

The first serological report for genotype C bovine parainfluenza 3 virus in ruminant species of mid-northern Turkey: Traces from the past

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Abstract. Bovine parainfluenza 3 virus (BPI3V) is one of the most important respiratory pathogens and a leading cause of serious respiratory illnesses in cattle, both independent of and in connection with other pathogens involved in the bovine respiratory disease complex (BRDC). In this study, we aimed to identify the historical circulation of genotype C bovine BPI3V (BPI3Vc) in Turkey using the archival serum samples of domestic ruminants that had been collected from six provinces of northern Anatolia in Turkey between 2009-2010. A total of 896 sera from cattle (n=442), sheep (n=330), and goats (n=124) were randomly selected and screened with a virus neutralization test in order to detect antibodies for BPI3Vc. The overall seropositivity rate was 21.09%, with seropositivity rates for cattle, sheep, and goats of 21.04%, 20.00%, and 24.19%, respectively. Neutralizing antibody titers for selected samples ranged between 1/4 to 1/512. This study represents the first serological study conducted using the first BPI3V isolate of Turkey.

INTRODUCTION

The bovine respiratory diseases complex (BRDC) consists of many viruses and bacteria and is one of the major health issues for the cattle industry worldwide due to its negative effects on milk and meat production (Zhu *et al.*, 2011). Bovine parainfluenza 3 virus (BPI3V) is an important member of BRDC, together with other viruses including Bovine herpesvirus-1 (BHV-1), Bovine respiratory syncytial virus (BRSV), Bovine viral diarrhoea virus (BVDV) (Fulton *et al.*, 2016) and some bacterial species including *Mannheimia haemolytica*, *Pasteurella multocida*, *Mycoplasma* spp., and *Histophilus somni* (Griffin *et al.*, 2010). Besides cattle, BRDC also affects sheep

and goats (Veljovic *et al.*, 2016). BPI3V, also known as bovine respirovirus 3 (BRV3), is an enveloped, non-segmented, negative-sense RNA virus belonging to the *Respirovirus* genus under the *Paramyxoviridae* family (Horwood *et al.*, 2008; Neil *et al.*, 2015). BPI3V has three genotypes referred to as A (BPI3Va), B (BPI3Vb) and C (BPI3Vc) (Zhu *et al.*, 2011). Studies have reported that the nucleotide identity for full genome sequences of BPI3Vs ranges between 91% and 99% within genotypes and from 81% to 84% between genotypes (Newcomer *et al.*, 2017). Furthermore, there are large differences in the geographical distribution of BPI3V genotypes across the world (Newcomer *et al.*, 2017). Generally, genotype A is the most

common worldwide (Newcomer *et al.*, 2017); genotype B is found in Australia and the US (Newcomer *et al.*, 2017; Wen *et al.*, 2017), whereas genotype C is found in South America, Asia and Africa including countries like Argentina (Maidana *et al.*, 2012), Korea (Oem *et al.*, 2013), Japan (Konishi *et al.*, 2014), China (Zhu *et al.*, 2011) and Egypt (Sobhy *et al.*, 2017).

In infected cattle, clinical signs of BPI3V infection may vary considerably, ranging from subclinical to acute respiratory disease symptoms, such as nasal discharge, pyrexia and coughing (Maidana *et al.*, 2012; Newcomer *et al.*, 2017). Moreover BPI3V may result in tissue damage and immunosuppression, which may create the conditions for a secondary infection with common BRDC.

To the best of our knowledge, it has not been possible to date to perform any serological study in Turkey using any kind of indigenous isolate of BPI3V due to the fact that there was no BPI3V isolation. All of the previous serological studies on BPI3V in Turkey were conducted using the genotype A SF-4 strain (Alkan *et al.*, 2000; Ozdarendeli and Kandil, 2001; Yazici *et al.*, 2007; Okur-Gumusova *et al.*, 2007; Yesilbag and Gungor, 2008; Yesilbag and Gungor, 2009). The aim of

this serological study was to investigate the historic circulation of indigenous BPI3V in Turkey using archived cattle, sheep and goat serum samples

MATERIALS AND METHODS

Samples

A total of 896 samples consisting of cattle (n=442), sheep (n=330) and goat (n=124) sera, randomly selected from archival samples collected from six provinces in northern Anatolia between 2009-2010 were used after obtaining permission from the Ministry of Food, Agriculture, and Livestock (Approval No. E-3336566 dated 27.12.2017). Cattle samples were obtained from all provinces, but sheep samples from Rize and goat samples from Ordu and Giresun were not available (Figure 1). Samples were collected from animals >1 year old which had not been vaccinated against any of the viral pathogens which contribute to BRDC. Serum samples were heat-inactivated for 30 min at 56°C prior to testing.

Cells and viruses

The cell cultures and viruses required for this study were provided from the Cell and Virus



Figure 1. A map indicates the six different provinces in Turkey, where cattle (▲), sheep (●) and goat (■) serum samples were previously collected.

collection of the Virology Department of the School of Veterinary Medicine operating under the auspices of Ondokuz Mayıs University. All cell culture reagents and cell lines were checked using RT-PCR for possible contamination with non-cytopathogenic (NCP) pestiviruses. Virus cultivation, infectivity assays and virus neutralization tests were performed in Madin Darby Bovine Kidney (MDBK) cells. MDBK cells were grown in Dulbecco's modified Eagle medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco), 1% antibiotics and 1% L-glutamine).

In this study, the first Turkish BPI3V isolate (GenBank accession no: MH357343) reported by Albayrak *et al.* (2018) was grown on MDBK cells in DMEM supplemented with 2% FBS. When obvious cytopathic effect (CPE) was observed, the supernatant was collected, clarified by centrifugation at 3000 rpm for 5 min, aliquoted and stored at -80°C until time for use.

Infectivity assay

The virus was titrated as described elsewhere (Yazici *et al.*, 2015). Briefly, the virus was diluted ten-fold in DMEM supplemented with 2% FBS. 100 μl of each dilution was put into quadruplicates in 96 well plates (TPP, Switzerland). Then 50 μl of a cell suspension containing 3.0×10^4 MDBK cells were added to each well and plates were incubated at 37°C for 72 hours inside a humidified incubator with 5% CO_2 . Following incubation, the 50% tissue culture infective dose (TCID_{50}) was calculated as $\text{TCID}_{50}/\text{ml}$.

Neutralization assays

A standard virus neutralization assay was used to detect neutralizing antibodies against BPI3Vc as previously described (Yazici *et al.*, 2015). Briefly, 1/2 dilutions of all serum samples were made in 96 well plates using 50 μl of each serum diluted in DMEM containing 2% FBS. 100 TCID_{50} of the virus was then added to each well and incubated at 37°C for 1 hour in a humidified incubator with 5% CO_2 . Finally, 50 μl of a cell suspension containing 3.0×10^4 MDBK were added to each well followed by incubation at 37°C

for 72 hours. Protection was evaluated according to the presence of CPE. Serum samples having an antibody titer $\leq 1/2$ were considered seronegative.

In order to determine antibody titers of positive sera, a 50% serum neutralization test (SN_{50}) was performed. Using an otherwise identical procedure to that described above, two-fold dilutions of all positive samples were prepared across a range of 1/4 to 1/1024.

Statistical analysis

Data were analyzed with the GENMOD (Generalized Linear Model) procedure. Differences in the groups were compared by using Chi-square test. The results were considered statistically significant at $P < 0.01$.

RESULTS

This study showed that 21.09 % (189/896) of samples were positive for neutralizing antibody against the first BPI3Vc isolate, with 21.04% (93/442), 20.00% (66/330) and 24.19% (30/124) seropositivity for cattle, sheep and goats, respectively.

As detailed in Table 1, the province of Samsun had the highest overall BPI3Vc seropositivity followed by Tokat with rates of 26.61% and 25.37%, respectively. While Trabzon had the highest seropositivity for sheep and goats, (36.00% and 29.16, respectively), the highest seropositivity for cattle was found in Tokat at 29.62%. Furthermore, no statistically significant difference could be found with regard to seropositivity in samples from the provinces of Samsun, Giresun and Tokat ($P > 0.05$). On the other hand, significant differences were determined in Ordu between cattle and sheep, in Rize between goats and cattle and also in Trabzon among sheep, goats, and cattle.

The distributions of neutralizing antibody titers in positive samples ranged between 1/4 and 1/256 as shown in Table 2. We determined a 1/256 antibody titer in 19 samples consisting of 9 cattle, 7 sheep, and 3 goat sera, whereas a 1/4 antibody titer was detected in 93 samples consisting of 50 cattle, 30 sheep, and 13 goat sera. No

Table 1. The distribution of BPI3VC-3 seropositivity in ruminant species according to provinces

Provinces	Positives / Total (%)			P value	Grand Total
	Cattle	Sheep	Goat		
Samsun	28/98 (28.57)	35/130 (26.92)	3/20 (15.00)	>0.05 ^d	66/248 (26.61)
Ordu	10/71 (14.08) ^a	3/60 (5.00) ^b	–	<0.01	13/131 (9.9)
Giresun	12/61 (19.67)	8/55 (14.54)	–	>0.05 ^d	20/116 (17.24)
Trabzon	10/78 (12.82) ^c	9/25 (36.00) ^a	7/24 (29.16) ^b	<0.01	26/127 (20.47)
Rize	9/53 (16.98) ^b	–	4/20 (20.00) ^a	<0.01	13/73 (17.80)
Tokat	24/81 (29.62)	11/60 (16.66)	16/60 (20.66)	>0.05 ^d	51/201 (25.37)
Total	93/442 (21.04)	66/330 (20.00)	30/124 (24.19)		189/896 (21.09)

^{a,b,c}: It indicates that there is a significant difference among species in the province ($p < 0.01$).

^d: It also refers to no statistical difference could be determined among species in the province ($p > 0.05$).

Table 2. The distribution of neutralizing antibody titers according to ruminant species

Species	Antibody Titers							Total
	1:4	1:8	1:16	1:32	1:64	1:128	1:256	
Cattle	50	17	9	4	2	2	9	93
Sheep	30	11	7	6		5	7	66
Goat	13	6	1	4	3		3	30
Total	93	34	17	14	5	7	19	189

statistically significant differences could be detected between species and the neutralizing antibody titer ($p > 0.05$).

DISCUSSION

Bovine respiratory diseases have a significant impact on the livestock industry around the world. BPI3V is one of the causative agents that causes sporadic outbreaks among cattle in many parts of the world either alone or in association with other viral and bacterial pathogens, most of whom belong to the BRDC (Horwood *et al.*, 2008; Oem *et al.*, 2013).

Unlike previous studies in Turkey, our recent study was carried out using the first indigenous BPI3V isolate from Turkey, which was isolated in 2016 and then submitted to Genbank with accession no: MH357343 (Albayrak *et al.*, 2018).

Previous studies revealed that non-A genotypes of BPI3V arise as result of

geographical isolation (Newcomer *et al.*, 2017). Of particular relevance to this study, initial reports indicated that BPI3Vc was confined to China. However, it was subsequently reported in the US, Argentina, and Korea (Oem *et al.*, 2013). Turkey imports livestock (especially cattle) from more than 15 countries spread across the three continents of America, Europe, and Australia, in particular from Argentina, Brazil and Uruguay, but there is no trade in livestock and animal products between Turkey and China. These facts suggest that BPI3Vc may have originally entered Turkey through livestock imports from Argentina. Nevertheless, further virus isolation and phylogenetic studies are necessary to clarify the origin of Turkish BPI3Vc.

In the current study, the overall seroprevalence as assessed using the Turkish BPI3Vc isolate was found to be 21.09%. Seropositivity rates in a selected sample from cattle, sheep and goats were 21.04%, 20.00% and 24.19%, respectively. Prevalence

of BPI3Va in Turkey was previously reported to vary between 41.02% and 88.82% in serological studies (Alkan *et al.*, 2000; Yazici *et al.*, 2007; Okur-Gumusova *et al.*, 2007; Yesilbag and Gungor, 2009). Furthermore, the rates of BPI3Va infection increased due to mixed infections whereas the rates for single BPI3Va infection were reported to be between 0.17% and 6.5% (Alkan *et al.*, 2000, Okur-Gumusova *et al.*, 2007; Yesilbag and Gungor, 2008). Data presented in earlier studies clearly demonstrated that other pathogens are contributing to the severity and pathogenesis of BPI3Va infections. Moreover, although BPI3Va is peculiar to cattle, cross-transmissions between species like cattle and sheep or cattle and goats has also been reported (Yesilbag and Gungor *et al.*, 2009; Maidana *et al.*, 2012). The results obtained in our current study can also be viewed as supporting evidence for the presence of cross-species transmission. In our current study, seroprevalence of 24.01% in goat samples was higher than in both cattle and sheep while the seroprevalence rate in sheep samples were similar to that in cattle. Additionally, the prevalence of neutralizing antibody established in our study for sheep and goats is higher than those reported by Yesilbag and Gungor (2009) (8.8% and 19.7% for sheep and goats respectively). Consequently, this could be interpreted as the BPI3Vc infection being common in small ruminants as is the case with BPI3Va. Therefore, both sheep and goats might have played a role in transmitting respiratory diseases stemming from BPI3Vc to cattle due to cross-species transmission.

BPI3Va is regarded as the most prevalent genotype worldwide. However, the comparatively high prevalence of BPI3Va may depend upon both commercial diagnostic kits and geographical isolation. The commercial kits employed for the diagnosis of BPI3V are produced using the sequences and antisera based on genotype A, which in turn results in scientific disputes as to whether diagnostic kits are an obstacle for the routine diagnosis of other genotypes (Neil *et al.*, 2015). The B and C genotypes were initially thought to be strains restricted to only a few continents.

However, this perception has begun to change rapidly with not only genotype C but also genotype B spreading across continents (Newcomer *et al.*, 2017; Wen *et al.*, 2017).

In conclusion, although it is not obligatory, some combined vaccines for BRDC pathogens that also include Bovine respiratory diseases have a significant impact on the livestock industry around the world. BPI3V is one of the causative agents that causes sporadic outbreaks among cattle in many parts of the world either alone or in association with other viral and bacterial pathogens, most of whom belong to the BRDC (Horwood *et al.*, 2008; Oem *et al.*, 2013). The BPI3Va are administered to cattle reared for dairy and meat production on some farms in Turkey in order to protect them from BRDC diseases. However, it is important to consider whether or not these vaccines can protect animals against BPI3Vc. In this context, we recommend that the relative efficacy of BPI3V vaccines against BPI3Vc should be investigated to find out if the vaccine is the main problem in the circulation of genotype C or not. In addition, a comprehensive serological screen of recent ruminant samples should be planned across Turkey to further define the presence of BPI3Vc and also check whether it has an effect on BPI3V epidemiology

Conflict of Interests

The authors do not have any conflict of interest to declare.

Authors Contribution

ZY, SG, HA formulated the plan and designed the research. CT, BM, EO and OB performed the experiments. SA performed the statistical analysis. ZY, AEE wrote the manuscript.

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