Eurasia Specialized Veterinary Publication

International Journal of Veterinary Research and Allied Science

ISSN:3062-357X

2021, Volume 1, Issue 2, Page No: 93-98

Available online at: www.esvpub.com/

Link between Dog Erythrocyte Antigens (DEA) and Canine Susceptibility to Babesiosis

Nora Lindholm[1]*, Aada Koivisto[1]

[1]Department of Animal Health, Faculty of Veterinary Medicine, University of Helsinki, Helsinki, Finland.

***E-mail** ✉ n.lindholm.vet@outlook.com

ABSTRACT

Babesiosis in dogs is a life-threatening condition primarily characterized by hemolytic anemia. Although certain canine blood groups have been suggested to influence resistance or vulnerability to infections, the role of blood type in determining susceptibility to babesiosis remains poorly understood. This study aimed to determine the distribution of Dog Erythrocyte Antigen (DEA) 1 blood groups among dogs in Abeokuta, Nigeria, and to examine their possible association with Babesia infection. Blood samples (1 mL each) were obtained from 200 client-owned dogs. DEA 1.1, DEA 1.2, and DEA 1.0 genotypes were identified using the Alvedia® assay, while Babesia DNA was detected via PCR after extraction. Statistical analysis with Chi-square tested associations between DEA 1 types and infection status. DEA 1 positive dogs comprised 63.5% of the population, significantly outnumbering DEA 1 negative dogs (36.5%). Breeds including Boerboel, Rottweiler, Caucasian, and local dogs exhibited a high prevalence of DEA 1 positivity (73.3–86.4%), whereas German Shepherds showed nearly equal proportions of DEA 1 positive and negative dogs (51.2% vs. 48.8%). No sex-related differences were observed in DEA 1 distribution. The occurrence of Babesia infection was similar between DEA 1 positive (63.0%) and DEA 1 negative (60.3%) dogs, indicating no significant correlation ($p > 0.05$). These findings suggest that DEA 1 blood type does not appear to influence canine susceptibility or tolerance to Babesia infection.

Keywords: Dog Erythrocyte Antigen, Dog, Blood group, Babesiosis

Received: 26 May 2021
Revised: 19 October 2021
Accepted: 21 October 2021

How to Cite This Article: Lindholm N, Koivisto A. Link between Dog Erythrocyte Antigens (DEA) and Canine Susceptibility to Babesiosis. Int J Vet Res Allied Sci. 2021;1(2):93-8. https://doi.org/10.51847/FR45U23x2H

Introduction

Babesiosis is among the most economically important tick-borne infections, affecting domestic animals, wildlife, and humans, particularly in humid and subtropical regions worldwide [1, 2]. In dogs, the disease is predominantly caused by Babesia canis and B. gibsoni and is recognized as a life-threatening piroplasmosis, with hemolytic anemia being a hallmark clinical feature [3]. Koster *et al.* [4] classify canine babesiosis into uncomplicated forms, where anemia alone accounts for clinical signs, and complicated forms, where anemia is accompanied by dysfunction in other organs.

The pathophysiology and clinical features of canine babesiosis share notable similarities with human malaria caused by Plasmodium falciparum, an intraerythrocytic parasite akin to Babesia species [5, 6]. Previous studies indicate that human ABO blood groups influence malaria outcomes, with individuals of blood group 'O' experiencing reduced severity due to decreased rosetting activity [7, 8]. Given these parallels, it is plausible that canine blood groups might similarly affect the clinical course of Babesia infections.

Understanding blood group systems is critical in veterinary medicine, particularly for transfusions, as incompatible blood can provoke severe or fatal reactions [9]. Blood typing and compatibility testing prior to transfusion help minimize such risks, and knowledge of breed-specific blood types supports the selection of suitable donor dogs.

Among the various canine blood group systems, the Dog Erythrocyte Antigen (DEA) system is considered the most clinically relevant [10, 11]. Antibodies against DEA 1.1 can provoke acute hemolytic transfusion reactions, and dogs typically become sensitized after a single incompatible transfusion [12]. To date, eight DEA types have been identified (DEA 1.1, 1.2, 3, 4, 5, 6, 7, and 8) [13].

In Nigeria, studies on canine blood groups are limited. The foundational work by Nottidge *et al.* [14] established baseline DEA distributions in indigenous dogs, but little research has addressed the relationship between DEA types and disease susceptibility, particularly to babesiosis, which is highly prevalent and contributes substantially to canine morbidity and mortality in the region. No studies have yet evaluated whether DEA phenotypes influence susceptibility or resistance to Babesia infection in Nigerian dogs. This study therefore aimed to investigate the prevalence of DEA 1 blood types and their potential association with canine babesiosis in Nigeria.

Materials and Methods

Study population and sample collection

A cross-sectional study was conducted on dogs presented for treatment at two government veterinary hospitals and two private veterinary clinics in Abeokuta (Ogun State) and Lagos (Lagos State), Nigeria. Two hundred client-owned dogs exhibiting clinical signs suggestive of babesiosis—including fever, pale mucous membranes, tick infestations, enlarged lymph nodes or spleen, jaundice, and anorexia—were enrolled. Dogs that had received antibiotics or antiprotozoal treatment within two weeks prior to presentation were excluded. For each dog, age, sex, breed, and name were recorded.

Venous blood (1 mL) was collected from either the cephalic or jugular vein into EDTA-containing tubes. These samples were used both for DEA typing and for genomic DNA extraction.

DEA typing

The DEA 1 status of each dog was determined using the Lab TEST DEA 1 Alvedia® immunochromatographic assay (Alvedia Veterinary Diagnostics, France), following the manufacturer's instructions. Briefly, 10 µL of fresh anticoagulated whole blood was placed in the sample well of the test cassette, followed by 200 µL of the provided dilution buffer. The sample was allowed to migrate along the test strip at room temperature for 2 minutes. The control band's appearance confirmed test validity, while the presence or absence of the DEA 1 test band indicated the genotype.

Dogs showing both control and test bands were classified as DEA 1-positive, while those with only the control band were classified as DEA 1-negative. Tests without a visible control band were repeated with fresh samples. Among positive samples, band intensity was used to differentiate genotypes: a strong red line indicated DEA 1.1, a faint or weak line indicated DEA 1.2, and absence of the test line indicated a DEA-negative dog.

Molecular Detection of Babesia spp. Using Polymerase Chain Reaction (PCR)

DNA Extraction and Amplification

Genomic DNA was isolated from 100 µL of whole blood using the Quick-gDNA™ MiniPrep Plus kit (Zymo Research, Irvine, CA, USA). Frozen blood samples (−20 °C) were thawed at room temperature. Lysis was performed by mixing 100 µL of blood with 400 µL of genomic lysis buffer (4:1 ratio), followed by vortexing for 6 seconds and incubation for 10 minutes. The lysate was then passed through a Zymo-Spin™ IIC column and centrifuged at 10,000 × g for 1 minute in a Spectrafuge 24D centrifuge (Labnet International, USA). Purified DNA was eluted according to the manufacturer's protocol and stored at −20 °C for 24–48 hours prior to use.

PCR was carried out in a BioRad MyCycler® thermocycler (USA) in a total reaction volume of 25 µL. Each reaction contained 20 ng of template DNA, 12.5 µL of 2× PCR Master Mix (Bioneer®), 7.5 µL nuclease-free water, and 0.5 µL (40 ng each) of PIRO-A and PIRO-B primer pair. Cycling conditions consisted of initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 57.8 °C for 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 10 min.

A known Babesia-positive canine blood sample (from the Veterinary Teaching Hospital, Federal University of Agriculture, Abeokuta) served as the positive control, while nuclease-free water was used as the negative control. Amplicons (10 µL) were separated by electrophoresis on 1% agarose gels stained with ethidium bromide in 1× TAE buffer and visualized under UV light using a Spectroline® transilluminator (USA). Samples displaying a band at 400 bp, identical to the positive control, were considered positive for Babesia spp.

Statistical analysis

Prevalence of Babesia infection and its subspecies was reported as percentages with 95% confidence intervals. The association between Dog Erythrocyte Antigen 1 (DEA 1) status and Babesia infection was evaluated using the chi-square (χ^2) test. Observed frequencies of DEA 1-positive and DEA 1-negative dogs among Babesia-positive cases were compared with expected frequencies. A p-value < 0.05 was regarded as statistically significant. All analyses were performed using SPSS version 20.0 (IBM Statistics).

Results

PCR detection

Of the 200 blood samples tested, 124 (62%) produced the expected 400 bp amplicon, confirming the presence of Babesia spp. DNA.

Distribution of DEA 1 Blood Types

Using the ALVEDIA DEA 1 rapid test kit:

- 70 dogs (35.0%) were DEA 1.1 positive,
- 57 dogs (28.5%) were DEA 1.2 positive,
- 73 dogs (36.5%) were DEA 1 negative.

Overall, 127 dogs (63.5%) were DEA 1 positive (combining DEA 1.1 and 1.2). The proportion of DEA 1-positive dogs was significantly higher than DEA 1-negative dogs ($p < 0.05$).

Table 1. Distribution of DEA 1 blood group types across different dog breeds in the study population (n = 200)

Breed	Total No. of dogs (%)	DEA 1 + ve* (%)	DEA 1.1 (%)	DEA 1.2 (%)	DEA 1 Negative (%)
German Shepherd	80 (40.0%)	41 (51.2%)	23 (28.8%)	18 (22.5%)	39 (48.8%)
Boerboel	35 (17.5%)	26 (74.3%)	14 (40.0%)	12 (34.3%)	9 (25.7%)
Rottweiler	22 (11.0%)	19 (86.4%)	9 (40.9%)	10 (45.5%)	3 (13.6%)
Caucasian	15 (7.5%)	11 (73.3%)	8 (53.3%)	3 (20.0%)	4 (26.7%)
Local (Nigerian indigenous)	16 (8.0%)	13 (81.3%)	6 (37.5%)	7 (43.8%)	3 (18.8%)
Mixed breed	23 (11.5%)	15 (65.2%)	9 (39.1%)	6 (26.1%)	8 (34.8%)
Others**	9 (4.5%)	2 (22.2%)	1 (11.1%)	1 (11.1%)	7 (77.8%)
Total	**200 (100%)**	**127 (63.5%)**	**70 (35.0%)**	**57 (28.5%)**	**73 (36.5%)**

+: positive, −: negative *DEA 1 + ve includes both DEA 1.1 and DEA 1.2 subtypes. **Others include Doberman, Samoyed, Lhasa Apso, and Cane Corso.

Association between Dog Breed and DEA 1 Blood Group

As shown in **Table 1**, German Shepherd Dogs (GSD), the most represented breed (40% of the study population), exhibited a significantly higher frequency of DEA 1-negative status (48.8%) compared to DEA 1.1 (28.8%) or DEA 1.2 (22.5%) when considered separately ($p < 0.05$). However, when DEA 1.1 and DEA 1.2 were combined, the difference between DEA 1-positive (51.2%) and DEA 1-negative (48.8%) GSDs was not statistically significant ($p > 0.05$).

In contrast, Boerboel, Rottweiler, Caucasian, and Local (Nigerian indigenous) breeds showed a clear predominance of DEA 1-positive blood types, ranging from 73.3% to 86.4%, which was significantly higher ($p < 0.05$) than the DEA 1-negative proportion (13.6–26.7%) in these breeds.

Association between Sex and DEA 1 Blood Group

Across the entire study population **(Table 2)**, no significant difference ($p > 0.05$) was observed in the overall prevalence of DEA 1-positive status (combining DEA 1.1 and 1.2) between males (64.3%) and females (62.4%). However, notable sex-related differences were found within the positive subtypes:

Male dogs had a significantly higher proportion of the strongly positive DEA 1.1 type (40.0%) than females (28.2%) ($p < 0.05$).

Conversely, female dogs showed a significantly higher frequency of the weakly positive DEA 1.2 type (34.1%) compared to males (24.3%) ($p < 0.05$).

Table 2. Distribution of DEA 1 blood group types according to sex of dogs (n = 200)

Sex	Total No. of dogs	DEA 1.1 + 1.2)	DEA 1.1 (strong positive)	DEA 1.2 (weak positive)	DEA 1.0
Male	115	74 (64.3%)	46 (40.0%)	28 (24.3%)	41 (35.7%)
Female	85	53 (62.4%)	24 (28.2%)	29 (34.1%)	32 (37.6%)
Total	**200**	**127 (63.5%)**	**70 (35.0%)**	**57 (28.5%)**	**73 (36.5%)**

Out of the 200 dog samples tested using PCR, 124 (62 percent) were positive for Babesia species, whereas 76 (38%) tested negative **(Table 3)**. The prevalence of Babesia species did not differ significantly ($p > 0.05$) between dogs that were DEA 1 positive and those that were DEA 1.

Table 3. Association between DEA Blood Group and Babesia Infection in Dogs

Blood Type	Number of Dogs	Babesia+ve	Babesia-ve
DEA 1.1	70	41 (58.6%)	29 (41.4%)
DEA 1.2	57	39 (68.4%)	18 (31.6%)
DEA 1.1 & 1.2	127	80 (63.0%)	47 (37.0%)
DEA Negative	73	44 (60.3%)	29 (39.7%)
Total	**200**	**124 (62%)**	**76 (38%)**

DEA: Dog Erythrocyte Antigen

Relationship between DEA Types and Babesia Prevalence

The rates of Babesia species infection across blood types were: DEA 1.1 (58.6%), DEA 1.2 (68.4%), and DEA 1-negative (60.3%). The infection rate for the combined DEA 1-positive groups (63.0%) showed no significant difference ($p > 0.05$) compared to DEA-negative blood type (60.3%). Therefore, DEA blood groups did not appear to markedly affect the dogs' vulnerability to babesiosis in this investigation.

Discussion

Gaining a thorough grasp of how DEA types affect dogs' vulnerability to Babesia infections is crucial for improving disease risk evaluation, ensuring safe blood transfusions, and shaping selective breeding approaches. It may also offer key perspectives on host-pathogen relationships that influence disease patterns. Accordingly, this research examined the effects of DEA types on babesial infection rates in dogs.

The elevated Babesia species detection rates in this study stand out as considerably higher and inconsistent with the results from Takeet *et al.* [15], who similarly used molecular methods to detect Babesia in naturally infected dogs in Ogun State, Nigeria. This variation in prevalence could stem from differences in sample collection—Takeet *et al.* [15] gathered samples randomly from dogs attending routine health checks, whereas the current work targeted dogs brought for clinical assessment and care at the Veterinary Teaching Hospital and veterinary clinics. This highlights the value of employing highly sensitive diagnostic methods for precise identification and analysis of canine babesiosis.

Variations in DEA blood type prevalence across dog breeds in different regions have been documented [17]. Here, the distribution of the three DEA genotypes—DEA 1.1, DEA 1.2, and DEA 1-negative—showed no notable differences, probably due to the varied breed makeup of the dogs sampled. Yet, combining the two DEA 1-positive types (1.1 and 1.2) resulted in a combined frequency that was markedly greater than that of DEA-negative dogs. Spada *et al.* [17] noted that DEA 1 prevalence is generally around 50% worldwide, with Corso dogs exhibiting a notably higher rate of DEA 1-negative types compared to most previously studied canine groups.

The German Shepherd Dog (GSD) was the most common breed in this study. Earlier work in South Africa by van der Merwe *et al.* [18] highlighted breed-specific differences in DEA 1 blood group distributions. In contrast, GSDs here displayed almost balanced rates of DEA 1-positive (51.2%) and DEA 1-negative (48.8%) types. This contrasts with the South African findings and a Portuguese study by Ferreira *et al.* [19], which reported a 100% DEA-negative prevalence in GSDs. Importantly, the majority of GSDs in both this and the South African study were hospital patients without confirmed pedigrees, indicating that such differences might arise from inconsistencies in breed authenticity. On the other hand, the elevated DEA 1-positive rates in Boerboels,

Caucasians, and Rottweilers (73.3% to 86.4%) in this research are consistent with reports from South Africa [20], Portugal [19], and California, USA [20].

In an earlier Ibadan, Nigeria, investigation, Nottidge *et al.* [14] found a 39.9% DEA 1-positive rate among local breeds, which differs from the 81.3% observed for the same breed in the present study.

No notable differences emerged in the proportions of DEA 1-positive or DEA 1-negative types between male and female dogs, indicating that sex does not impact DEA 1 genotypes. Comparable outcomes were reported in studies from Brazil [21], Portugal [19], and California, USA [20]. However, a Zimbabwean study [22] identified a greater DEA-positive prevalence in females than males.

The Babesia species infection rate in DEA 1-positive dogs (63.0%) did not differ significantly from that in DEA 1-negative dogs (60.3%). Likewise, Dhliwayo *et al.* [22] detected no meaningful link between DEA 1 blood group and babesiosis. These results imply that the DEA 1 group might not affect susceptibility to Babesia. Nevertheless, since a one-time parasitemia screening may not fully capture infection progression or severity, additional studies are required to clarify if this applies only to infection rates or also to disease pathogenicity in dogs.

Certain limitations of this study warrant recognition. The sampled dogs were limited to those attending specific veterinary hospitals and clinics in Abeokuta and Lagos, potentially not representing the wider dog population, especially stray or rural dogs with minimal veterinary access. Focusing solely on dogs showing clinical babesiosis signs might have overlooked asymptomatic or mild cases, thereby influencing the evaluation of DEA status and infection susceptibility. Moreover, excluding dogs with recent antiprotozoal or antibiotic therapy could have skewed the sample toward acute, untreated instances. As a cross-sectional design, it could only capture associations between DEA types and babesiosis at one moment, ignoring potential temporal or seasonal fluctuations. Finally, depending on owner-provided histories might have introduced reporting biases. Notwithstanding these constraints, the research offers valuable initial observations on DEA's potential involvement in canine babesiosis susceptibility in Nigeria and stresses the necessity for larger-scale, multi-site, and longitudinal research.

In summary, this investigation sheds light on DEA blood type distributions across various dog breeds at veterinary facilities and their connection to Babesia infections. Although DEA 1-positive dogs predominated among the studied animals, no significant link existed between DEA type and Babesia prevalence. Breed and sex variations were apparent, pointing to intricate genetic factors. The results underscore the importance of deeper genetic profiling of local breeds and more extensive research into DEA-linked disease risks. Such efforts will improve diagnostic precision, therapeutic approaches, and tailored health management for dog breeds.

Acknowledgments: Dr. Oyinkansola Fadiji, Dr. Taiye Adewumi and Mr Olugbogi (of the Federal University of Agriculture, Abeokuta) for assistance during the laboratory work.

Conflict of Interest: None

Financial Support: None

Ethics Statement: Ethics approval was granted by the Ethics Committee of the Federal University of Agriculture, Abeokuta Ogun State Nigeria. (FUNAAB/COLVET/ CREC/2021/09/02). All protocols (clinical, experimental and laboratory) implemented were in accordance with international principles and protocols for animal usage. Consent to participate was not applicable as dogs were client owned and the owners indicated interest in patient care and treatment.

References

1. Ali KM, Marif H. Babesiosis in cattle. Int J Agric Biosci. 2023;3:114–21.
2. Yusuf JJ. Review of bovine babesiosis and its economical importance. J Vet Med Res. 2017;4(5):1090.
3. Scheepers E. The haematological kinetics of canine babesiosis in South Africa. Thesis, University of Pretoria; 2008. Available from: http://hdl.handle.net/2263/26347
4. Koster L, Lobetti R, Kelly P. Canine babesiosis: a perspective on clinical complications, biomarkers and treatment. Vet Med Res Rep. 2015;6:119–28.

5. Bloch EM, Kasubi M, Levin A, Mrango Z, Weaver J, Munoz B, et al. Babesia microti and malaria infection in Africa: a pilot serosurvey in Kilosa district, Tanzania. Am J Trop Med Hyg. 2018;99(1):51–6.
6. Krause PJ, Daily J, Telford SR, Vannier E, Lantos P, Spielman A. Shared features in the pathobiology of babesiosis and malaria. Trends Parasitol. 2007;23(12):605–10.
7. Rowe JA, Handel IG, Thera MA, Deans A, Lyke KE, Kone A, et al. Blood group O protects against severe Plasmodium falciparum malaria through the mechanism of reduced resetting. Proc Natl Acad Sci U S A. 2007;104(44):17471–6.
8. Zerihun T, Degarege A, Erko B. Association of ABO blood group and Plasmodium falciparum malaria in Dore Bafeno Area, Southern Ethiopia. Asian Pac J Trop Biomed. 2011;1:289–94.
9. Tiwari AJ, Balekar NS, Jain DK. Blood group systems and blood transfusion of animals. Int J Pharm Pharm Sci. 2009;1:50–4.
10. Giger U, Stieger K, Palos H. Comparison of various canine blood-typing methods. Am J Vet Res. 2005;66:1386–92.
11. Goggs R. BSAVA manual of canine and feline emergency and critical care. J Small Anim Pract. 2009;50(8):440–5.
12. Carli E, Carminato A, Ravagnan S, Capello K, Antognoni MT, Miglio A, et al. Frequency of DEA 1 antigen in 1037 mongrel and purebreed dogs in Italy. BMC Vet Res. 2017;13(1):364.
13. Arikan S, Guzel M, Mamak N, Ograk YZ. Frequency of blood types DEA 1.1, 3, 4, 5 and 7 in Kangal dog. Rev Med Vet. 2009;160(4):180–3.
14. Nottidge HO, Omobowale TO, Washio M, Ajadi RA, Toizumi S, Takahashi K. The prevalence of the dog erythrocyte antigen 1 (DEA 1.1 and 1.2) in Nigerian indigenous dogs. Folia Vet. 2006;5:60–8.
15. Takeet MI, Oyewusi AJ, Abakpa SAV, Daramola OO, Peters SO. Genetic diversity among Babesia rossi detected in naturally infected dogs in Abeokuta, Nigeria, based on 18S rRNA gene sequences. Acta Parasitol. 2017;62(1):192–8.
16. Mangiaterra S, Rossi G, Antognoni MT, Cerquetella M, Marchegiani A, Miglio A, et al. Canine blood group prevalence and geographical distribution around the world: an updated systematic review. Animals. 2021;11(2):342.
17. Spada E, Proverbio D, Priolo V, Ippolito D, Baggiani L, Perego R, et al. Dog erythrocyte antigens (DEA) 1, 4, 7 and suspected naturally occurring anti-DEA 7 antibodies in Italian Corso dogs. Vet J. 2017;222:17–21.
18. Van der Merwe LL, Jacobson LS, Pretorius GJ. The breed prevalence of dog erythrocyte antigen 1.1 in the Onderstepoort area of South Africa and its significance in selection of canine blood donors. J S Afr Vet Assoc. 2002;73:53–6.
19. Ferreira RRF, Ruiz de Gopegui R, Matos AJF. Frequency of dog erythrocyte antigen 1.1 expression in dogs from Portugal. Vet Clin Pathol. 2011;40(2):198–201.
20. Bank AS, Farrell KS, Epstein SE. Prevalence of dog erythrocyte antigen 1 in a population of dogs tested in California. J Vet Emerg Crit Care. 2022. (Brief clinical communication).
21. Ferreira RRF, Ruiz de Gopegui R, Matos AJF. Frequency of dog erythrocyte antigen 1.1 expression in dogs from Portugal. Vet Clin Pathol. 2011;40(2):198–201.
22. Dhliwayo S, Makonese TA, Whittall B, Chikerema SM. A study on the prevalence of dog erythrocyte antigen 1.1 and detection of canine Babesia by polymerase chain reaction from apparently healthy dogs in a selected rural community in Zimbabwe. J S Afr Vet Assoc. 2016;87(1).