of information forthe effectiveness of policies taken by the government in planning appropriate rabies prevention programs.Therefore, it is necessary to conduct research on rabies antibody titers in dogs in Mapanget District, Manado City.

II. METHODS

This study was an observational study with a cross-sectional design. The study was conducted in Mapanget District, Manado City, from October to November 2025. Laboratory testing was conducted at the Maros Veterinary Center.This research uses***purposive sampling*****as much as**97 Samples withprevalence assumption formula according to Thrusfield (2018) Because there is no data on rabies antibody titers in dogs in Mapanget District and the prevalence of protective antibody titers is unknown, the focus was on obtaining representative samples with varying characteristics for analysis. Blood sampling was carried out on the dogs that were the study subjects using standard procedures. The dogs were first physically examined. If the dogs were healthy, then blood was drawn from the cephalic vein. Blood was drawn using a 3 ml syringe and then placed into a tube without anticoagulant. The tube was left for 20-30 minutes until the blood clotted and the serum separated. The serum was then separated and transferred into a microtube. The serum was stored at room temperature.– 20°Cup to – 80°C. The samples are then sent to the BBVet Maros laboratory using a coolbox containing ice gel or ice packs to maintain antibody stability.

III. RESULT AND DISCUSSION

This study involved 97 dogs from Mapanget District, Manado City. Sample characteristics showed that the majority of dogs were local (76.3%), male (56.7%), mature (>1 year) (60.8%), and had not received a rabies vaccination (79.4%). This reflects low rabies vaccination coverage among community-owned dogs.

Table 1. Rabies Elisa Test Results

No	Antibody Titer	Number of Samples	%
1	Seropositive (≥ 0.5 IU/ml)	8	8.2
2	Seronegative (< 0.5 IU/ml)	89	91.8
	Total	97	100

ELISA test results showed that 91.8% of dogs had seronegative rabies antibody titers (<0.5 IU/mL), while only 8.2% had seropositive antibody titers (≥0.5 IU/mL). The low proportion of protective antibody titers indicates that the level of immunity of the dog population to rabies in the study area is still not optimal and has the potential to increase the risk of rabies transmission.

Table 2. Relationship between Rabies Vaccination Status and Antibody Titer

Vaccination Status	Seropositive		Seronegative		p-value
	Number of Samples	%	Number of Samples	%	
Not vaccinated against rabies	1	1.3	76	98.7	< 0.001
Have you had a rabies vaccination?	7	35	13	65.0	
Total	8	8.2	89	91.8	

Bivariate analysis showed a highly significant correlation between rabies vaccination status and antibody titers (p < 0.001). Vaccinated dogs showed a seropositive antibody titer of 35%, significantly higher than unvaccinated dogs (1.3%). These results confirm that vaccination plays a crucial role in the formation of protective antibodies against rabies and is a major factor in enhancing population immunity.In contrast, dog breed, sex, and age did not show a significant association with rabies antibody titer status (p > 0.05). Although descriptively, purebred, male, and adult dogs had slightly higher proportions of protective antibody titers than the other groups, the difference was not statistically significant. These findings indicate that demographic factors do not significantly influence the rabies immune response in dogs.

Table 3. Logistic Regression Analysis

Variables	B	p-Value	OR (Exp9B))	95% CI for EXP(B)	
				Lower	Upper
Vaccination status	3,575	0.002	35.71	3,888	328,026
Types of Dogs	1,037	0.247	2,822	0.488	16.33
Dog Age	-0.152	0.887	0.859	0.105	7,051
Dog Gender	-0.727	0.469	0.483	0.068	3,451

The results of the logistic regression analysis showed that vaccination status was the only variable with a significant effect on rabies antibody titers (p = 0.002). Vaccinated dogs were 35.7 times more likely to have protective antibody titers than unvaccinated dogs (OR = 35.71; 95% CI: 3.89–328.03). The regression model was able to explain 44.2% of the variation in antibody titer status (Nagelkerke $R^2 = 0.442$), while the variables of dog breed, age, and sex did not provide a significant contribution.This is in accordance with WHO (2018) and WOAH/OIE (2019) which emphasized that vaccination status is the main determinant of rabies antibody formation, and dogs that are not vaccinated or do not receive adequate boosters tend to have antibody titers below the protective limit. Another study by Hampson et al. (2015) also emphasized that the success of rabies elimination is highly dependent on vaccination coverage capable of producing a protective antibody response in at least 70% of the dog population.Overall, the study results indicate that the low proportion of dogs with protective rabies antibody titers is closely related to low rabies vaccination coverage. Vaccination has been shown to be a major determinant of rabies immunity in dogs, while demographic factors have no significant effect. Therefore, increasing the coverage and consistency of rabies vaccination programs in dogs is crucial to reduce the risk of rabies transmission and support regional rabies elimination efforts.

IV. CONCLUSION

The level of rabies immunity in dogs in Mapanget District, Manado City, remains low, with 91.8% of dogs having seronegative antibody titers and only 8.2% achieving protective titers. Vaccination status is the main factor significantly associated with the formation of rabies antibody titers, where vaccinated dogs have a 35.7 times greater chance of achieving protective titers compared to unvaccinated dogs (OR = 35.71; p = 0.002). The variables of dog breed, sex, and age did not show a significant association with antibody titer status. Low vaccination coverage is the main cause of low population immunity, so increasing rabies vaccination coverage in dogs needs to be prioritized to support rabies control.

V. ACKNOWLEDGMENTS

The author would like to thank the Head of the Agriculture and Animal Husbandry Service of North Sulawesi Province, the Head of the Animal Health Division of the Agriculture and Animal Husbandry Service of Manado City, the Maros Veterinary Center Laboratory, and the community who have given permission to take samples of their pet dogs in Mapanget District, Manado City and all parties who have helped and supported this research.

REFERENCES

[1] Akoso, Budi Tri. 2011. Prevention and Control of Rabies, 5th Edition, Kanisius, Yogyakarta.

[2] Cahyana Tirta IP, Parmawati M, Narayani I. 2021. Detection of Antibody Levels in Kintamani Dog Blood Serum After Rabies Vaccination Using Direct ELISA. ***Journal of Biological Sciences*** ISSN: 2302-5697*http://ojs.unud.ac.id/index.php/metamorfosa*.

[3] Chopy, D., Pothlichet, J., Lafage, M., Megret, F., Fiette, L., Si-Tahar, M., & Lafon, M. 2011. Ambivalent Role of the Innate Immune Response in Rabies Virus Pathogenesis. Journal of https://doi.org/10.1128/JVI.00302-11 Virology, 85(13), 6657–6668.

[4] Devira, D., Tyagita, & Mariani, H. (2023). Knowledge, Attitude, and Practice of Dog Owners on Rabies in the Province of West Java. ***Journal of Veterinary Science***, 41(2), 144.https://doi.org/10.22146/jsv.80495

[5] Dibia IN, Sumiarto B, Susetya H, Putra AAG, Scott-Orr H. 2015. Analysis of risk factors for rabies cases in dogs in Bali. BBVet Denpasar Veterinary Bulletin 27(86): 1–16.https://doi.org/10.4142/jvs.2015.16.4.459

[6] Dietzschold, B., & Schnell, M. 2009. Concepts in the pathogenesis of rabies, 3(5), 481–490.https://doi.org/10.2217/17460794.3.5.481

[7] North Sulawesi Provincial Health Office. 2025. North Sulawesi GHPR Incident Data 2024.

[8] Department of Agriculture and Animal Husbandry of North Sulawesi Province. 2025. Population Data of Animals Transmitting Rabies in North Sulawesi in 2024.

[9] Fooks, AR, Banyard, AC, Horton, DL, Johnson, N., McElhinney, LM, & Jackson, AC 2014. Current status of rabies and prospects for elimination. The Lancet, 384(9951), https://doi.org/10.1016/S0140-6736(13)62707-5 1389–1399.

[10] Hellgren F, Cagigi A, Arcoverde Cerveira R, Ols S, Kern T, Lin A, Eriksson B, Dodds MG, Jasny E, Schwendt K, Freuling C, Müller T, Corcoran M, Karlsson-Hedestam GB, Petsch B, Loré K. (2023). Unmodified rabies mRNA vaccine elicits high cross-neutralizing antibody titers and diverse B cell memory responses. Nature Communications, 14, 3713.https://doi.org/10.1038/s41467-023-39421-5

[11] Ji, C., Li, Y., Zhang, X., Wang, H., Liu, J., Chen, Q & Zhao, Z. (2023). Factors associated with dog rabies immunization in Changsha, China: Results of a cross-sectional cluster survey, 2015–2021. Viruses, 15(1), 138.https://doi.org/10.3390/v15010138

[12] Kaila, M., Marjoniemi, J., & Nokireki, T. (2019). Comparative study of rabies antibody titers of dogs vaccinated in Finland and imported street dogs vaccinated abroad. Acta Veterinaria Scandinavica 1–7.

[13] ary Drug Pharmacopoeia (FOHI). Volume 1. Edition 5. Directorate General of Animal Husbandry and Animal Health.

[14] Ministry of Agriculture. 2019. National Masterplan for Rabies Eradication in Indonesia. Directorate General of Animal Husbandry and Animal Health.

[15] Ministry of Health of the Republic of Indonesia. 2017. Rabies situation in Indonesia. Infodatin 2017. Jakarta: Data and Information Center of the Ministry of Health of the Republic of Indonesia.

[16] Ministry of Health of the Republic of Indonesia. 2019. Pocket Book of Technical Guidelines for the Management of Rabies-Transmitting Animal Bites. Jakarta. Directorate General of Disease Prevention and Control, Directorate of Prevention and Control of Vector-Borne and Zoonotic Diseases.

[17] Ministry of Health of the Republic of Indonesia. 2024. Indonesian Health Profile 2023. Infodatin 2017. Jakarta: Data and Information Center of the Ministry of Health of the Republic of Indonesia 2024.

[18] Ministry of Health of the Republic of Indonesia. 2025. Rabies Case Alert. Circular Letter of the Minister of Health of the Republic of Indonesia.

[19] Khan, M., Ahmed, S., Rauf, A., Ullah, N., Yousaf, M., & Ali, F. (2024). mRNA vaccine development against zoonotic diseases. Vaccines, 12(4), 485.https://doi.org/10.1016/j.vetvac.2025.100105

[20] Knobel, D., Shakespeare, M., Townsend, S. E., Brunker, K., & Hampson, K. (2022). Non-specific effects of rabies vaccination in dogs. Vaccine, 40(1),76–84

[21] MacLachlan, N. J., & Dubovi, E. J. (Eds.). 2011. Rabdhoviridae. In Fenner's Veterinary Virology (Fourth Edition) (4th ed., pp. 179–201). Tokyo: Academic Press.https://doi.org/https://doi.org/10.1016/B978-0-12-375158-4.00009-2

[22] Maharani Sekar Ayu, Hilmi Indah Laily & Salma, et al. (2023). Immune response to rabies vaccination in dogs. https://doi.org/10.5281/zenodo.7684314

[23] Phuyal, S., Acharya, S., Regmi, P., & Khanal, P. (2024). Maternal immunity interference with rabies vaccination. Frontiers in Veterinary Science, 11, 1354978.https://doi.org/10.3389/fvets.2024.1354978

[24] Prasatya, IGMK, Suardana, IBK, & Suartha, IN (2018). Immune Response of Local Male Dogs Aged Over One Year After Rabies Vaccination. Indonesia Medicus Veterinus, 69. https://doi.org/10.19087/imv.2018.7.1.69 Sebunya TK, Ndabambi N, Mpuchane S. A serosurvey of rabies antibodies in dogs in Gaborone, Botswana. 2007. ***Journal of Animal and Veterinary Advances*** 6:549–52.

[25] Rieder, M., & Conzelmann, KK 2011. Interferon in Rabies Virus Infection. Advances in Virus Research (1st ed., Vol. 79). Elsevier Inc.https://doi.org/10.1016/B978-0-12-387040-7.00006-8

[26] Salomao C, Nacima A, Cuamba L, Gujral L, Amiel A. Baltazar C, Cliff J. Gudo ES. 2017. Epidemiology, clinical features and risk factors for human rabies and animal bites during an outbreak of rabies in Maputo and Matola cities, Mozambique, 2014: Implications for public health interventions for rabies control. PLoS Neglected Tropical Diseases. 11(7): 1–16.

[27] Savaliya Bhumika F, Mathakiya RA, Bhanderi. B & Jhala MK 2015. Evaluation of phenotypic factors for anti-rabies antibodiesin vaccinated pet dogs.

[28] Singh, R., Batra, G., & Sood, M. (2017). Rabies—epidemiology, pathogenesis, and diagnosis. Advances in Human Biology, 7(4), 125–131.DOI:10.1080/01652176.2017.1343516

[29] Sudarmayasa, IN, Suardana, IBK, & Suartha, IN (2020). Antibody Titer of Local Dogs Six Months Post Rabies Vaccination. Udayana Veterinary Bulletin, 50.https://doi.org/10.24843/bulvet.2020.v12.i01.p09

[30] Utami, S., & Sumiarto, B. (2012). Level and Risk Factors of Protective Immunity to Rabies in Dogs in Makassar City. ***Veterinary Journal***, 13(1), 77–85.https://ojs.unud.ac.id/index.php/jvet/article/view/2142

[31] Thrusfield M. 2018. Veterinary Epidemiology Fourth Edition. Oxford (GB): John Willey & Sons Ltd.

[32] Wallace RM, Pees A, Blanton JB, Moore SM. 2017. Risk factors for inadequate antibody response to primary rabies vaccination in dogs under one year of age. PLOS Neglected Tropical Diseases. 11(7).

[33] World Health Organization (WHO). 2018. WHO expert consultation on rabies. Third report. WHO technical report series (1012) 1-139p.

[34] World Organization of Animal Health (WOAH). 2023. Rabies (Infection With Rabies Virus and Other Lyssaviruses). Terrestrial Manual. Chapter 3.1.18

[35] Wera E, Warembourg C, Bulu PM, Siko MM, Dürr S. (2022). Immune Response After Rabies Vaccination in Owned Free-Roaming Domestic Dogs in Flores Island, Indonesia. Frontiers in Veterinary Science, 9:868380. DOI: 10.3389/fvets.2022.868380.