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Seroprevalence and risk factors assessment of the three main infectious agents associated with abortion in dairy cattle in Isfahan province, Iran

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Abstract

This study aimed to determine the seroprevalence and identify the risk factors associated with *Neospora caninum*, Bovine herpesvirus type 1 (BHV-1), and Bovine viral diarrhea virus (BVDV) infection on industrial Holstein dairy cattle farms in Isfahan province, Central Iran. Blood samples were taken from 216 apparently healthy cattle from 16 randomly selected Holstein dairy farms in the North, South, East, and West of Isfahan in the summer of 2017. The antibodies to *N. caninum*, BHV-1, and BVDV were detected using a commercially available ELISA kit. The overall seroprevalence for *N. caninum*, BHV-1, and BVDV was 19%, 72.2%, and 52.8%, respectively. The significant major risk factors of BHV-1 in cattle were identified as farm direction, age groups, parity, and milk yield by the univariate analysis ($p < 0.05$). The significant major risk factors of BVDV in cattle were identified as age groups, parity, milk yield, and stage of pregnancy ($p < 0.05$). The only significant major risk factor of *N. caninum* was farm direction ($p < 0.05$). A significant association of concurrent infection with BVDV and BHV-1 has shown in the current study ($p < 0.05$). This study is the first to report the risk factors for *N. caninum*, BHV-1, and BVDV infection in the central part of Iran and allows us to conclude that these agents are widely distributed in this region.

Keywords *Neospora caninum* · BHV-1 · BVDV · Risk factors · Iran

Introduction

Iran is one of the largest dairy producers in the Middle East. In recent years, reduced fertility in dairy cattle is a global problem (Royal et al. 2000). Several agents interfere with the reproductive process of dairy cows. Infectious agents such as viruses, bacteria, and parasites are the most important cause of infertility in dairy cattle. These agents can lead to abortion, retained placenta, early embryonic death, delay in conception, anestrus, repeat breeding, and delayed return to estrus. These will ultimately lead to calving and milk reduction in dairy farms. According to the research, the economic loss of abortion and delay in conception is 500–900 US$ per case and 4.5–6.7 US$ per day, consequently (Carpenter et al. 2006; Kafi et al. 2007).

Neosporosis, infectious bovine rhinotracheitis (IBR) and bovine viral diarrhea (BVD) are the three main causes of reproductive disorder in dairy cattle (Kirkbride 1992).

Neosporosis has been reported from many countries in the world as one of the major causes of abortions in cattle, which has a wide global prevalence between 0.5% and 76.9% (Llano et al. 2018). Studies have shown that seropositive cows to neosporosis have 12 to 19 times increased risk for abortion compared to seronegative ones (Favero et al. 2017). The annual economic losses due to neosporosis for the dairy industry are estimated to be US$ 843 million worldwide (Reichel et al. 2013).

Bovine herpesvirus type 1 (BHV-1) is an important pathogen globally and has a significant impact on cattle health and welfare. The virus was estimated to be responsible for a loss of £4 million per annum in the UK farming industry (Bennett 2003). Reproductive and mixed clinical problems in infected cows lead to a milk yield loss of 2.6 kg/d in BHV-1 seropositive compared with seronegative dairy cows (Statham et al. 2015) as well as the risk of abortion in seropositive herds increased by 7 times compared to seronegative herds (Raaperi et al. 2012). In Turkey, the average economic loss due to the infection was estimated to be US$ 379 per infected cow (Can et al. 2016).

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Bovine viral diarrhea virus (BVDV) is endemic in many dairy cattle herds throughout worldwide. In different studies, the direct costs of a BVD infection in dairy herds ranged between €21 and €135 per cow but could be as high as € 340 per cow when simultaneously other infections occurred or in case of highly virulent strains (Santman-Berends et al. 2015).

In a meta-analysis study (include 19 studies) to estimate mean annual BVDV production losses, the mean annual direct losses were found to be €42.14 per animal (Pinior et al. 2019).

In Iranian dairy farms, although the prevalence of abortion by noninfectious agents is 10% to 20%, the abortion rate is up to 30% due to infectious agents including *Brucella abortus*, BVDV, BHV-1, *N. caninum, Listeria monocytogenes*, and *Leptospira interrogans* (Rafati et al. 2010).

Due to the variety of abortion factors, it is very difficult to determine the causative agent of abortion in the herd. Although effective vaccines against some of the factors affecting abortion are used in dairy farms, identification of abortion agents is essential for developing herd health programs or effective control measures and reducing the economic losses caused by abortion in dairy herds.

There is little information about the status of infectious causes of abortion in dairy farms in Iran. The goals of this study were to investigate some of the most important infectious agents of abortion and associated risk factors in dairy herds in central Iran via a seroepidemiological study with special emphasis on *N. caninum*, BHV-1, and BVDV.

Materials and methods

Study area

The study was carried out on industrial Holstein dairy cattle farms in Isfahan province (latitude 30° 43′-34° 27′ N, longitude 49° 36′55° 31′ E), Central Iran. The province of Isfahan covers an area of approximately 107,027 km^2 and is situated in the center of Iran. The province experiences a moderate and dry climate, on the whole, ranging between 40.6 °C and 10.6 °C on a cold day in the winter season. The average annual temperature has been recorded as 16.7 °C, and the annual rainfall on average has been reported as 116.9 mm. According to statistics released by the Iranian Ministry of Jahad-e-Agricultural (animal production department) in 2017, in Isfahan province, there are about 194,000 pure, 357,000 crossbreed, and 164,000 native cattle that produce more than 1.31 million tons of milk.

Blood sampling and serum preparation

Blood samples were taken via the middle coccygeal vein from 216 apparently healthy cattle from 16 randomly selected

Holstein dairy farms in the North, South, East, and West of Isfahan in the summer of 2017. The target population was cattle herds, and the sampling unit was cattle. According to the farmer statement, cattle were only vaccinated against brucellosis and foot and mouth disease. No abnormal abortions were reported in animal farms.

Cattle were grouped according to the farm direction (north, 61; south, 48; east, 55; and west, 52), age groups (calf, 55; heifer, 32; dairy, 108; and dry, 21), parity (non-calving, 89; 1–2, 85; and over 3 calving, 42) milk yield (Kg) per day (Over 30 kg, 50; 10–29 kg, 56; and non-milk yield, 110), stage of pregnancy (under 3 months, 50; 3–6 months, 10; and over 6 months, 28).

The samples were immediately sent to the serological laboratory, in ice at 4 °C. After complete clotting, the blood samples were centrifuged at 4000 rpm for 15 min. The serum samples were removed from the clot and stored at − 20 °C until analysis.

ELISA test

The antibodies to BHV-1 and BVDV were detected using a commercially available ELISA kit developed by Svanova Biotech (Uppsala, Sweden) according to the procedure provided in the kit. The corrected optical density (COD) level was calculated and the individual sera with CODs ≥ 0.2 and CODs ≥ 0.3 were considered positive for antibodies to BHV-1 and BVDV, respectively. The antibodies against *N. caninum* were detected using a commercially available ELISA kit (enzyme-linked immunosorbent assay; IDEXX Laboratories Inc., Westbrook, Maine, USA) according to the protocol provided by the manufacturer. Briefly, each serum sample (diluted 1:100) was added to the Neospora antigen-coated microplates, which incubated at room temperature for 30 min. The plates were washed before adding a substrate-chromogen solution. After incubation for another 30 min in the dark at room temperature, 100 μL of 1.5 N sulfuric acid was added to stop the reaction. The plates were read at a wavelength of 450 nm using a reference filter of 630 nm. The results were expressed as a sample to positive (S/P) ratios, as recommended by the manufacturers. An S/P ratio greater than or equal to 0.5 were classified as positive.

Statistical analysis

Chi-square (χ2) and logistic regression tests (SPSS software, version 18) were used to assess the association between the seroprevalence (*N. caninum*, BHV-1, and BVDV) and risk factors (farm direction, age groups, parity, milk yield, and stage of pregnancy). The factors with univariate *p* values less than 0.16 were considered in the multivariable logistic regression. Phi and Cramer's V test was used for the correlation of the concurrent presence of antibodies against BHV-1, BVDV,

and *N. caninum*. Results were considered statistically significant for $p < 0.05$.

Results

The overall seroprevalence for *N. caninum*, BHV-1, and BVDV was 19%, 72.2%, and 52.8%, respectively (Table 1). All sixteen farms were BHV 1- and BVD-positive, and antibodies against *N. caninum* were detected in twelve tested farms (Fig. 1).

Risk factor analysis for *N. caninum*, BHV-1, and BVDV

The results of univariable logistic regression analysis are summarized in Table 1. Only farm direction was significantly associated with *N. caninum* seropositivity status. No significant association was found between the age groups, parity, milk yield, stage of pregnancy, and *N. caninum*. A significant association was found between the risk factors (farm direction, age groups, parity, and milk yield) and BHV-1. No significant association was found between the farm direction and BVDV (Table 1).

In multivariable logistic regression analysis, farm direction was still significantly associated with *N. caninum* seropositivity ($p < 0.05$), and only farm direction and age groups were significantly associated with seropositivity status of BHV-1 and BVDV. In the farm direction group, a statistically significant relation of cattle kept in north direction (OR = 9.06, 95% CI: 2.41–34.06.34; $P = 0.001$) and south direction (OR = 0.39, 95% CI: 0.16–0.96; $P = 0.039$) was determined for seropositivity to BHV-1 and BVDV, respectively. In age groups, heifer was significantly less likely to be seropositive for both BHV-1 and BVDV than calf (OR = 0.19) (Table 2).

In Phi and Cramer's V, a significant association for the involvement of both BVD and BHV-1 infections was indicated within the cattle ($p < 0.05$) (Table 3).

Discussion

N. caninum

This study showed that the overall seroprevalence of *N. caninum* infection in dairy cattle was 19% which is a little higher, compared to worldwide *N. caninum* antibody prevalence (16.1%) in dairy cattle (Reichel et al. 2013). Variable values were reported for *N. caninum* antibody prevalence in cattle from several provinces of Iran: 38.8% in Tehran (Salehi et al. 2010), 61.2% in Hamedan (Gharekhani 2014), 24.3% in Khorasan Razavi (Mikhchi et al. 2013), 32% in Mazandaran (Youssefi et al. 2009), 12.6% in Kerman (Nourollahi-Fard et al. 2008), and 32.1% and 26.33% in Isfahan (Morovati

and Noaman 2016; Hosseininejad et al. 2017). Several factors such as history of reproduction problems, source of replacement heifers, presence of definitive hosts, farm size, cattle density, breed, age, fodder, drinking water, feeding colostrum or milk, season, stress, and body condition have been described as associated with seroprevalence of *N. caninum* infection in dairy cattle (Moore and Venturini 2018). The presence of different risk factors in each region may be due to different prevalence. The prevalence observed in this study was lower than the average rate of the infection reported in other studies in Iran, and *N. caninum*-positive animals were observed in 75% of the evaluated farms. In contrast, other studies found positive animals in all the evaluated farms (Salehi et al. 2010; Nazir et al. 2013; Llano et al. 2018).

In this study, participants were not asked about the presence of dogs on the farm, but the horizontal transmission of *N. caninum* or exposure from a point source seems to be responsible for a high seroprevalence within-herd (Barrett et al. 2018). In our study, the farm seroprevalence of *N. caninum* was relatively low compared to the other two pathogens studied, which probably reflects the fact that transmission of *N. caninum*, is, for the most part vertical (Bartels et al. 1999). Furthermore, if a postnatal infection has occurred, these must have been very rare in recent years, since no significant differences in the seroprevalences of different age groups could be observed.

Despite high seroprevalence of BVDV and BHV-1, antibodies to *N. caninum* were not observed in 4 farms in this study. The probable reasons for this difference are limiting access of definitive hosts of *N. caninum* to water and food supplies, selective culling through the use of diagnostic tests, embryo transfer, and good hygienic practices at the farms level (Dubey et al. 2007).

In relation to farm direction, the univariate analysis confirmed that cattle in west direction were 3.2 times more likely to be seropositive by *N. caninum* than others, where the geographical differences, rainfall, temperature, and climate vary considerably. This result may be explained by the fact that mild temperatures and humidity support sporulation and survival of oocysts (Moore and Venturini 2018).
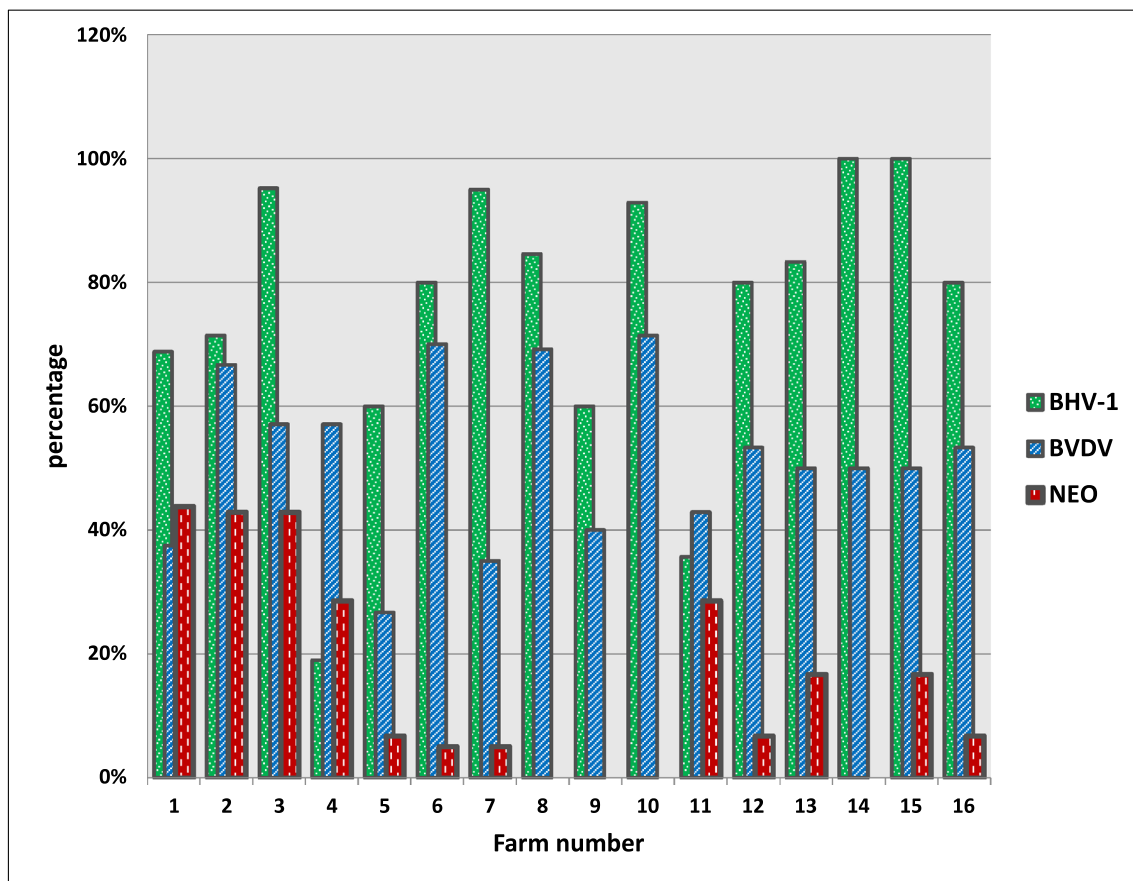
BHV-1

In this study, the prevalence of BHV-1 was identified 72.2% and 100% in cattle and farms, respectively. Worldwide seroprevalence has been reported 12–77.5% and 43–100% in cattle and farms, respectively (Raaperi et al. 2014). The high seroprevalence at the farm level indicates that all the dairy herds had at least one positive animal, and BHV1 infection is widely distributed in dairy cattle from the central part of Iran. The level of herd prevalence described here is probably related to the relatively large size of the participating herds. The overall BHV1 seroprevalence has been reported 31.9% in

Table 1 Univariate analysis of risk factors associated with the prevalence of the three main causes of abortion in cattle from Isfahan province, Iran

Factors	Category	Number tested	N. caninum P(%)	χ2	P value	OR	95% CI for OR Lower	Upper	BHV-1 P(%)	χ2	P value	OR	95% CI for OR Lower	Upper	BVDV P(%)	χ2	P value	OR	95% CI for OR Lower	Upper
All animals	Total	216	41(19%)						156(72.2%)						114(52.8%)					
Farm direction	East[1]	61	8(13.1%)	–	–	1	–	–	39(63.9%)	–	–	1	–	–	33(54.1%)	–	–	1	–	–
	North	48	9(18.8%)	0.00	0.423	1.53	0.54	4.32	44(91.7%)	11.63	0.002	6.21	1.97	19.58	31(64.6%)	3.45	0.271	1.55	0.71	3.37
	South	55	7(12.7%)	1.88	0.95	0.97	0.33	2.87	38(69.1%)	0.36	0.557	1.26	0.58	2.74	26(47.3%)	0.90	0.463	0.76	0.37	1.58
	West	52	17(32.7%)	8.37	0.015	3.22	1.25	8.26	35(67.3%)	0.83	0.707	1.16	0.53	2.53	24(46.2%)	1.21	0.4	0.73	0.35	1.53
Age groups	Calf[1]	55	10(18.2%)	–	–	1	–	–	36(65.5%)	–	–	1	–	–	26(47.3%)	–	–	1	–	–
	Dairy	108	20(18.5%)	0.03	0.958	1.02	0.44	2.37	91(84.3%)	15.60	0.007	2.83	1.32	6.04	66(61.1%)	6.02	0.093	1.75	0.91	3.38
	Dry	21	3(14.3%)	0.33	0.687	0.75	0.19	3.05	18(85.7%)	2.11	0.092	3.17	0.83	12.13	17(81.0%)	7.41	0.012	4.74	1.41	15.91
	Heifer	32	8(25.0%)	0.89	0.451	1.50	0.52	4.30	11(34.4%)	26.82	0.006	0.28	0.11	0.69	5(15.6%)	20.81	0.005	0.21	0.07	0.62
Parity	Non-calving[1]	89	19(21.3%)	–	–	1	–	–	49(55.1%)	–	–	1	–	–	33(37.1%)	–	–	1	–	–
	1–2	85	14(16.5%)	0.58	0.413	0.73	0.34	1.56	75(88.2%)	17.91	0	6.12	2.80	13.37	58(68.2%)	13.44	0	3.65	1.95	6.83
	≥3	42	8(19.0%)	0.00	0.761	0.87	0.35	2.18	32(76.2%)	0.41	0.022	2.61	1.15	5.95	23(54.8%)	0.08	0.058	2.05	0.98	4.33
Milk yield	Non-milk yield[1]	110	21(19.1%)	–	–	1	–	–	67(60.9%)	–	–	1	–	–	48(43.6%)	–	–	1	–	–
	≥30 kg	50	5(10.0%)	3.41	0.155	0.47	0.17	1.33	45(90.0%)	10.25	0.001	5.78	2.13	15.70	31(62.0%)	2.22	0.033	2.11	1.06	4.18
	10–29 kg	56	15(26.8%)	2.99	0.257	1.55	0.73	3.31	44(78.6%)	1.52	0.024	2.35	1.12	4.95	35(62.5%)	2.87	0.023	2.15	1.11	4.16
Stage of pregnancy[a]	<3m[1]	50	10(20.0%)	–	–	1	–	–	41(82.0%)	–	–	1	–	–	21(42.0%)	–	–	1	–	–
	3–6 m	10	3(30.0%)	0.48	0.487	1.71	0.37	7.84	7(70.0%)	0.73	0.392	0.51	0.11	2.37	9(90.0%)	5.32	0.021	12.43	1.46	105.74
	>6 m	28	4(14.3%)	0.39	0.530	0.67	0.19	2.36	25(89.3%)	0.72	0.397	1.83	0.45	7.40	20(71.4%)	5.97	0.015	3.45	1.28	9.33

[1] Reference value, N number of animals evaluated, P(%) prevalence of seropositivity, χ2 chi-square, OR odd ratio, CI confidence interval, m months, [a] Included 88 pregnant animals

Fig. 1 Percentage of seropositive cattle in tested farms for Bovine herpesvirus type 1 (BHV-1), Bovine viral diarrhea virus (BVDV), and *N. caninum* (NEO) in Isfahan province

Iran which is lower than the findings of the present study (Nikbakht et al. 2015). The seroprevalence identified in this study was high not only in cattle but also in the evaluated farms when compared with other studies in Iran (Ezzi et al. 2013; Erfani et al. 2019). Similar observations in Isfahan (Shirvani et al. 2012) and Kerman (Sakhaee and Khalili 2009) have been reported. Since the healthy cattle may be infected directly from secretions (respiratory, eye, reproductive) or indirectly through equipment or individuals (Ackermann and Engels 2006), it is necessary to establish

Table 2 Multiple logistic regression analysis of the major risk factors associated with prevalence of the three main causes of abortion in cattle from Isfahan province, Iran

Factors	Category	*N. caninum*				BHV-1				BVDV			
		P value	OR	95% C.I. for OR		*P* value	OR	95% C.I. for OR		P value	OR	95% C.I. for OR	
				Lower	Upper			Lower	Upper			Lower	Upper
Farm direction	East[1]	–	1	–	–	–	1	–	–	–	1	–	–
	North	0.218	2.04	0.66	6.32	0.001	9.06	2.41	34.06	0.725	1.18	0.47	2.92
	South	0.417	0.61	0.18	2.03	0.354	0.65	0.26	1.63	0.039	0.39	0.16	0.96
	West	0.03	3.18	1.12	9.04	0.217	1.86	0.70	4.98	0.277	0.60	0.24	1.50
Age groups	Calf[1]	–	1	–	–	–	1	–	–	–	1	–	–
	Dairy	0.999	0.00	0.00	.	1	3.90	0.00	.	1	0.45	0.00	.
	Dry	0.667	2.31	0.05	104.52	1	1.20	0.00	.	0.999	4.10	0.00	.
	Heifer	0.789	1.16	0.39	3.45	0.002	0.19	0.07	0.55	0.004	0.19	0.06	0.59

[1] Reference value, N number of animals evaluated, P(%) prevalence of seropositivity, χ2 chi-square, OR odd ratio, CI confidence interval

Table 3 Association between prevalence of antibodies to BVDV, BHV-1, and *N. caninum*

BVDV and BHV-1		BVDV and *N. caninum*		BHV-1 and *N. caninum*	
Antibody status	Rates	Antibody status	Rates	Antibody status	Rates
BVDV (+) & BHV-1(+)	41.2%	BVDV (+) & *N. caninum* (+)	10.7%	BHV-1 (+) & *N. caninum* (+)	12%
BVDV (−) & BHV-1(−)	16.2%	BVDV (−) & *N. caninum* (−)	38.9%	BHV-1 (−) & *N. caninum* (−)	20.9%
BVDV (+) & BHV-1(−)	11.6%	BVDV (+) & *N. caninum* (−)	42.1%	BHV-1 (+) & *N. caninum* (−)	60.2%
BVDV (−) & BHV-1(+)	31%	BVDV (−) & *N. caninum* (+)	8.3%	BHV-1 (−) & *N. caninum* (+)	6.9%
Correlation coefficient	0.138	Correlation coefficient	0.032	Correlation coefficient	0.095
P value	0.042*	*P* value	0.636	*P* value	0.162

$*P < 0.05$ was considered significant

prevention and control measures between animals of the same region and among regions.

Based on previous studies, large herd size, purchased cattle, older age, high density of herds within an area, dairy cattle farms with the presence of beef cattle, and dairy cattle farms located close to BHV-1 positive farms are the main risk factors for the presence of BHV1 among cattle farms. Differences in risk factors in various studies may be due to variations in farm, region, husbandry, and microclimate (Almeida et al. 2013).

In related to farm direction, the univariate analysis confirmed that cattle in north direction were 6.21 times more likely to be seropositive than other directions. It is well-known that BHV-1 seropositivity is higher at larger dairy farms compared with smaller farms (Raaperi et al. 2014). The largest dairy farms in Isfahan are located in the north of the province, and this result may be explained the most seropositivity in the north direction. With regard to age groups, BHV-1 seropositivity status was significantly higher in dairy cattle (OR = 2.83) and lower in heifer (OR = 0.28). Similar to our result, other authors have also reported the highest seroprevalence in dairy cattle and the lowest in heifers. They believe that heifers exposed to the virus at breeding and positive dairy cattle are responsible for the transmission of the virus to seronegative animals (Guarino et al. 2008; Romero-Salas et al. 2013).

Regarding the parity, cattle with 1–2 calving had more chance to be seropositive for BHV-1 than non-calving cattle. Contrary to our results, higher seroprevalence was reported in the cattle with more than 6 calving (Romero-Salas et al. 2013). The more susceptibility of young cattle and routine culling of unprofitable older cattle may explain the significant difference.

In the milk yield group, the risk of BHV-1 seropositivity status was significantly higher in cattle with over than 30 kg/day milk production followed by 10–29 kg/day milk production compared to non-milk yield cattle. Although a less milk yield in BHV-1 seropositive dairy cattle has been reported (Statham et al. 2015), there is no published information related to the BHV-1 seropositivity and milk yield group. The possible reason for higher BHV-1 seropositivity status in cattle with over than 30 kg/day milk production compared to non-milk yield cattle could be the presence of heifers and calves in the non-milk group. To assess the actual effect of milk yield group on BHV-1 seropositive, more research is needed to be particularly conducted.

No statistically significant association was observed between the BHV-1 and stage of pregnancy in the univariate logistic regression analysis. Similar to our observation, no clear association was observed between the BHV-1 seropositivity and pregnancy status in beef cows from western Canada (Waldner 2005).

BVDV

In this study, the BVDV prevalence was identified 52.8% and 100% in cattle and farms, respectively. Worldwide seroprevalence has been reported from 11.1% to 100% in cattle level (Ran et al. 2019) and from 70% to 100% in herd level (Houe 2008) which the findings of this research were in the global range. The overall BVDV seroprevalence reported in the current study was higher, compared to a recent report from Zanjan province in Iran (Erfani et al. 2019) and in agreement with previous reports from Sistan and Baluchestan, Semnan, and Arak province in Iran (Ghaemmaghami et al. 2014; Nikbakht et al. 2015).

The risk of BVDV seropositivity status was higher in dry cattle (OR = 4.74) and lower in heifer (OR = 0.21). This result was consistent with other studies that reported higher BVDV seropositivity in adult cattle than young age groups (Sayers et al. 2015; Daves et al. 2016; Tadesse et al. 2019). This result may be explained by the fact that young animals have less exposure to the BVDV during their lifetime compared with adult animals (Lanyon and Reichel 2014). On the other hand, if infection occurs during pregnancy, the virus may establish immunotolerance and persistent infection (PI) in the young animals which may cause the lack of antibody production against the virus and subsequent defect in detection by the ELISA Ab test (Daves et al. 2016).

Age, cattle density, herd size, housing systems, biosecurity, and management practices have been discussed as the most important BVDV risk factors in various studies (Uddin et al. 2017).

The risk for BVDV seropositivity status in cattle with 1–2 calving increased by 3.64 times compared to non-calving cattle. Similarly, higher seroprevalence was observed in cattle older than 2 years compared to younger ones from Zanjan province in Iran (Erfani et al. 2019). Age is the most common factor that is always mentioned to be associated with BVD which is likely related to the increased chance of exposure to the virus in the older (Ramirez Vasquez et al. 2016).

In the milk yield group, the risk of BVDV seropositivity status was significantly higher in cattle with 10–29 kg/day milk production (OR = 2.15) followed by over than 30 kg/day milk production (OR = 2. 11) compared to non-milk yield cattle. Similar to our observation, the higher seroprevalence of BVDV was reported in lactating cattle than non-lactating cattle in Malaysia (Daves et al. 2016). The risk of getting infection during the milking process by workers who milk the cows and direct transmission of virus from infected cow to the next through contaminated milking clothes or equipment and reuse of towels and dirty hands may be the explanation for the higher BVDV seropositivity in milk yield cattle in this study (Daves et al. 2016).

In the stage of pregnancy group, the risk of BVDV seropositivity status was significantly higher in cattle with 3–6 months pregnancy period (OR = 12. 42) followed by upper 6 months pregnancy period (OR = 3.45) compared to lower 3 months pregnancy period. Our findings are in agreement with the observations made in Malaysia, which suggested the importance of pregnancy status as contributing factors to the prevalence of BVDV. They suggest that most of the positive cattle can be imputed to natural change in the stress hormone (cortisol) level in the body which is well-known to increase about a few weeks before parturition (Daves et al. 2016). In contrast, another study in Bangladesh found no association between BVDV seropositivity and the stage of pregnancy (Uddin et al. 2017).

A significant association of concurrent infection with BVDV and BHV-1 has shown in the current study. A previous study indicated that the interaction between IBR and BVD viruses could increase abortion and infertility problems in dairy cattle (Aslan et al. 2015). Our findings are in agreement with the previous studies in Iran (Ghaemmaghami et al. 2014; Nikbakht et al. 2015; Erfani et al. 2019). Both BVDV and BHV-1 are the most significant immunosuppressive agents and can suppress not only the innate but also adaptive immune responses. In addition, both viral infections help to the reappearance of other viral, bacterial, and parasitic pathogens (Erfani et al. 2019).

In the present study, no significant correlation between *N. caninum* seropositivity and either BHV-1 seropositivity or BVDV seropositivity was observed. Contrary to our observation, there was a statistically significant association between antibodies against BVDV and *N. caninum* in Swedish dairy cows (Bjorkman et al. 2000). The other study has shown that concurrent infection with BVDV and *N. caninum* may be conducive to caused abortion in cattle (Konnai et al. 2008). With regard to the association of *N. caninum* seropositivity with BHV-1 seropositivity, the results of the studies are contradictory. Rinaldi et al. (2007) found a positive association of *N. caninum* with BHV-1 in Italia, whereas Bartels et al. (1999) did not find any association of antibodies to *N. caninum* seropositivity and BHV-1 seropositivity in the Netherlands. Further studies are needed to explore the underlying immunological mechanisms, in order to identify the synergistic relevance of these two pathogens.

In conclusion, the results of this study demonstrate the potential importance of BHV-1, BVDV, and *N. caninum* as possible causes of reproductive disorder of cattle in Isfahan Province, where it is one of the most important dairy cattle production areas of Iran. The clinical significance of these infectious diseases still needs to be fully investigated through isolations from aborted fetuses or demonstration of active infection. Government disease control practices have in the past only focused on the control of brucellosis among the reproductive diseases in Iran. Based on the high seroprevalence of other reproductive diseases in this study, other infectious causes of reproductive failures also need attention. The main risk factors were common to both BHV-1 and BVDV and included farm direction, age groups, parity, and milk yield. A comprehensive epidemiological study of bovine reproductive agents including *Brucella abortus*, BVDV, BHV-1, *N. caninum, Listeria monocytogenes*, and *Leptospira interrogans* in all regions of Iran is supposed. Besides, some preventive measures such as quarantine, mass vaccination, and biosecurity can help to reduce bovine reproductive infections in dairy farms.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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