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RESEARCH ARTICLE

First Detection and Prevalence of Bovine Rhinitis A and B Viruses in Cattle in Türkiye: Risk Assessment and Association with Bovine Respiratory Disease

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ABSTRACT

Bovine respiratory disease (BRD) represents a multifactorial syndrome causing significant economic losses in cattle worldwide. Among viral pathogens, bovine rhinitis A virus (BRAV) and bovine rhinitis B virus (BRBV) have been implicated in BRD etiology; however, to our knowledge, no data are available for Türkiye. This study aimed to determine the prevalence of BRAV and BRBV in cattle from Kırıkkale and Ankara provinces. Samples were collected from 200 cattle (100 BRD-affected and 100 asymptomatic) and BRAV and BRBV detection was carried out via multiplex real-time RT-PCR from nasal swabs, and hematological parameters were analyzed from blood samples. In addition, data on age, body temperature, respiratory signs, and the presence of ocular and nasal discharge were recorded for all animals. BRAV was detected in 24.5% (49/200), BRBV in 12.5% (25/200), and co-infections in 7% (14/200). Infection rates were significantly higher in BRD-affected animals for both BRAV (40% vs. 9%, OR: 6.7, 95% CI: 3.05–14.90, P<0.0001) and BRBV (19% vs. 6%, OR: 3.7, 95% CI: 1.40–9.64, P=0.0082). BRAV positivity was significantly associated with younger age (1–2 months) and fever (P<0.05), while no significant clinical sign or age-related differences were observed for BRBV. Hematological analysis revealed significant differences in neutrophils, lymphocytes and monocytes among virus-positive animals (P<0.05). This study provides the first evidence of BRAV and BRBV circulation in Türkiye, underscoring their potential involvement in BRD pathogenesis.

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INTRODUCTION

Bovine respiratory disease (BRD) is a multifactorial syndrome involving viral and bacterial infections, management, environmental factors and host-related factors such as age, breed, underlying infections, immunological status, etc. (Taylor *et al*., 2010; Risalde *et al*., 2015; Zeineldin *et al*., 2020; Peel, 2020; Smith, 2020). BRD incidence may reach 54.6% in calves, particularly within the first 45 days (Sasaki *et al*., 2022). BRD causes significant economic losses in the cattle industry due to production losses, high treatment costs, increased mortality rates, lower slaughter weights, etc. (Blakebrough-Hall *et al*., 2020; Johnson *et al*., 2017; Speer *et al*., 2001; White and Larson, 2020). BRD related viral etiology includes agents such as herpesvirus, pestivirus, coronavirus, parainfluenza virus, respiratory syncytial virus, influenza C and D viruses, bovine rhinitis A and B viruses, adenovirus, parvovirus, astrovirus, picobirnavirus (Zhang *et al*., 2020; Ng *et al*., 2015; Özbek *et al*., 2024). Clinical signs of BRD include fever, coughing, abnormal lung sounds, dyspnea, nasal discharge, ocular discharge and increased respiratory rate (Sasaki *et al*., 2022). Several studies have reported differences in serum biochemistry, acute phase proteins and complete blood counts between BRD-affected and healthy animals (Almujalli *et al*., 2015; Omar *et al*., 2024).

Bovine rhinitis viruses are classified in family *Picornaviridae,* subfamily *Caphthovirinae*, genus *Aphthovirus* and consist of two virus species: bovine rhinitis A virus (BRAV; currently renamed as *Aphthovirus bogeli*) and bovine rhinitis B virus (BRBV; currently renamed as *Aphthovirus reedi*). BRAV and BRBV are non-enveloped, approximately 30 nm in diameter, and have 7.2–7.5 kb of positive-sense, single-

stranded RNA genome (International Committee on Taxonomy of Viruses, 2024). Bovine rhinitis viruses cause mild upper respiratory tract infections and fever in cattle. In addition, BRBV has demonstrated neurotropic effects, as viral presence in brain tissue has been confirmed by real-time RT-PCR, virus isolation, and immunohistochemistry in experimentally infected calves, although no neurological symptoms were observed (Bhattarai *et al*., 2022). Although BRAV and BRBV had been identified previously, widespread application of metagenomics increased and underlined their role in the etiology of BRD, consequently the number of studies focusing on rhinitis viruses has been increasing. Several studies have detected BRAV and BRBV in BRD-affected cattle and these limited available studies on bovine rhinitis viruses have primarily been conducted in the United States, Canada, Mexico, China, Japan, Australia, and Sweden (Hause *et al*., 2015; Ng *et al*., 2015; Mitra *et al*., 2016; Blomström *et al*., 2017; Zhang *et al*., 2019; Zhang *et al*., 2020; Zhai *et al*., 2021; Zhang *et al*., 2021; Bhattarai *et al*., 2022; Zhou *et al*., 2023a; Ambrose *et al*., 2023). To the best of our knowledge, no comprehensive data on bovine rhinitis viruses in Turkish cattle populations currently exist in published literature. This pioneering study aims to: (i) investigate BRV prevalence in cattle across Kırıkkale and Ankara provinces, (ii) evaluate associations between viral detection rates, hematological parameters, and clinical manifestations.

MATERIALS AND METHODS

Study Groups and Samples: This study was approved by the Kırıkkale University Animal Experiments Local Ethics Committee (Approval number: 2023/09/32). Cattle from farms in Kırıkkale and Ankara provinces were clinically examined for respiratory symptoms indicative of bovine respiratory disease (BRD). Animals exhibiting BRD-related symptoms (fever, cough, dyspnea, nasal/ocular discharge) without prior treatment were classified as "BRD-affected group" (n=100), while cattle without any BRD-related signs and with rectal temperatures <39°C comprised the "asymptomatic group" (n=100). Animals developing BRD symptoms within 40 days post-sampling were excluded from asymptomatic group. Sterile swabs were used for sampling, placed in viral transport media which contains gentamicin and amphotericin B. Blood samples were collected via jugular venipuncture into EDTA-treated anticoagulant tubes and analyzed within 4 hours of collection. Swab samples were transported to the laboratory under cold-chain conditions; subsequently, they were vortexed briefly and the viral transport medium was transferred to sterile microcentrifuge tubes for storage at −20°C pending RNA isolation.

RT-PCR and multiplex real-time RT-PCR: RNA isolation was performed using the High Pure Viral RNA kit (Roche, Germany) according to the manufacturer's protocol, and RNA samples were stored at -80°C until further use. The multiplex real time RT-PCR was carried out in two steps: cDNA synthesis followed by multiplex real-time PCR. cDNA was synthesized from isolated RNA samples using random hexamer primers and stored at -20°C until used. Prior to multiplex real-time PCR, cDNA samples were verified via conventional PCR targeting the bovine glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene with specific primers (Aksoy *et al*., 2021; Ji-Hye *et al*., 2023). Multiplex real-time PCR assays were performed using cDNA samples with specific primers and probes for BRAV and BRBV as described by Ambrose *et al.* (2023).

Hematological Analysis: Complete blood counts (CBC) were performed on collected blood samples using the BC-5000 Vet veterinary hematology analyzer (Mindray, China) at the diagnostic laboratory of Kırıkkale University Veterinary Faculty Animal Hospital. A total of 23 blood parameters were analyzed: white blood cell (WBC), neutrophil (NEU), lymphocyte (LYM), monocyte (MON), eosinophil (EOS), basophil (BAS), neutrophil percentage (NEU%), lymphocyte percentage (LYM%), monocyte percentage (MON%), eosinophil percentage (EOS%), basophil percentage (BAS%), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width-coefficient of variation (RDW-CV), red blood cell distribution width-standard deviation (RDW-SD), platelet (PLT), mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT).

Statistical Analysis: Statistical analyses were conducted using IBM SPSS version 30 software (IBM, USA). Statistical significance was set at $P<0.05$. Associations between viral positivity and clinical findings were analyzed using Chi-square and Fisher's exact tests. Blood parameters were compared using independent T-test or Mann-Whitney U test. Odds ratio (OR), 95% confidence interval (95% CI) and P value were calculated via MedCalc software version 23 to analyze the association between virus positivity, clinical BRD status and BRD-associated clinical signs.

RESULTS

In this study, nasal swab and blood samples were collected from 200 cattle in farms located in Ankara and Kırıkkale provinces, Türkiye. The animals were separated into two groups: asymptomatic (n=100) and BRD-affected group (n=100). Clinical examination comprised assessment of rectal temperature, pulmonary auscultation, and observation for respiratory signs like coughing, nasal and ocular discharge. Following clinical examination, nasal swab and whole blood samples were collected from all animals for subsequent laboratory analyses. All nasal swab samples were subjected to multiplex real-time RT-PCR to detect viral RNA (n=200); however, 3 blood samples cannot be analyzed due to hemolysis (n=98 for BRD group, n=99 for asymptomatic group, total n=197 for blood samples).

Following RNA isolation and cDNA synthesis, cDNA was assessed by conventional RT-PCR targeting the bovine GAPDH gene and all samples tested positive.

In our cohort of 200 cattle, multiplex real-time RT-PCR confirmed that BRAV and BRBV are prevalent in both healthy and clinically affected animals, but markedly so in those exhibiting clinical respiratory disease. Overall, 24.5% (49/200) of nasal swabs were BRAV-positive and 12.5% (25/200) were BRBV-positive. When stratified by clinical status, BRAV was detected in 40% of BRD-affected cattle versus only 9% of asymptomatic animals, corresponding to a 6.7-fold increase in the odds of disease (OR 6.7; 95% CI 3.05–14.90; P<0.0001). Similarly, BRBV infection conferred a 3.7-fold higher risk of BRD (19% vs. 6%; OR 3.7; 95% CI 1.40–9.64; P=0.0082) (Table 1).

Importantly, dual infection with BRAV and BRBV was observed in 7% of all cattle but was predominantly a feature of clinical disease: 13% of BRD cases harbored both viruses compared to just 1% of asymptomatic counterparts, translating to a 14.8-fold elevated risk for BRD (OR 14.8; 95% CI 1.90–115.40; P=0.0102) (Table 1). These findings underscore not only the individual contributions of BRAV and BRBV to BRD but also the synergistic impact of co-infection, highlighting the need to include both agents in diagnostic panels and risk-assessment models.

The information on age and breed and clinical signs such as fever, respiratory signs, ocular discharge and nasal discharge of the sampled animals have recorded. The animals were distributed in 3 age groups: 1-2 months (*n*=10), 2-6 months (*n*=82) and over 6 months (*n*=108). The breeds of the animals were Simmental (*n*=60), Holstein (*n*=88), brown Swiss (n=19), cross-bred (*n*=32) and Charolais (*n*=1).

There is statistically significant association between age groups and BRAV positivity according to chi-square test (P=0.037), with the highest positivity rate in the 1-2 months age group (50%), indicating that calves are the most susceptible to BRAV infections compared to older ages. After chi-square testing, risk assessment between age groups were calculated. Calves aged 1-2 months BRAV positivity rate was significantly higher and approximately fivefold greater than in 2-6 months-old age group (OR: 4.86, 95% CI: 1.24-19.05, P=0.0234) (Table 2), suggesting that BRAV could be a significant respiratory pathogen in younger animals. No significant association was found between positivity of BRBV positivity, co-infection status and age groups (P>0.05).

Rectal temperature, respiratory signs, nasal and ocular discharge were clinically evaluated in all sampled animals. Animals in the asymptomatic group had no fever, respiratory signs, or nasal/ocular discharge. In BRD-affected group, rectal temperature, respiratory signs, nasal discharge were statistically analyzed based on virus positivity/negativity (Table 2). There is no ocular discharge detected in BRD-affected group.

According to the results, the odds of fever were approximately 5 times higher in BRAV-positive animals (OR: 4.7; 95% CI: 1.18–19.18; P=0.0287). In this study, no statistically significant difference was found between BRAV or BRBV positivity and the presence of nasal discharge or cough severity. No statistically significant association was determined between BRBV positivity or co-infection and any clinical signs. These findings indicate that BRAV infection is more likely in animals with fever and in younger animals aged 1–2 months (Table 2).

A total of 197 blood samples were analyzed in this study. According to comparison of complete blood count results of the BRD-affected and asymptomatic animals, there are statistically significant differences were detected in NEU, LYM, MON, EOS, NEU%, LYM%, MON%, EOS%, RBC, HGB, HCT, MCV, MCH, RDW-CV, RDW-SD, PLT, PDW, and PCT parameters (P<0.05) (Table 3). When the hematological parameters of all BRAV-positive and -negative animals were compared, regardless of the study group (BRD-affected and asymptomatic animals were evaluated together), statistically significant differences were determined in NEU, LYM, MON, EOS, NEU%, LYM%, MON%, EOS%, HGB, HCT, and MPV (P<0.05) (Table 4).

When the hematological parameters of all BRBV-positive and -negative animals were compared, regardless of the study group (BRD-affected and asymptomatic animals were evaluated together), statistically significant differences were found in NEU, LYM, NEU%, and LYM% (P<0.05) (Table 5). Regardless of the study group, when the hematological parameters of all co-infected (only BRAV and BRBV positive animals) and other animals were compared, statistically significant differences were determined in LYM, NEU% and LYM% (P<0.05) (Table 6).

The analysis of hematological parameters was also carried out between BRD-affected and asymptomatic groups for BRAV and BRBV positive animals. Statistically significant differences in EOS, EOS%, MCV, and PDW parameters were determined when BRAV positive BRD- and asymptomatic-groups are compared (P<0.05) (Table 7). In BRBV-positive animals, significant differences were found between the BRD-affected and asymptomatic groups in terms of MON, MCV, and MCH (P<0.05) (Table 8). The hematological results of co-infected animals could not be statistically analyzed between groups as only one co-infected animal was identified in the asymptomatic group.

Table 1: Risk assessment of viruses in the BRD-affected and asymptomatic animals. Multiplex real-time RT-PCR detection results of viruses in the nasal swab samples were summarized, and odds ratio (OR), 95% confidence interval (95% CI) and P values were calculated.

Risk factors	Multiplex real-time RT-PCR result	BRD-affected (%)	Asymptomatic (%)	OR	95% CI	P value
Bovine rhinitis A virus (BRAV)	Positive	40 (40%)	9 (9%)	6.7	3.05–14.90	< 0.0001
	Negative	60 (60%)	91 (91%)			
Bovine rhinitis B virus (BRBV)	Positive	19 (19%)	6 (6%)	3.7	1.40–9.64	0.0082
	Negative	81 (81%)	94 (94%)			
Co-infection with BRAV and BRBV	Positive	13 (13%)	1 (1%)	14.8	1.90–115.40	0.0102
	Negative	87 (87%)	99 (99%)			

Table 2: Risk factors associated with clinical signs and age in the BRD-affected group. The distribution of multiplex real-time RT-PCR detection results of viruses in the nasal swab samples according to clinical signs and age, and odds ratio (OR), 95% confidence interval (95% CI) and P values were calculated. Factors with statistical significance (P<0.05) are shown with asterisk.

Risk factors	Multiplex real-time RT-PCR result	Fever (%)	Normal rectal temperature (%)	OR	95% CI	P value
Bovine rhinitis A virus (BRAV)	Positive	8 (8%)	32 (32%)	4.7	1.18–19.18	0.0287*
	Negative	3 (3%)	57 (57%)			
Bovine rhinitis B virus (BRBV)	Positive	3 (3%)	16 (16%)	1.7	0.41–7.17	0.4626
	Negative	8 (8%)	73 (73%)			
Co-infection with BRAV and BRBV	Positive	3 (3%)	10 (10%)	2.9	0.67–13.02	0.1507
	Negative	8 (8%)	79 (79%)			
Risk factors	Multiplex real-time RT-PCR result	Nasal discharge present (%)	Nasal discharge absent (%)	OR	95% CI	P value
Bovine rhinitis A virus (BRAV)	Positive	6 (6%)	34 (34%)	1.9	0.55–6.85	0.3028
	Negative	5 (5%)	55 (55%)			
Bovine rhinitis B virus (BRBV)	Positive	1 (1%)	18 (18%)	0.39	0.05–3.28	0.3897
	Negative	10 (10%)	71 (71%)			
Co-infection with BRAV and BRBV	Positive	1 (1%)	12 (12%)	0.64	0.08–5.47	0.6850
	Negative	10 (10%)	77 (77%)			
Risk factors	Multiplex real-time RT-PCR result	Cough and respiratory distress (%)	Cough (%)	OR	95% CI	P value
Bovine rhinitis A virus (BRAV)	Positive	9 (9%)	31 (31%)	2.2	0.74-6.49	0.1539
	Negative	7 (7%)	53 (53%)			
Bovine rhinitis B virus (BRBV)	Positive	3 (3%)	16 (16%)	0.98	0.25-3.85	0.9778
	Negative	13 (13%)	68 (68%)			
Co-infection with BRAV and BRBV	Positive	2 (2%)	11 (11%)	0.95	0.19-4.75	0.9483
	Negative	14 (14%)	73 (73%)			
Risk factors	Multiplex real-time RT-PCR result	1-2 months age (%)	2-6 months age (%)	OR	95% CI	P value
Bovine rhinitis A virus (BRAV)	Positive	5 (5%)	14 (14%)	4.86	1.24-19.05	0.0234*
	Negative	5 (5%)	68 (68%)			
Bovine rhinitis B virus (BRBV)	Positive	1 (1%)	8 (8%)	1.03	0.11-9.19	0.9804
	Negative	9 (9%)	74 (74%)			
Co-infection with BRAV and BRBV	Positive	1 (1%)	4 (4%)	2.17	0.22-21.55	0.5095
	Negative	9 (9%)	78 (78%)			
Risk factors	Multiplex real-time RT-PCR result	1-2 months age (%)	Over 6 months age (%)	OR	95% CI	P value
Bovine rhinitis A virus (BRAV)	Positive	5 (5%)	30 (30%)	2.60	0.70-9.63	0.1526
	Negative	5 (5%)	78 (78%)			
Bovine rhinitis B virus (BRBV)	Positive	1 (1%)	16 (16%)	0.64	0.08-5.39	0.6806
	Negative	9 (9%)	92 (92%)			
Co-infection with BRAV and BRBV	Positive	1 (1%)	9 (9%)	1.22	0.14-10.77	0.8565
	Negative	9 (9%)	99 (99%)			
Risk factors	Multiplex real-time RT-PCR result	2-6 months age (%)	Over 6 months age (%)	OR	95% CI	P value
Bovine rhinitis A virus (BRAV)	Positive	14 (14%)	30 (30%)	0.54	0.26-1.09	0.0858
	Negative	68 (68%)	78 (78%)			
Bovine rhinitis B virus (BRBV)	Positive	8 (8%)	16 (16%)	0.62	0.25-1.53	0.3017
	Negative	74 (74%)	92 (92%)			
Co-infection with BRAV and BRBV	Positive	4 (4%)	9 (9%)	0.56	0.17-1.90	0.3556
	Negative	78 (78%)	99 (99%)			

Table 3: Mean and standard deviation of hematological parameters of animals in the BRD-affected and asymptomatic groups and statistical significance results between the study groups. (Mann-Whitney U test and independent T test were used in statistical analysis)

	Study Groups		
Hematological Parameters	BRD-affected (n=98)	Asymptomatic (n=99)	P value
WBC (10^9/L)	15.52±17.59	10.48±3.36	0.971
NEU (10^9/L)	6.11±6.19	3.03±2.12	0.000*
LYM (10^9/L)	8.33±13.91	6.88±2.36	0.000*
MON (10^9/L)	0.89±1.06	0.32±0.23	0.000*
EOS (10^9/L)	0.16±0.15	0.24±0.17	0.000*
BAS (10^9/L)	0.03±0.07	0.01±0.01	0.903
NEU%	39.78±17.77	27.69±13.37	0.000*
LYM%	53.19±18.51	66.92±14.08	0.000*
MON%	5.74±3.48	2.98±2.04	0.000*
EOS%	1.33±1.19	2.30±1.52	0.000*
BAS%	0.25±0.46	0.11±0.12	0.359
RBC (10^{12}/L)	8.23±1.63	7.72±1.34	0.012*
HGB (g/dL)	9.67±1.60	10.44±1.64	0.001*
HCT (%)	28.22±4.83	29.84±5.06	0.039*
MCV (fL)	34.81±5.01	39.20±6.48	0.000*
MCH (pg)	11.94±1.77	13.71±2.06	0.000*
MCHC (g/dL)	34.48±3.58	35.02±1.14	0.244
RDW-CV (%)	24.70±4.46	23.06±2.31	0.010*
RDW-SD (fL)	29.89±5.17	31.57±5.45	0.008*
PLT (10^9/L)	539.59±290.02	380.90±220.56	0.000*
MPV (fL)	5.64±0.90	5.67±0.61	0.479
PDW (10GSD)	12.11±3.57	14.88±0.76	0.000*
PCT (mL/L)	3.00±1.67	2.14±1.13	0.000*

* Parameters with statistical significance (P<0.05) are shown with asterisk.

Table 4: Mean and standard deviation of hematological parameters of bovine rhinitis A virus (BRAV) positive animals in all blood samples (*n*=197), and statistical significance results between the positive-negative groups. (Mann-Whitney U test and independent T test were used in statistical analysis)

	Multiplex real-time RT-PCR results for BRAV§		
Hematological Parameters	Positive (*n*=49)	Negative (*n*=148)	P value
WBC (10^9/L)	15.24±17.40	12.24±10.92	0.855
NEU (10^9/L)	6.54±6.88	3.90±3.78	0.011*
LYM (10^9/L)	7.67±13.37	7.58±8.59	0.000*
MON (10^9/L)	0.84±1.08	0.53±0.69	0.020*
EOS (10^9/L)	0.16±0.14	0.21±0.18	0.037*
BAS (10^9/L)	0.03±0.07	0.02±0.05	0.066
NEU%	41.82±20.22	31.01±14.63	0.001*
LYM%	51.20±20.49	63.03±15.78	0.000*
MON%	5.33±3.57	4.03±2.95	0.012*
EOS%	1.42±1.15	1.95±1.51	0.016*
BAS%	0.23±0.53	0.16±0.25	0.160
RBC (10^{12}/L)	7.86±1.61	8.01±1.48	0.278
HGB (g/dL)	9.61±1.69	10.20±1.63	0.018*
HCT (%)	27.65±4.75	29.49±5.01	0.034*
MCV (fL)	35.69±5.03	37.45±6.48	0.212
MCH (pg)	12.40±1.85	12.97±2.18	0.127
MCHC (g/dL)	34.85±3.19	34.72±2.48	0.154
RDW-CV (%)	24.88±5.03	23.54±2.98	0.116
RDW-SD (fL)	30.81±5.76	30.71±5.25	0.882
PLT (10^9/L)	526.63±311.51	437.73±250.41	0.063
MPV (fL)	5.46±0.87	5.72±0.72	0.045*
PDW (10GSD)	12.97±3.26	13.68±2.79	0.228
PCT (mL/L)	2.80±1.61	2.49±1.44	0.163

§ Positive and negative groups consist of both BRD-affected and asymptomatic animals. * Parameters with statistical significance (P<0.05) are shown with asterisk.

Table 5: Mean and standard deviation of hematological parameters of bovine rhinitis B virus (BRBV) positive animals in all blood samples (*n*=197), and statistical significance results between the positive-negative groups. (Mann-Whitney U test and independent T test were used in statistical analysis)

	Multiplex real-time RT-PCR results for BRBV§		
Hematological Parameters	Positive (n=25)	Negative (n=172)	P value
WBC (10^9/L)	10.80±4.66	13.30±13.62	0.577
NEU (10^9/L)	4.95±3.33	4.50±5.04	0.021*
LYM (10^9/L)	5.15±1.70	7.96±10.59	0.019*
MON (10^9/L)	0.51±0.40	0.62±0.86	0.541
EOS (10^9/L)	0.16±0.13	0.20±0.17	0.443
BAS (10^9/L)	0.02±0.05	0.02±0.05	0.903
NEU%	43.22±11.24	32.32±17.06	0.000*
LYM%	50.18±11.50	61.53±18.08	0.001*
MON%	4.70±2.87	4.30±3.20	0.386
EOS%	1.64±1.26	1.85±1.47	0.664
BAS%	0.24±0.47	0.17±0.32	0.636
RBC (10^{12}/L)	8.47±1.92	7.90±1.43	0.350
HGB (g/dL)	9.89±1.36	10.08±1.70	0.613
HCT (%)	29.67±4.76	28.94±5.04	0.569
MCV (fL)	35.78±5.28	37.19±6.30	0.351
MCH (pg)	12.01±2.11	12.95±2.09	0.085
MCHC (g/dL)	33.71±4.02	34.91±2.38	0.135
RDW-CV (%)	24.46±4.43	23.79±3.51	0.439
RDW-SD (fL)	30.46±5.43	30.77±5.37	0.787
PLT (10^9/L)	484.00±253.23	456.33±271.56	0.604
MPV (fL)	5.51±0.75	5.68±0.77	0.378
PDW (10GSD)	12.52±3.60	13.65±2.79	0.191
PCT (mL/L)	2.60±1.35	2.56±1.51	0.774

§ Positive and negative groups consist of both BRD-affected and asymptomatic animals. * Parameters with statistical significance (P<0.05) are shown with asterisk.

Table 6: Mean and standard deviation of hematological parameters of animals that are co-infected with bovine rhinitis A virus (BRAV) and bovine rhinitis B virus (BRBV) and others, and statistical significance results between the positive-negative groups. (Mann-Whitney U test was used in statistical analysis)

	Multiplex real-time RT-PCR results for BRAV+BRBV§		
Hematological Parameters	Co-infected (n=14)	Others (n=183)	P value
WBC (10^9/L)	11.01±5.28	13.14±13.25	0.688
NEU (10^9/L)	5.50±3.93	4.49±4.92	0.051
LYM (10^9/L)	4.80±1.76	7.81±10.29	0.013*
MON (10^9/L)	0.50±0.25	0.61±0.84	0.344
EOS (10^9/L)	0.19±0.16	0.20±0.17	0.688
BAS (10^9/L)	0.03±0.06	0.02±0.05	0.698
NEU%	46.56±12.85	32.72±16.69	0.001*
LYM%	46.64±12.65	61.12±17.71	0.001*
MON%	4.72±2.33	4.33±3.22	0.337
EOS%	1.74±1.52	1.82±1.44	0.825
BAS%	0.29±0.61	0.17±0.31	0.530
RBC (10^{12}/L)	8.31±1.90	7.95±1.48	0.842
HGB (g/dL)	9.86±1.58	10.07±1.67	0.481
HCT (%)	28.84±3.82	29.05±5.08	0.842
MCV (fL)	35.64±5.62	37.12±6.23	0.457
MCH (pg)	12.11±1.69	12.88±2.13	0.186
MCHC (g/dL)	34.11±2.16	34.80±2.70	0.148
RDW-CV (%)	25.86±5.50	23.72±3.42	0.064
RDW-SD (fL)	32.07±6.28	30.63±5.29	0.350
PLT (10^9/L)	537.43±284.50	453.91±267.50	0.221
MPV (fL)	5.33±0.74	5.68±0.76	0.150
PDW (10GSD)	11.67±4.20	13.64±2.77	0.098
PCT (mL/L)	2.80±1.52	2.55±1.48	0.432

§ Co-infected animals only consist of double positive results from both BRD-affected and asymptomatic animals. * Parameters with statistical significance (P<0.05) are shown with asterisk.

DISCUSSION

Bovine respiratory disease (BRD) is one of the significant concerns in the cattle industry globally. Bovine rhinitis A virus (BRAV) and bovine rhinitis B virus (BRBV) have been screened by several studies conducted in different countries but not in Türkiye. In this study, we investigated the presence of BRAV and BRBV in cattle with and without clinical respiratory disease in Türkiye. Our findings initially indicated that both viruses are circulating in cattle populations in Kırıkkale and Ankara provinces, in Türkiye, and the detection rates of BRAV and BRBV were significantly higher in BRD-affected animals compared to asymptomatic animals. This suggests that these viruses may play a role in the development of respiratory disease in cattle in Türkiye.

Table 7: Mean and standard deviation of hematological parameters of bovine rhinitis A virus (BRAV) positive animals in the BRD-affected and asymptomatic groups and statistical significance results between the study groups. (Mann-Whitney U test and independent T test were used in statistical analysis)

	BRAV Positive Animals		
Hematological Parameters	BRD-affected (n=40)	Asymptomatic (n=9)	P value
WBC (10^9/L)	16.34±19.06	10.33±3.43	0.849
NEU (10^9/L)	7.11±7.44	3.99±2.12	0.567
LYM (10^9/L)	8.14±14.77	5.58±1.70	0.245
MON (10^9/L)	0.91±1.17	0.50±0.43	0.468
EOS (10^9/L)	0.14±0.13	0.24±0.14	0.027*
BAS (10^9/L)	0.03±0.07	0.01±0.01	0.829
NEU%	43.12±21.23	36.03±14.50	0.348
LYM%	49.97±21.49	56.64±15.06	0.383
MON%	5.42±3.31	4.92±4.79	0.326
EOS%	1.22±1.06	2.33±1.18	0.002*
BAS%	0.27±0.58	0.07±0.05	0.889
RBC (10^{12}/L)	8.03±1.73	7.12±0.49	0.107
HGB (g/dL)	9.62±1.81	9.56±1.03	0.922
HCT (%)	27.55±5.07	28.09±3.11	0.762
MCV (fL)	34.85±5.00	39.47±3.19	0.011*
MCH (pg)	12.17±1.90	13.43±1.19	0.063
MCHC (g/dL)	35.03±3.48	34.04±1.14	0.339
RDW-CV (%)	25.20±5.48	23.48±1.62	0.694
RDW-SD (fL)	30.48±6.04	32.24±4.26	0.133
PLT (10^9/L)	531.20±330.17	506.33±224.11	0.831
MPV (fL)	5.51±0.94	5.24±0.32	0.154
PDW (10GSD)	12.54±3.47	14.89±0.55	0.019*
PCT (mL/L)	2.84±1.72	2.63±1.10	0.730

* Parameters with statistical significance (P<0.05) are shown with asterisk.

Table 8: Mean and standard deviation of hematological parameters of bovine rhinitis B virus (BRBV) positive animals in the BRD-affected and asymptomatic groups and statistical significance results between the study groups. (Mann-Whitney U test and independent T test were used in statistical analysis)

	BRBV Positive Animals		
Hematological Parameters	BRD-affected (n=19)	Asymptomatic (n=6)	P value
WBC (10^9/L)	10.77±5.18	10.89±2.79	0.437
NEU (10^9/L)	5.21±3.75	4.11±1.21	0.926
LYM (10^9/L)	4.81±1.59	6.23±1.68	0.59
MON (10^9/L)	0.57±0.44	0.34±0.16	0.274
EOS (10^9/L)	0.15±0.13	0.20±0.13	0.138
BAS (10^9/L)	0.03±0.05	0.01±0.01	0.437
NEU%	44.91±11.98	37.85±6.64	0.185
LYM%	47.94±11.78	57.27±7.48	0.083
MON%	5.23±3.09	3.03±0.89	0.011*
EOS%	1.59±1.36	1.80±0.91	0.333
BAS%	0.29±0.53	0.05±0.08	0.246
RBC (10^{12}/L)	8.77±2.06	7.54±0.98	0.175
HGB (g/dL)	9.72±1.43	10.42±0.99	0.283
HCT (%)	29.52±5.23	30.17±3.13	0.437
MCV (fL)	34.34±4.64	40.35±4.82	0.012*
MCH (pg)	11.39±1.89	13.98±1.53	0.003*
MCHC (g/dL)	33.42±4.58	34.63±0.80	0.733
RDW-CV (%)	24.95±4.96	22.90±1.33	0.333
RDW-SD (fL)	29.96±5.88	32.05±3.57	0.138
PLT (10^9/L)	513.00±270.18	392.17±178.03	0.318
MPV (fL)	5.51±0.84	5.52±0.44	0.987
PDW (10GSD)	11.83±3.88	14.73±0.51	0.121
PCT (mL/L)	2.75±1.45	2.13±0.87	0.333

* Parameters with statistical significance (P<0.05) are shown with asterisk.

In this study, BRAV positivity was detected in 40% of cattle with BRD symptoms, while it was only 9% in asymptomatic animals (Table 1). This significant result shows that BRAV may be related to respiratory disease in cattle. Previous studies have reported varying BRAV positivity rates worldwide. A study conducted in China indicated BRAV positivity in nasal swabs from symptomatic cattle at 22.09% (Zhou *et al*., 2023a). In the United States, a metagenomic study found a BRAV positivity rate of 24% in calves with BRD (Ng *et al*., 2015). Similarly, a Canadian study using metagenomics showed a BRAV positivity of 12% in symptomatic calves compared to just 3% in healthy ones (Zhang *et al*., 2019). Metagenomic analysis involving both symptomatic and asymptomatic cattle from the United States and Mexico reported an overall BRAV positivity rate of 52.7%. Specifically, in Mexico, symptomatic cattle showed an 18.5% positivity, while asymptomatic animals had a higher rate of 26.9%. Notably, in the United States, positivity was considerably higher: 90% among symptomatic cattle and 95% among asymptomatic cattle (Mitra *et al*., 2016). In this study we found a higher rate of positivity in BRD-affected group than asymptomatic animals (40% and 9%, respectively) (Table 1), in contrary to the results of Mitra *et al*. (2016). These results show that BRAV is common in cattle population globally, but detection rates can be very different in different studies, countries and disease situations.

Our results revealed that BRBV positivity was 19% in BRD-affected cattle compared to 6% in the asymptomatic group (Table 1). In the United States, nasal swabs from cattle with BRD revealed a BRBV positivity rate of 40.8% (Bhattarai *et al*., 2022). Another study in China reported a lower BRBV positivity rate of 9.2% in cattle showing symptoms of BRD (Zhou *et al*., 2023a). Additionally, a US-based metagenomic study detected BRBV positivity at rates of 8-10% in calves with BRD (Ng *et al*., 2015). A study from Canada showed 28% BRBV positivity in symptomatic calves and 10% in healthy calves (Zhang *et al*., 2019). In Mexico, BRBV positivity was 25.9% in symptomatic and 11.5% in asymptomatic cattle. In the US, rates were 20% in symptomatic and 40% in asymptomatic cattle. Combined, overall positivity was 23.7% in both groups (Mitra *et al*., 2016). Considering these findings of previous studies conducted other countries, BRBV positivity rate appears to be lower in cattle population in Türkiye.

Risk factor assessment in several viral diseases is important to understand, analyze and predict the potential predisposing risks. The risk factors for BRD development include a variety of component such as bacterial and viral infections, host immunity, environmental and weather conditions, stress, transportation. Although risk assessments have been performed in studies on different BRD-associated viruses such as bovine viral diarrhea virus, bovine parainfluenza virus-3, bovine respiratory syncytial virus, bovine herpesvirus-1 (İnce *et al*., 2021; Zhou *et al*., 2023b), no such comprehensive risk assessment has been found in any study on BRAV and BRBV. We analyzed some risk factors in BRD-affected animals in this study. Our results indicated that younger age and fever were significantly associated with BRAV infection, suggesting that suckling period may represent a critical risk window for BRAV-associated respiratory disease. BRAV positivity was nearly five times higher in 1–2-month-old calves compared to 2–6-month-old animals (OR: 4.86, 95% CI: 1.24-19.05, p=0.0234). Additionally, BRAV-positive animals had approximately five times higher odds of exhibiting fever compared to BRAV-negative animals (OR: 4.7, 95% CI: 1.18–19.18, p=0.0287) (Table 2). These findings suggest that BRAV may act as a significant respiratory pathogen in young calves. In contrast, no statistically significant associations were identified between BRBV or co-infection positivity and any clinical signs or age groups. Overall, BRAV appears to contribute to clinical disease expression, particularly in calves aged 1–2 months, whereas the role of BRBV remains uncertain. In a previous experimental study, Bhattarai *et al*. (2022) inoculated three 7-day-old calves intranasally with 10^7 $TCID_{50}$ of BRBV strain 6900 and monitored them daily. Only one calf developed transient fever (>39.4°C) on day 2, which resolved by day 3, and no other clinical signs were observed. Our field study demonstrated that BRAV infection was significantly associated with fever, while BRBV infection showed no significant relationship with fever or other clinical signs, showing compatible findings with the study of Bhattarai *et al*. (2022). These clinical differences between BRAV and BRBV infection may reflect the influence of viral strain, infectious dose, host immunity, and sampling time on the clinical expression of fever in naturally infected animals compared to experimentally infected calves. Further studies with larger sample sizes are needed to confirm these associations and better define the epidemiological and clinical significance of BRAV and BRBV in bovine respiratory disease.

While hematological changes may indicate infection, they are not standalone for clinical diagnosis. In our previous study, we have reported that infections with BVDV, BHV-1, and BHV-4 significantly altered hematological parameters as MCV, HCT, MCHC, and MPV (Aslan *et al*., 2016). Consistent with these findings the present study revealed that BRD-affected animals showed significant hematological differences compared to asymptomatic animals, particularly in NEU, LYM, MON, EOS, their percentages, and several red blood cell indices ($P<0.05$) (Table 3). In the BRD group, marked neutrophilia indicates an inflammatory response associated with acute bacterial infection or tissue damage. Neutrophils constitute the first line of defense in pulmonary infections (Ackermann *et al*., 2010; Bielamowicz *et al*., 2024; Omar *et al*., 2024). Although lymphocyte counts may be within physiological ranges in both groups, absolute lymphocyte elevation is numerically higher in the BRD group due to increased total WBC. This elevation may be associated with viral infections and viral-bacterial co-infections are prevalent in BRD (Ackermann *et al*., 2010) BRD pathogenesis consistently demonstrates leukocytosis with neutrophilia and lymphopenia, hallmarks of acute infection. Elevation of the neutrophil / lymphocyte ratio (NLR) beyond 0.6 provides a clinically validated metric for objectively quantifying disease severity, as it encapsulates both the inflammatory surge and compensatory immunosuppression (Carlos-Valdez *et al*., 2016; Burciaga-Robles *et al*., 2010). In their study on calves,

Cuevas-Gómez *et al*. (2020) reported that NLR on the day of bovine respiratory disease (BRD) diagnosis was 73% higher than in healthy calves. Consistent with the literature, the present study also demonstrates a 69.51% greater NLR in BRD-affected calves compared to asymptomatic controls. BRAV positivity was associated with significant changes in NEU, LYM, MON, EOS, HGB, HCT, and MPV ($P<0.05$) (Table 4), while BRBV positivity was linked to NEU and LYM counts and percentages ($P<0.05$) (Table 5). Additionally, BRAV-positive BRD animals exhibited significant alterations in EOS, EOS%, MCV, and PDW compared to asymptomatic animals ($P<0.05$) (Table 7). Overall, these findings suggest that BRAV infection may lead to more extensive hematological alterations than BRBV, possibly reflecting a stronger host immune response.

Compared to devices such as the Rayto RT-7600Vet (China), which have shown variability in precision for veterinary species, the BC-5000 Vet (Mindray, China) used in this study offers multi-species capability; however, hematological analyzer optimization in animals remains more challenging than in humans due to species-specific differences in cell morphology and distribution. Therefore, species-specific calibration and validation remain essential for accurate interpretation of veterinary hematology results (Farooq *et al.* 2025).

In conclusion, this first nationwide survey of bovine rhinitis A and B viruses in Türkiye fills a critical epidemiological gap and advances the global understanding of their role in bovine respiratory disease (BRD). Our findings indicate that BRAV and BRBV infections—and especially their co-infection—significantly contribute to BRD pathogenesis, that calves aged 1–2 months are particularly susceptible to BRAV and that BRAV positivity is strongly associated with fever, underscoring the necessity of incorporating these viral agents both individually and in combination into diagnostic and risk-assessment protocols for bovine respiratory disease.

Authors contribution: EA and AKA conceived and designed the study. YŞ examined the animals, collected nasal swab and blood samples and carried out analysis of whole blood samples. EA and AKA executed the RNA isolation, cDNA synthesis and multiplex real-time PCR experiments and analyzed the results and the statistical data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

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