

## A two-year serological study of bovine viral diarrhoea virus, bovine alphaherpesvirus 1 and bovine parainfluenza virus type 3 in Qazvin dairy cattle farms, Northwestern of Iran

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### ABSTRACT

Infections with bovine viral diarrhoea virus (BVDV), bovine alphaherpesvirus 1 (BoHV-1), and bovine parainfluenza virus type 3 (BPIV-3) cause diseases in cattle with serious economic consequences worldwide. The objective of the present study was to determine of herd-level and animal-level BVDV, BoHV-1, and BPIV-3 seroprevalence, and evaluate some of the associated risk factors on farms in Qazvin province, Northwestern Iran. A total of 1036 cattle in 16 herds were randomly selected, and their serum samples were tested to detect antibodies to these viruses in a cross-sectional study over 2 years. The results showed the seroprevalence of BVDV, BoHV-1, and BPIV-3 was 100%, 56.3%, and 100% at herd-level and 55.1%, 5.1%, and 95.2% at animal-level, respectively. Statistical analysis revealed that the farm was a strong risk factor for all the studied viruses, while the year was determined as a risk factor for only BVDV ( $P < 0.001$ ). The seroprevalence of BVDV and BPIV-3 was significantly ( $P < 0.01$ ) affected by season. The proportion of seropositive cows increased with age for BVDV and BoHV-1 ( $P < 0.001$ ). Concurrent infection was the highest in mixed infections with BVDV and BPIV-3 (53.2%), and there was a positive correlation between BVDV and BoHV-1 seropositivity ( $R^2 = 0.106$ ,  $P < 0.001$ ). The present study shows that infections of BVDV and BPIV-3 are common in cattle in Northwestern Iran and which implies the need to implement control programs to reduce the risk of the spread of these viruses.

**Key words:** seroprevalence; BVDV; BoHV-1; BPIV-3; Iran

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## Introduction

Bovine respiratory disease (BRD) complex is a major cause of economic losses in the cattle industry caused primarily by viral agents and secondarily by bacteria and mycoplasmas. Among the viral agents of BRD, Bovine viral diarrhea virus (BVDV), bovine alphaherpesvirus 1 (BoHV-1), and bovine parainfluenza virus type 3 (BPIV-3) are the best known, causing damage to the respiratory tract of cattle, and creating opportunities for settlement of bacteria in the lungs (PARDON et al., 2011).

BVDV is a member of the genus Pestivirus, of the family Flaviviridae, and affects animal health by decreased fertility and milk production, slow fetal growth, diarrhea, respiratory symptoms, reproductive dysfunctions such as abortion, teratogenesis, embryonic resorption, fetal mummification and stillbirth, immunological dysfunctions, concurrent infections, and impaired herd performance, resulting in significant economic losses. The ability of the virus to cross the placenta during early pregnancy can result in the birth of persistently infected (PI) calves who shedding large quantities of the virus throughout their lives, and are considered the primary reservoirs for BVD (KHODAKARAM-TAFTI and FARJANIKISH, 2017).

BoHV-1 belongs to the Varicellovirus genus of the Herpesviridae family. BoHV-1 is an important agent causing livestock losses globally due to several clinical conditions, including infectious bovine rhinotracheitis (IBR) in the respiratory system, infectious pustular vulvovaginitis (IPV) in the genital system of female cattle, infectious pustular balanoposthitis (IPB) in the genital system of male cattle, conjunctivitis, and generalized disease in newborn calves (COMAKLI et al., 2019). Reductions in milk-related performance and minor effects on herd fertility and mortality were identified in subclinical BoHV-1 in Irish dairy herds (SAYERS, 2017).

BPIV-3 is in the genus Respirivirus of the family Paramyxoviridae, and like other respiratory viruses spreads primarily by large droplet transmission. It causes various results, ranging from subclinical infections to severe pneumonia (ELLIS, 2010).

Prevalence studies have an important role to play in highlighting the necessity, or otherwise, for disease control and eradication schemes (SAYERS et al., 2015). Several studies have already demonstrated a wide variation in seroprevalence of BVDV and BoHV-1 infections in several regions of Iran, while seroprevalence reports for BPIV-3 are rare (ERFANI et al., 2019; NIKBAKHT et al., 2015; SAKHAAEE et al., 2009; SHIRVANI et al., 2012). There is no published data on any concurrent study of BVDV, BoHV-1, and BPIV-3 prevalence in dairy herds located in Qazvin province, Iran. Due to the considerable economic losses caused by these viruses, the present study was conducted to determine the seroprevalence of BVDV, BoHV-1, and BPIV-3 infection in dairy cattle herds of Qazvin province in different seasons, and the association of potential risk factors with these infections.

## Materials and methods

The study was reviewed and approved by the Research Committee of the Razi Vaccine and Serum Research Institute, and all applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

*Study area.* Qazvin province is located in the northwest region of Iran. In total, about 358,000 head of cattle, mainly crossbred, are raised under industrial, semi-industrial, and traditional livestock systems in the province (AGRICULTURE STATISTICS OF IRAN, 2018). Cattle are generally not vaccinated against BVDV, BoHV-1, and BPIV-3 in the study area.

*Sample collection and serum preparation.* A cross-sectional seroprevalence study was conducted to investigate the herd and animal prevalence levels of antibodies against BVDV, BoHV-1, and BPIV-3 infections in different seasons in Qazvin Province during 2011 and 2012. For serological screening, 8 intensively managed dairy herds were randomly selected each year (16 farms in total) and a blood sample was taken from a total of 1036 cattle. The health status of the selected animals was good and they had no history of BVD, IBR, or BPI3 diseases. Blood samples were collected from the jugular vein (5 mL) into

vacutainer tubes (VACUETTE®, Greiner Bio-One GmbH, Kremsmünster, Austria) and serum was separated by centrifugation at 3000 rpm for 10 min from 1017 blood samples. Serum samples were stored in a 1.5 mL microtube at -20 °C until analysis. The age of the animal (1 year and up to 2 years, 2 years and up to 3 years, 3 years and up to 4, and 4 years and above ) and the season (spring, summer, autumn or winter) were also recorded during sampling.

*Serological diagnosis.* Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to test sera samples for the presence antibodies against: (i) BVDV, (HerdChek BVDV Ab, IDEXX Switzerland AG, Bern, Switzerland); (ii) IBR/IPV, (HerdChek IBRgB, IDEXX Switzerland AG, Bern, Switzerland) and (iii) PI3 (POURQUIER® ELISA PI3, Montpellier, France). The tests were performed according to the manufacturer's instructions and the final plate reading was carried out at 450 nm using an Elisa plate reader (Bio Tek, Vermont, USA).

A ratio of sample/positive control (S/P) equal or greater to 0.3 was considered positive for BVDV Ab, and a percentage of inhibition of sample/negative control (S/N) equal to or higher than 55% was considered positive for BoHV-1 Ab. For BPIV-3. The corrected OD was calculated for each sample by subtracting the OD value obtained in a uncoated well from the OD of a coated well. The ratio between the mean corrected OD value of the sample and the mean corrected OD value of positive controls (S/P) was calculated and multiplied by the coefficient 0.8 to obtain the corrected percentage value. A S/P ratio greater than or equal to 20% was considered positive. A herd was considered positive when at least one positive animal was detected.

*Statistical analysis.* All statistical procedures were conducted with SPSS version 21 software in three steps. Data were analyzed descriptively in the first step. The relationship between the seroprevalence rates and year, herd, season, and age were then analyzed using chi-square analysis, and finally, all variables with a P value  $\leq 0.05$  were considered for logistic regression analysis to obtain the adjusted odds ratios (OR) and 95% confidence

interval (CI). The goodness of fit of the model was determined using the Hosmer & Lemeshow test.

## Results

A total of 1036 samples were taken but serological results of 994, 1017, and 1014 samples were recorded for BVDV, BoHV-1, and BPIV-3 respectively. The results of the prevalence of antibodies of viruses at herd and animal levels are shown in Table 1. Antibodies to BVDV and BPIV-3 were found in all the herds, but antibodies to BoHV-1 were discovered in 56.3% herds. The rates of seropositivity at cow level were 55.1, 5.1 and 95.2% for BVDV, BoHV-1, and BPIV-3 respectively. The seroprevalence of BVDV in the first year was significantly ( $P < 0.01$ ) higher than in the second year. The associations of other independent variables, including the farm, season, and age with BVDV, BoHV-1, and BPIV-3 prevalence were also statistically significant ( $P < 0.01$ ), except for season and age which showed no significant correlation with BoHV-1 and BPIV-3 infections, respectively (Table 2). The logistic regression models were statistically significant for BVDV ( $\chi^2(3) = 20.66$ ,  $P < 0.01$ ), BoHV-1 ( $\chi^2(3) = 44.92$ ,  $P < 0.001$ ) and BPIV-3 ( $\chi^2(3) = 20.95$ ,  $P < 0.01$ ). The model explained 45.6%, 45.0%, and 15.1% (Nagelkerke  $R^2$ ) of the variance and correctly classified 76.6%, 96.1%, and 95.2% of the cases for BVDV, BoHV-1, and BPIV-3 infections. A final logistic regression model for seroprevalence of BVDV, BoHV-1, and BPIV-3 is shown in Table 3. The year of life was found to be a risk factor for BVDV infection (OR = 0.389, 95% CI: 0.282-0.537,  $P < 0.001$ ). Seroprevalence of BVDV (OR = 5.684, 95% CI: 3.541-9.124,  $P < 0.001$ ) and BoHV-1 (OR = 19.507, 95% CI: 5.374-70.816,  $P < 0.001$ ) was significantly increased with older age. Antibodies against BVDV were significantly higher in autumn than other seasons (OR = 3.838, 95% CI: 2.415-6.098,  $P < 0.001$ ). Concurrent seropositivity for BVDV, BoHV-1, and BPIV-3 is reported in Table 4.

Table 1. Herd-level and animal-level antibodies to bovine viral diarrhoea virus (BVDV), bovine alphaherpesvirus 1 (BoHV-1), and bovine parainfluenza virus type 3 (BPIV-3) in cattle

Level Antibody	Herd Number		Pre*%	Cow Number		Pre %	$\chi^2$	P-value
	Tested	Positive		Tested	Positive			
BVDV							23.30	0.001
Year								
First	8	8	100	510	319	62.5		
Second	8	8	100	484	229	47.3		
Total	16	16	100	994	548	55.1		
BoHV-1							2.17	0.14
Year								
First	8	4	50	505	31	6.1		
Second	8	5	62.5	512	21	4.1		
Total	16	9	56.3	1017	52	5.1		
BPIV-3								
Year							0.01	0.917
First	8	8	100	504	480	95.2		
Second	8	8	100	510	485	95.1		
Total	16	16	100	1014	965	95.2		

\* Pre=Prevalence, \*\*  $\chi^2$ =Chi-square

Table 2. The relationship between potential risk factors and bovine viral diarrhoea virus (BVDV), bovine alphaherpesvirus 1 (BoHV-1), and bovine parainfluenza virus type 3 (BPIV-3) antibodies

Virus	BVDV				BoHV-1				BPIV-3			
	Number tested	Pre* (%)	$\chi^2$ **	P-value	Number tested	Pre (%)	$\chi^2$	P-value	Number tested	Pre (%)	$\chi^2$	P-value
Farms			237.79	0.001			165.26	0.001			26.24	0.001
1	102	47.1			125	0.8			128	96.6		
2	126	55.6			127	0.8			125	98.4		
3	126	37.3			127	5.5			125	96.8		
4	128	82.8			127	3.9			127	97.6		
5	128	11.7			128	0.0			128	89.1		
6	128	90.6			129	27.9			128	97.7		
7	128	44.5			128	0.0			127	89.8		
8	128	69.5			126	2			126	95.2		
Season			27.68	0.001			1.678	0.642			11.90	0.008
Spring	256	46.5			255	5.5			254	97.2		
summer	255	50.2			257	4.3			255	93.7		
Autumn	241	68.5			245	4.3			254	92.1		
Winter	242	56.2			233	6.4			251	97.6		
Age (years old)			54.43	0.001			37.25	0.001			7.24	0.065
1 and up to 2	247	45.3			247	1.2			244	92.2		
2 and up to 3	249	43.8			245	2.0			248	95.6		
3 and up to 4	252	59.5			252	5.6			249	96.0		
4 and above	244	72.5			245	12.2			245	97.1		

\* Pre=Prevalence, \*\*  $\chi^2$ =Chi-square

Table 3. Final logistic regression models for the seroprevalence of bovine viral diarrhoea virus (BVDV), bovine alphaherpesvirus 1 (BoHV-1), and bovine parainfluenza virus type 3 (BPIV-3)

Virus	Category	BVDV		BoHV-1		BPIV-3	
		OR*	P-value	OR	P-value	OR	P-value
Constant		0.458	0.008	0.002	0.001	48.986	0.001
Years	First	1	-	-	-	-	-
	Second	0.389	0.001	-	-	-	-
Farms	1	1	-	1	-	1	-
	2	1.492	0.183	0.893	0.937	1.337	0.775
	3	0.591	0.085	6.722	0.081	0.620	0.590
	4	7.270	0.001	4.646	0.169	0.882	0.893
	5	0.100	0.001	0	0.996	0.164	0.020
	6	15.403	0.001	59.659	0.001	0.878	0.889
	7	0.854	0.598	0	0.996	0.177	0.027
	8	3.031	0.001	1.695	0.671	0.422	0.303
Season	Spring	1	-	-	-	1	-
	Summer	1.727	0.271	-	-	0.407	0.056
	Autumn	3.838	0.001	-	-	0.304	0.009
	Winter	1.714	0.016	-	-	1.739	0.387
Age (years old)	1 and up to 2	1	-	1	-	-	-
	2 and up to 3	0.912	0.676	1.751	0.463	-	-
	3 and up to 4	2.365	0.001	6.092	0.008	-	-
	Above 4	5.684	0.001	19.507	0.001	-	-

\* OR= Odds ratio



Table 4. Frequency of concurrent seropositivity of bovine viral diarrhoea virus (BVDV), bovine alphaherpesvirus 1 (BoHV-1), and bovine parainfluenza virus type 3 (BPIV-3) in cattle

Mixed viruses	Number		Prevalence (%)
	Tested	Positive	
BVDV + BoHV-1	984	39	3.96
BVDV + BPIV-3	979	521	53.2
BoHV-1 + BPIV-3	998	48	4.81
BVDV + BoHV-1 + BPIV-3	972	38	3.91

The frequency of concomitant seropositivity was the highest in concurrent infection with BVDV and BPIV-3 (53.2%), and 2.78% of sera were free from all of the viruses studied. Also, no correlation was observed between BPIV-3 seropositivity and either BVDV or BoHV1 seropositivity, while there was a positive correlation between BVDV and BoHV1 seropositivity ( $P < 0.01$ ).

### Discussion

In recent years, the seroprevalence study of viral bovine respiratory diseases has been established in different regions of Iran, and the results show a considerable range of variation in the prevalence of the infections. For BVDV, the prevalence of seropositive animals in different regions of Iran has been reported as 28.6%, 28.5%, 49.2%, 59.5%, 66.0% and 73.3% in Zanjan, Ahwaz, Esfahan, Fars, Arak and Kerman, respectively (HAJIHAJIKOLAEI and SEYFIABADSHAPOURI, 2007; SAKHAEI et al., 2009; BADIEI et al., 2010; SHIRVANI et al., 2012; GHAEMMAGHAMI et al., 2013; ERFANI et al., 2019). In a study conducted in four provinces of Iran (NIKBAKHT et al., 2015), the average rate of seropositive animals for BVDV was reported as 64.4% which is higher than the result of the present study (51.5%). The increased density of cattle in the area, herd size, animal purchasing and adding to herds, pasture rental and contact with PI animals could play a role in the regional differences in the rate of infection, which was also reported in other countries (FERNANDES et al., 2016; HOUE, 1995). The herd- and animal-level prevalences of BVDV were reported as 65.5 % and 39.1 % in the State of Paraíba, Brazil, 95.6% and 51.7% in Jimma

town, Ethiopia and 80.0% and 32.2% in Selangor, Malaysia, respectively (DAVES et al., 2016; FERNANDES et al., 2016; TADESSE et al., 2019).

The overall seroprevalence of BoHV-1 in this study was 5.1%, indicating that BoHV-1 infection was not widely distributed among the bovine population in Qazvin province. Although the herd-level seroprevalence of BoHV-1 in Qazvin province (56.3%) is in the range found in other regions of Iran, the animal level prevalence of the virus is lower than reports from Isfahan, Hamedan, Chaharmahal Bakhtiari, Khouzestan, Sistan and Baluchestan, Kerman, Khorasan, Semnan and Zanjan provinces, which were 72%, 58.74%, 57.7%, 48.67%, 32.9%, 30.39%, 25.6%, 21.7 and 11.4%, respectively (ADELI et al., 2017; BAHARI et al., 2013; ERFANI et al., 2019; NIKBAKHT et al., 2015; SAKHAEI et al., 2009; SHIRVANI et al., 2012). The difference between herd and cow levels of BoHV-1 seroprevalence revealed that a few seropositive cows were in half of the studied farms. A wide variation in the prevalence of BoHV-1 has been seen in reports from other parts of the world (BARRETT et al., 2018; KADDOUR et al., 2019). Although the geographical variations may explain the differences between the results, stress also can be a reason (LEMAIRE et al., 2001).

Infection by BPIV-3 has been long-recognized and currently underappreciated, but nevertheless is significantly correlated with respiratory diseases in cattle (ELLIS, 2010). The most prevalent virus in the present study was BPIV-3. Similar results were also observed in Kerman (100%), Khorasan Razavi (90%), and Esfahan (84.4 %) provinces of Iran (ROSHTKHARI et al., 2012; SAKHAEI

et al., 2009; SHIRVANI et al., 2012). Despite the high seroprevalence, BPIV-3 was less commonly identified in livestock farms, which may be due to the similarity of its symptoms with other respiratory diseases and the lack of use of diagnostic kits.

The moderate and low seropositivity for BPIV-3 observed in Saudi Arabia (67.6%), Western Kenya (20.1%) and Grenada (13.4%) are lower than the results from Iran (CALLABY et al., 2016; MAHMOUD and ALLAM, 2013; TIWARI et al., 2016).

As vaccination against BVDV, BoHV-1, and BPIV-3 with commercial vaccines was not practical in the studied herds, the presence of specific antibodies indicates a natural exposure of cattle to wild viruses. Many authors introduced age as a potential risk factor for BVDV (DAVES et al., 2016; TADESSE et al., 2019) and BoHV-1 (ADELI et al., 2017; ERFANI et al., 2019; RAMÍREZ et al., 2016). The higher odds ratio of BVDV and BoHV-1 with increasing age in the present study may be due to the increase in exposure to these respiratory viruses during life (SHIRVANI et al., 2012). Although older age was mentioned to be associated with BPIV-3 in some surveys (CALLABY et al., 2016; SOLIS-CALDERON et al., 2007), increased age was not found to be a risk factor for seroprevalence of BPIV-3 in the present study.

The results of this study show that individual farms had varied seroprevalence, ranging from 11.7% to 90.6% for BVDV and 0% to 27.9% for BoHV-1. Variations in seroprevalence between one farm and other farms in a region may be explained by microclimatic changes, management differences, and stock densities (ALMEIDA et al., 2013). The production type, herd size, housing and management practices, such as animal movement and hygiene, which were introduced as main risk factors for bovine respiratory disease, may vary between farms (GAY and BARNOUIN, 2009). Introducing new cattle to a farm without testing for viremia, and livestock trade that involves purchasing transiently or persistently infected cattle can increase the risk of exposure of farms to BVD and IBR (RAMÍREZ et al., 2016). Although a seasonal variation was observed in the seroprevalence of both BVDV and

BoHV-1 in Sudan, we did not see it in BoHV-1 seroprevalence (ELHASSAN et al., 2011).

In contrast to earlier studies on Iranian cattle, we did not find any positive correlation between BVDV and BoHV1 seropositivity (NOAMAN and NABINEJAD, 2020; NIKBAKHT et al., 2015; SHIRVANI et al., 2012) and the highest number of concurrent infections observed were infections with BVDV and BPIV-3.

The results of the current study show the high seroprevalence of BVDV and BPIV-3 in unvaccinated cattle in Qazvin province, Northwestern Iran, so it may be suggested that control programs should be implemented to prevent further spread. Some preventive measures, such as biosecurity, quarantine, mass vaccination, and detection of PI animals at early stages are key for control programs. The lack of disease prevention and low awareness of herd biosecurity may result in the continuing spread of BVDV and BPIV-3 and subsequently silent economic losses. The study of other important viruses in the BRD, such as bovine respiratory syncytial virus and bovine adenovirus type 3 may be suggested in the region.

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**HASHEMI M., M. BAKHSHEH, M. KHEZRI, M. M. GHARAGOUZLOUIAN, G. TAVAKOLI: Dvogodišnje serološko istraživanje virusa govede virusne dijareje, govedeg alfa-herpesvirusa 1 i virusa govede parainfluence tipa 3 na farmama mliječnih krava u Qazvinu, sjeverozapadni Iran. *Vet. arhiv* 92, 1-10, 2022.**

#### **SAŽETAK**

Infekcije virusom govede virusne dijareje (BVDV), govedeg alfa-herpesvirusa 1 (BoHV-1) i virusa govede parainfluence tipa 3 (BPIV-3) uzrokuju pobol u goveda širom svijeta, sa znatnim ekonomskim posljedicama. Cilj ovog istraživanja bio je odrediti seroprevalenciju BVDV-a, BoHV-1 i BPIV-3, na razini stada i na razini životinje, te procijeniti rizične čimbenike povezane s tim virusima na farmama u pokrajini Qazvin u sjeverozapadnom Iranu. U presječnom istraživanju, koje je trajalo više od dvije godine, nasumično je odabrano ukupno 1036 goveda iz 16 stada čiji su uzorci seruma testirani kako bi se pronašla antitijela na tri navedena virusa. Rezultati su pokazali da je seroprevalencija BVDV-a 100 %, BoHV-1 56,3 %, a BPIV-3 100 % na razini stada, dok je na razini životinje seroprevalencija BVDV-a bila 55,1 %, BoHV-1 5,1 %, a BPIV-3 95,2 %. Statistička je analiza pokazala da je farma znatan rizični čimbenik za sve istraživane viruse, dok se kombinacija godine i sezone pokazala rizičnim faktorom samo za BVDV ( $P < 0,001$ ). Na seroprevalenciju BVDV-a i BPIV-3 znakovito je utjecala sezona ( $P < 0,01$ ). Omjer seropozitivnih krava za BVDV i BoHV-1 znakovito je rastao s dobi ( $P < 0,001$ ). Najčešća je koinfekcija bila virusima BVDV i BPIV-3 (53,2 %), a ustanovljena je i pozitivna korelacija između seropozitivnosti BVDV-a i BoHV-1 ( $R^2 = 0,106$ ,  $P < 0,001$ ). Ovo je istraživanje pokazalo da su infekcije BVDV-om i BPIV-3 česte u goveda u sjeverozapadnom Iranu što upućuje na potrebu uvođenja programa nadzora kako bi se smanjio rizik od širenja ovih virusa.

**Ključne riječi:** seroprevalencija; BVDV; BoHV-1; BPIV-3; Iran

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