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Antigenic detection of bovine parainfluenzavirus- 3 in cattle with respiratory system infection and some risk factors

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Abstract

Bovine Parainfluenzavirus-3 is one of the major viral pathogens that causes the respiratory system disease complex of cattle. In our study, it was aimed to detect the antigenicity of bovine parainfluenzavirus-3 by direct immunofluorescence method in cattle and the effect of some risk factors on the presence of Bovine Parainfluenzavirus-3 in the herd were statistically analyzed. For this purpose, nasal swab samples were collected from sixty cattle from six different ranches. On the other hand, the farms were evaluated in terms of production type, production purpose, quarantine regulations and animal welfare. In the immunofluorescent examination, Bovine Parainfluenzavirus-3 antigen was detected in seven (7/60) preparations. It was determined that Bovine Parainfluenzavirus-3 plays an etiological role in respiratory system infections of cattle. On the other hand, in our statistical evaluation, there were statistically significant results between the presence of BPIV3 infection, quarantine practices and suitable with animal welfare, but no significant results were found between the breeding type and breeding purpose. Statistical evaluations were made to reveal the relationship between general characteristics of the animals and the presence of BPIV3, and no statistically significant result could be reached. It was concluded that especially when evaluated in terms of herd health the implement of biosafety rules in enterprises where animal circulation is frequent and bringing the enterprises in accordance with animal welfare that will provide minimum zootechnical conditions are important in protection and control. Our study is the first in Turkiye in which the risk factors of Bovine Parainfluenzavirus-3 were evaluated statistically.

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Introduction

Bovine respiratory system diseases complex are multifactorial infections that negatively affect the welfare of animals in large-capacity herds and cause direct or indirect economic losses to breeders. Bovine Parainfluenzavirus-3 (BPIV3), which is one of the factors involved in the etiology of this disease complex, is classified in the *Respirovirus* genus within the *Paramyxoviridae* family, *Orthoparamyxovirinae* subfamily (1-3). BPIV3 is an upper respiratory tract pathogen that causes infection mostly in autumn and winter sessions in Turkiye, and its prevalence is high, mostly in 2-8 months old animals (4,5). Although the primary transmission route of the virus is in the form of nasal secretions, aerosols and droplets, it is known that it is also spread by contaminated fomites. Although it usually causes a subclinical infection in cattle, dyspnea, cough, high fever, mucoprulent nasal discharge and conjunctival discharge can be observed in case of co-infection with other pathogens, while in cases of coinfection, it causes fatal pneumonias, especially in calves (5,6). Many antigenic (DIF, ELISA, IHC), serological (HI, VN) and molecular (RT-PCR, RT-qPCR) laboratory methods are used in the diagnosis of infection (7-10). However, one of the most important steps in the protection and control of herd health against BPIV3 infection is the studies and protective practices aimed at

eliminating the stress factors that predispose animals to infection (3,4).

This study aimed to the antigenic detection of BPIV3 using direct immunofluorescence (DIF) method in nasal swab samples obtained from different breeds, ages and genders with respiratory system infection symptoms cattle on the other hand it was aimed to reveal statistically the relationship between the presence of BPIV3 in herds and animal feeding and production purposes, quarantine practices and animal welfare factors.

Materials and methods

Ethical Statement

This study was approved by Burdur Mehmet Akif Ersoy University Local Ethics Committee (Approval No: MAKU-HADYEK E-93773921-020-260889 1044/29.03.2023).

Animals Used in the Study and Sampling

In this study, cattle bred in Burdur province with clinical signs of respiratory system infection (40°C > fever, dyspnea, mucopurulent nasal discharge, conjunctival discharge, depression) and unvaccinated against BPIV3 were used. Nasal swab samples were obtained from 60 animals of different ages, genders and breeds. Sampling was carried out from six different ranches to form 10% of the herd population. Although the maintenance and feeding conditions of the farms where the samples obtained were far from modern herd management techniques, they were in a position to provide minimum animal welfare conditions. However, none of them had a regular vaccination schedule for respiratory system infections. It is known that the possibility of detecting BPIV3 is higher in these periods, since the local immune response cannot reach a sufficient level in the early stages of infection (11). For this reason, the samplings were carried out in the first 5 days when the clinical signs were seen in line with the information given by the animal owners. On the other hand, the ranches where the samples were obtained were evaluated under the headings of animal welfare suitability determined by the examination of the animals' breeding types, production purpose, circulation/quarantine practices in the herd and the number of animals coexisting in the shelter, ventilation type, litter type, drinker type, etc. zootechnical conditions. The number of obtained samples, the general characteristics of the ranches where the samples were obtained and the animals sampled were given in Table 1 and Table 2, respectively.

Preparation of samples and direct ımmunofluorescence test (DIF)

Preparation of nasal swab samples and DIF testing were performed with a few minor modifications to the methods reported by Kucuk and Yildirim (11) and Alkan *et al.* (12). Samples obtained with sterile swab sticks were placed in tubes containing 1.5 mL antibiotic DMEM (Dulbecco's Modified Eagles Medium, 2500 IU/mL penicillin, 20 mg/mL streptomycin) and brought to the diagnostic laboratory in accordance with the cold chain procedure. Swabs were discarded after vortexing. The remaining cell and DMEM mixture was transferred to 2 mL microcentrifuge tubes and centrifuged at +4°C at 1200 rpm for 10 min. After centrifugation, the supernatant was discarded and the pellet obtained at the bottom of the tube was collected. The collected pellet was diluted by adding 50 µl of DMEM and spread on a slide using a coverslip. After the slides were dried at room temperature, they were treated with acetone cooled at -20°C for 15 min to ensure fixation of the cells on the slide. Slides were washed twice with PBS to remove residues. Then, the slides were covered with 50 µl of anti-BPIV3 polyclonal antiserum conjugate (VMRD, USA) solution and incubated in ıncubator (Nuve, Turkiye) for 30 min at 37°C in a humid and dark environment. After incubation, the slide was washed 3 times with PBS in a dark room to remove conjugate residues. The preparations, which were dried at room temperature after washing, were examined under a fluorescent microscope (Olympus Co., Japan).

Statistical evaluation

Chi-Square test was used to determine the statistical significance of the relationship between the quarantina practices, welfare conditions and breeding type and purpose of the sampled herds and the presence of BPIV3 infection in the animals.

Results

Antigenic results

BPIV3 antigen was detected in nasal swap samples collected from ranches I, II, III and VI. On the other hand, positivity could not be detected in ranch IV and ranch V. Considering the total number of samples, positivity was determined in a total of seven (7/60) nasal swab samples (Tables 1 and 2). DIF test results are given in table 3 and BPIV3 specific immunofluorescence images are given in figure 1.

Statistical Results

In order to reveal the relationship between the breeding style of the sampled herds, their suitability for animal welfare, the quarantine process for the newly joined animals to herds and the production purposes and the presence of BPIV3 in the herd statistical evaluations were carried out. The relationship between quarantine practices and compliance with animal welfare and the presence of BPIV3 was found to be statistically significant ($P<0.05$). On the other hand, a statistically significant result could not be obtained between the breeding type of the animals and the presence of the agent for production purposes. Additionaly, statistical evaluations were made to reveal the relationship

between general characteristics such as age, breed and gender of the sampled animals and the presence of BPIV3, and no statistically significant result could be reached (Tables 4-10).

Table 1: The total number of cattle and sampling amount in the farms where the samples were collected, the type of feeding of the farms, the purpose of feeding, their compliance with quarantine processes and animal welfare

Ranches	Total (n)	samples (n)	Type	Purpose	Quarantine	Suitable
I	116	11	Intensive	Beef	No quarantine	Suitable
II	75	7	Extansive	Beef	No quarantine	Nonsuitable
III	102	10	Extansive	Dairy	No quarantine	Nonsuitable
IV	95	9	Intensive	Beef	Quarantine	Suitable
V	136	13	Extansive	Dairy	Quarantine	Suitable
VI	109	10	Intensive	Beef	Quarantine	Nonsuitable

Table 2: Age, Gender and Breed information about sampled animals

	Criteria	n (%)
Age	< 1 age	34 (%56.7)
	> 1 age	26 (%43.3)
Gender	Female	24 (%40)
	Male	36 (%60)
Breed	Montofon (Brown Swiss)	17 (%28.4)
	Simmental	22 (%36.6)
	Holstein	21 (%35)

Table 3: Sampled ranches and DIF test results

Ranches	n	DIF Test	
		Positive (%)	Negative (%)
I	11	1 (9.1)	10 (90.0)
II	7	3 (42.9)	4 (57.0)
III	10	2 (20)	8 (80)
IV	9	-	9 (100)
V	13	-	13 (100)
VI	10	1 (10)	9 (90)
Total	60	7 (11.7)	53 (88.3)

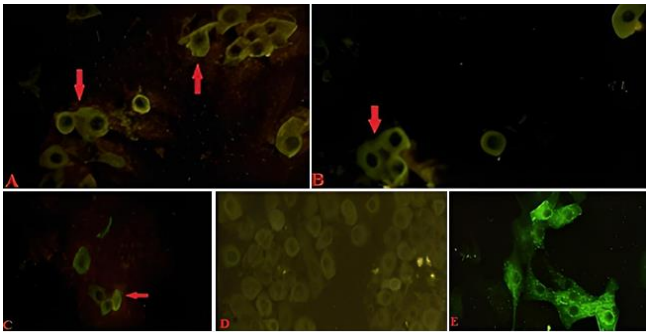

Figure 1: A- BPIV3-specific fluorescence image in nasal swab sample of animal #5 sampled in the I. herd (Red cursors) B- BPIV3-specific fluorescence image in nasal swab sample of animal #9 sampled in the II. Herd (Red cursor) C- BPIV3-specific fluorescence image in nasal swab sample of animal #33 sampled in the VI. herd (Red cursor) D- Negative control E- Positive control (Cat No: CJ-F-PI3, VMDR, ABD).

Table 4: Statistical evaluation between BPIV3 presence and quarantine practise

DIF Results	Quarantine Practise	Not Quarantine Practise	Evalution
BPIV3 positive	1	6	X^2 4. 8546
BPIV3 negative	31	22	P Value 0.027, P< 0.05*

Table 5: Statistical evaluation between BPIV3 presence and Suitable for animal welfare

DIF Results	Suitable for Animal Welfare	Not suitable for Animal Welfare	Evalution
BPIV3 positive	1	6	X^2 5.307
BPIV3 negative	32	21	P Value 0.021, P< 0.05*

Table 6: Statistical evaluation between BPIV3 presence and breeding type

DIF Results	Intensive Breeding	Extansive Breeding	Evalution
BPIV3 positive	2	5	X^2 1.4555
BPIV3 negative	28	25	P Value 0.2276 P>0.05

Table 7: Statistical evaluation between BPIV3 presence and breeding purpose

DIF Results	Dairy	Beef	Evalution
BPIV3 positive	2	5	X^2 0.3195
BPIV3 negative	21	32	P Value 0.5719 P>0.05

*P<0.05 values are statistically significant.

Table 8: Statistical evaluation between BPIV3 presence and animals ages

DIF Results	< 1 age	> 1 age	Evalution
BPIV3 positive	5	2	X^2 0.7032
BPIV3 negative	29	24	P Value 0.4016 P>0.05

*P<0.05 values are statistically significant.

Table 9: Statistical evaluation between BPIV3 presence and genders

DIF Results	Female	Male	Evalution
BPIV3 positive	3	4	X^2 0.027
BPIV3 negative	21	32	P Value 0.8695 P>0.05

*P<0.05 values are statistically significant.

Table 10: Statistical evaluation between BPIV3 presence and breeds

	Holstein	Simmental	Swiss Brown	
BPIV3 positive	4	2	1	X^2 0.7032
BPIV3 negative	17	20	16	P Value 0.4016, P>0.05

*P<0.05 values are statistically significant.

Discussion

Bovine respiratory system disease complex (BRDC) is one of the multifactorial infections reported worldwide. There are contains many viral and bacterial factor in its etiology such as BHV-1, BPIV3, Bovine Coronavirus (BCoV), BRSV, Bovine Adenovirus (BAV), Bovine Viral Diarrhea Virus (BVDV), Pasteurella multicidae, Mannheimia haemolytica, Histophilus somni (7,13). In particular, BPIV3 is one of the most important viral pathogens that predisposes cattle to respiratory system infections.

Studies on risk assessments of viral agents causing respiratory system infections in Turkiye are lacking. Ince and Sevik (14) evaluated the seroprevalence and risk factors of BoHV-1 in their serological study in cattle in western Turkiye. Our study is the first in Turkiye in which the risk factors of BPIV3 were evaluated statistically.

On the antigenic detection of BPIV3 in cattle with the DIF technique, studies have been carried out using different sample in many regions of the world. In these studies, the prevalence of BPIV3 infection was determined as 3,15-37,3% (8-18). In this study, we found the prevalence of BPIV3 among cattle to be 11.7%. Our study results Alkan *et al.* (12), Ceribasi *et al.* (8), Saeed *et al.* (17) show similarities with the results of their research.

On the other hand, in this study, a higher level of antigen positivity was detected compared to our previous research on the molecular and antigenic detection of BPIV3 Kucuk and Yildirim (11) and another research Kamdi *et al.* (18) study. The reason for this was thought to be the limitation of the sampling region and the number of herds in this study and the use of animals with clinical symptoms of the respiratory system for a few days instead of sampling from all animals. In addition, it was thought that not obtain samples from animals with chronic respiratory system infection, developed local and systemic immunization against the agent, or in the convalescent period increased the chance of BPIV3 detection. In addition, it has been concluded that the prevalence results may vary due to reasons such as the semi-professional or public breeding enterprises do not pay attention to the biosecurity rules and the animal mobility of the region is high.

Another aimed of our research is to make statistical evaluations between the suitability of the ranches for animal welfare, the presence/absence of quarantine, the type and purpose of breeding and the presence of BPIV3 infection in animals. There are studies on the effects of risk factors such as zootechnical factors, stress situations, appropriate welfare levels on respiratory system infections in large-scale cattle herds on the basis of pathogen or epidemic.

Hay *et al.* (19) carried out a statistical-based study in which they revealed the effects of some risk factors on respiratory system infections in cattle with respiratory system infections in Australia. Accordingly, among the most important risk factors, they reported situations such as mixing with newly joined animals without a waiting period, sharing drinkers, and having a history of BVDV infection in the past. In the study conducted by Van der Fels-Klerx *et al.* (20) in the Netherlands, on calves with respiratory system infection, newly recruited cattle or directly added to the herd and conditions affecting animal welfare such as air circulation, litter types, group size were among the most important risk factors. Again in Mexico, Solis-Calderon *et al.* (21) found a higher seropositivity rate for BPIV3 in newly added animals to the herd compared to those born in the barn, in their study on serological diagnosis of BPIV3 and BRSV in beef cattle, and determination of risk factors on the basis of herd and animal. Additionaly, it was emphasized that the effect of factors such as the number of animals kept together and herd size on BPIV3 seropositivity was statistically significant. Carbonero *et al.* (22) studied the seroprevalence of BVDV, BPIV, BRSV and BHV-1 and statistical evaluation of some risk factors in calves in Spain. They revealed that contact with animals from different farms and the lack of adaptation process to new animals in the herd are a risk factor for BPIV3. In the study conducted by Pardon *et al.* (9) in cattle with acute respiratory system infection

outbreak and statistical risk assessments specific to pathogens were carried out, it was revealed that there is a statistically significant relationship between the presence of BPIV3 in the herd and ill animals recently purchase and herd size. Hashemi *et al.* (23) studied the seroprevalence of BPIV3 and BHV-1 and statistical evaluation of some risk factors in calves in Iran. They revealed that there is a statistically significant difference between farm conditions and the presence of BPIV3. Cusack (3) In their serological study on cattle in Australia, they revealed the statistical relationship between the causative agents of BRDC and some risk factors. Accordingly, it has been shown that the risk of BRD increases with the serological increase of respiratory viruses, BHV1, PI3, BRSV and BVDV alone or in combination between the entrance to the barn and the 42nd day. Additionaly it has been shown feeding and sheltering conditions is the important risk factor.

In our study, it was revealed that there is a statistically significant difference between the quarantine practices and the compliance of the ranches with animal welfare and the detection of BPIV3 from the samples in the herd when new animals are added to the herd, on the other hand, there is no significant difference between the animals' feeding patterns and production purposes and factor detection. The results of this study, in which we conducted risk assessments of BPIV3 among cattle with respiratory system infection, are similar to other research data carried out on the subject (3,9,19-23).

On the other hand, statistical evaluations were made between the age, breed and gender of the sampled animals and the presence of BPIV3. No statistically significant value could be determined between these values. Betancur *et al.* (24) stated in their study in Colombia that there was no statistically significant difference between the gender of cattle and the seropositivity of BPIV3 in cattle. Additionaly Leon *et al.* (25) could not detect a statistically significant difference between BPIV3 seropositivity and age of cattle in their study in Colombia. Our results are also similar to this researchs results.

In the light of studies, it is seen that BPIV3 plays an important role in the etiology of respiratory system infection in cattle. For this reason, producers should consider risk factors in order to prevent BPIV3-based economic losses. In this research we have carried out, we have revealed that enterprises that cannot provide optimal zootechnical conditions and do not quarantine procedures are at risk for BPIV3 infection. For this reason in order to protect animals from BPIV3 infection, it will be beneficial to carry out animal circulations in the herds in a controlled manner and to practise a quarantine for 7-10 days to the new animals entering the herd. In addition, ensuring minimum zootechnical and biosecurity conditions in animal shelters and making them suitable for animal welfare will make a significant contribution to protection from infection.

Conclusion

As a result, in this study, a substantial amount of BPIV3 was detected in unvaccinated cattle with respiratory system infection symptoms. Especially in herd-based infections, it was concluded that nasal swab samples obtained from animals in the early stages of the disease and transported to the diagnosis laboratory using the correct transport fluid in accordance with the cold chain protocol are more suitable for the diagnosis of the causative agent. It was thought that the DIF test in the diagnosis of BPIV3 could provide economically more convenient and much faster results than the diagnostic techniques.

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Conflict of Interests

The authors declare that there are no conflicts of interest regarding the publication and/or funding of this manuscript

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الكشف المستضدي لفيروس نظير الأنفلونزا البقري - ٣ في الأبقار المصابة بعدوى الجهاز التنفسي وبعض عوامل الخطر

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الخلاصة

يعد فيروس نظير الأنفلونزا البقري -٣ أحد مسببات الأمراض الفيروسية الرئيسية التي تسبب مرض الجهاز التنفسي المركب في الابقار. هدفت دراستنا إلى الكشف عن مستضدية فيروس نظير الأنفلونزا البقري -٣ باستخدام تقنية التألق المناعي المباشر في الابقار، كما اعتمد التحليل الاحصائي لايجاد تأثير بعض عوامل الخطورة على تواجد فيروس نظير الأنفلونزا البقري -٣ في القطيع. تم جمع عينات من مسحات الأنف من ستين بقرة متواجدة في ستة مزارع مختلفة. من ناحية أخرى، تم تقييم المزارع من ناحية نوع الإنتاج والغرض من الإنتاج وأنظمة الحجر الصحي ورعاية الحيوان. من خلال استخدام الفحص المناعي التألقي، تم الكشف عن مستضد فيروس نظير الأنفلونزا البقري -٣ في سبعة عينات (٦٠/٧). تم تحديد أن فيروس نظير الأنفلونزا البقري -٣ له دور كمسبب لالتهابات الجهاز التنفسي في الابقار. كما بينت نتائج التحليل الإحصائي وجود علاقة معنوية بين عدوى BPIV3 واعتماد الحجر الصحي والرعاية المناسبين، ولكن لم يتم العثور على نتائج علاقة معنوية بين نوع والغرض من التكاثر. تم إجراء تحليل إحصائي للكشف عن العلاقة بين الخصائص العامة للحيوانات ووجود BPIV3، ولم يظهر وجود علاقة بينهما. أستنتج من الدراسة أن تنفيذ قواعد السلامة الأحيائية في المؤسسات ستوفر الحد الأدنى من الظروف المهمة في الحماية والمكافحة ضد الاصابات. تعد دراستنا هذه الأولى في تركيا التي تم فيها تقييم عوامل خطر الإصابة بفيروس نظير الأنفلونزا البقري -٣ إحصائيا.