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The first study of bovine immunodeficiency virus (BIV) and brucellosis co-infection in west-central Iran

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Abstract:

BACKGROUND: BIV is a well-known bovine immunosuppressive cause, but its pathogenesis has not been well characterized. In recent years, it has been hypothesized that infection with BIV might predispose cattle to be infected by other agents. **OBJECTIVE:** This study was performed to investigate of BIV and Brucella co-infection so that in the future more studies will be done on the issue of predisposing cattle to other microorganisms like Brucella after BIV infection. **METHODS:** Blood samples were collected from a total of 2290 cattle in Iran (490 and 1800 cattle in non-industrial and industrial dairy farms, respectively from Isfahan and Chaharmahal and Bakhtiari provinces). The BIV-positive animals were detected by Lab-ELISA and nested PCR tests. **RESULTS:** In this study, the overall prevalence of BIV in Iran was 1.61% (4.5% and 0.83% in non-industrial and industrial dairy farms, respectively). **CONCLUSIONS:** There was a statistically significant relationship between BIV status and Brucella infection using Chi square and Pearson's correlation coefficient test for all of the samples (p=0.0001, r=0.24), samples from Chaharmahal and Bakhtiari (p=0.044, r=0.13) and from industrial farms in Isfahan (p=0.001, r=0.074).

Introduction

Bovine immunodeficiency virus (BIV) is a lentivirus of the family Retroviridae. BIV infections are lifelong and generally subclinical (Amborski et al., 1989; Belloc et al., 1996). Serological investigations have shown wide distribution with differing prevalence (1.4% to 80%) of BIV infections around the world (Amborski et al., 1989; Belloc et al., 1996; Baron et al., 1998; Burkala et al., 1999). Serologic evidence for BIV infection has been reported in many countries around the world such as the Netherlands, France, Japan, Canada, Australia, Brazil, Costa Rica, Venezuela, and Turkey (Baron et al., 1998; Gonda et al., 1987; Gonda et al., 1994; Horzinek et al., 1991; McNab et al., 2010; Meas et al., 2003; Polack et al., 1996; Usui et al., 2003). The impact of BIV is controversial due to the difficulty in culturing new isolates in vitro and the complexity in identifying BIV-infected animals (Evermann et al., 1997; Gradil et al., 1999; Lew et al., 2004). Although several pathological changes, including monocyte dysfunction, encephalopathy, lymphadenopathy, and immunodeficiency have been reported in BIV-infected cattle, the detailed pathogenesis of BIV-infected cattle remains unclear (Burkala et al., 1999; Carpenter et al., 1992; Cyrcoats et al., 1994;Esmaeili et al., 2011; Evermann et al., 1997; Gonda et al., 1994). There is evidence that BIV can cause immunosuppression with increased incidence of secondary bacterial infections and encephalitis with high seroprevalences (Gonda et al., 1994). Following experimental infections, cattle may have transient increases in lymphocytes, lymphoid hyperplasia, atypical lymphosarcoma, and secondary bacterial infections such as *Mycobacterium bovis* (Yilmaz et al., 2008; Rola et al., 2011). Even though the virus has not been linked to any specific disease condition in cattle, it certainly can aggravate several illnesses in the animals, including impairment of the immune system (Carpenter et al., 1992). BIV seropositivity is associated with decreased milk production in dairy cattle, but no direct link has been found to clinical disease in naturally infected cattle (Burkala et al., 1999; Carpenter et al., 1992; Cyrcoats et al., 1994; Yilmaz et al., 2008). However, Walder et al. reported evidence for a possible association between bovine paraplegic syndrome and a viral agent related to BIV (Walder et al., 1995). Snider et al. determined that a herd with high seroprevalence of BIV had a high percentage of cows with encephalitis associated with depression and stupor, alteration of the immune system associated with secondary bacterial infections, and chronic inflammation of the feet and legs (Horzinek et al., 1991; Snider et al., 1996; Orr et al., 2003). The detailed pathogenesis in infected cattle still remains unclear. BIV seropositivity has been shown to be variably associated with decrease in animal production, weight loss, secondary diseases, and diminished milk production (McNab et al., 1994; Gonzalez et al., 2001).

Despite a control program being in place for over 30 years, brucellosis remains endemic in Iran and several Mediterranean countries where it is one of the most important zoonotic diseases (Esmaeili et al., 2011). The Veterinary Organization of Iran uses test and slaughter policy and vaccination against Brucella (Esmaeili, 2014). There are cases of Brucella and tuberculosis co-infection or Brucella and HIV co-infection. Therefore, the management of HIV and tuberculosis or any other potential risk factors may be of great clinical importance in the treatment of brucellosis infection in a brucellosis endemic country like Iran (Karsen et al, 2008; Abdollahi et al, 2010;Cadmus et al, 2008). Brucellosis incidence is influenced by management factors, herd size, population density, type of animal breed, and biological features such as herd immunity (Boukary et al., 2013). BIV infection in cattle may be associated with common bacteria such as *E. coli* and *Salmonella* spp. and may also co-infect with bovine viral diarrhea virus or infectious bovine rhinotracheitis virus or Brucella (Meas et al., 2004). Up to now, studies about HIV and Brucella co-infection have been done (Hajiabdolbaghi et al., 2011), but there aren't any available reports about the co-infection of BIV and Brucella. Systemic brucellosis is characterized by involvement of tissues rich in reticuloendothelial elements and profound activation of cell-mediated immunity. Similar to other zoonotic diseases, Iran is an endemic country for Brucella infection and symptomatic brucellosis. Among the affected populations, HIV-infected patients might be at a greater risk for Brucella infection. The dramatic decline of CD4 marker level in HIV-infected patients predisposes them to organisms that are mostly eradicated via cell-mediated immunity. Therefore, a frequent association could be anticipated within geographical areas in which both brucellosis and HIV are prevalent. In the early 1990s, the possible association between brucellosis and HIV infection was assessed only in a few endemic countries. There have been evaluations of Brucella infection prevalence in hospitalized patients, most of whom were asymptomatic HIV-positive patients with a partially preserved immune system. A few studies suggest that immune reactions are probably crucial for the development of brucellosis from Brucella infection. Hence, this immune response is phenotypically polymorphic in different cases with different immunological states, and the range of clinical manifestations widely varies among patients, so one may assume that brucellosis features are likely correlated with the state of the patient's immune system. Therefore, variable clinical responses to Brucella infection are expected in HIV-positive patients with varying CD4+ levels. However, one may conclude that CD4+ count would be inversely correlated with the severity of brucellosis complications (Hajiabdolbaghi et al., 2011).

The bovine immunodeficiency virus (BIV) and human immunodeficiency virus types 1 and 2 (HIV-1 and -2) are members of the lentivirus genus of retroviruses. Although the DNA sequences of these viruses have diverged considerably, the BIV genome organization, function of structural and regulatory genes, and replication cycle are very similar to that of HIV-1 (Tobin et al., 1996). So far, no reports on the importance of BIV as a predisposing factor for Brucella have been published. However, due to the biological similarities of BIV and HIV, the present study was designed according to the reports of the co-infection of HIV and Brucella. In fact, the purpose of this study was to determine the prevalence of BIV and brucella co-infection in Iranian cattle. Our study adds to the available data on BIV and Brucella and is the first report of BIV-Brucella co-infection in Iran. However, further studies should be done to determine the predisposing effects of BIV infection to other organisms like Brucella.

Materials and Methods

Herd management, blood sampling and DNA extraction: The samples were obtained from dairy industrial and non-industrial farms in Isfahan and Chaharmahal and Bakhtiari. These two provinces are among the regions with moderate incidence of brucellosis (Esmaeili, 2014). Two dairy cattle production systems are described in these areas. One is a system of small independent farms. The herd density is about 5-50 animal per farm with a low technology level and milk production (average milk yield about 3575 Kg/cow/year based on the reports of the local veterinary organization). The second system is the commercial industrial herds which use more advanced technology with average milk production from about 4300 to 7900 Kg/cow/year. The cow population of the tested industrial herd was 1800. All of the cows were Holstein breed. They were housed in an intensive system and were kept in individual boxes. They fed on milk, concentrate, and alfalfa. About 95% of the herds had free-stall system. All the animals were immunized against foot and mouth disease and clostridial diseases, and all female cows were vaccinated against brucellosis. All of the herds used artificial insemination. Nutrition and reproduction management of the herds were controlled using computerized herd health management.

Sera were isolated from 2990 peripheral blood samples (1800 cattle from an industrial dairy province in Isfahan and 490 cattle of 84 non- industrial dairy farms, in Isfahan (n=46) and Chaharmahal and Bakhtiari (n=38) areas of Iran) from 2008 to 2009. The sera were stored at -20 ºC until future use. Serum samples were analyzed to detect antibodies against BIV using Lab-ELISA as described by Scobie et al (1999). For PCR assay, 45 blood samples with EDTA were obtained from seropositive and seronegative dairy cows and genomic DNA was extracted from PBMC using the DNA isolation kit for mammalian blood (Roche Applied Science Co., Germany C. no:11 667 327 001) according to the manufacturer's directions within 48 h.

Labeled Avidin-Biotin enzyme-linked immunosorbent assay (Lab- ELISA): Serological analysis was performed on 2290 serum samples using a synthetic peptide derived from the available sequence of the transmembrane (TM) glycoprotein of BIV-FL112, produced at the Veterinary Laboratories Agency, Surrey, UK (Scobie et al., 1999). The detection of antibodies against this TM peptide was performed under the following conditions: a volume of 100 µl peptide (12 µg/ml in 0.05 M carbonate-bicarbonate buffer, pH 9.6) was added to each well of a microtitre plate (Immulon 2 HB) and the plates were incubated overnight at 4 ºC. The wells were washed three times with 200 µl TBS-T (138 mM NaCl, 2.6 mM of KCl, 24.8 mM Tris-Cl, 1% Tween-20, pH 7.5) and blocked with dried milk powder (2%) and goat serum (20%) in TBS-T for 1 h at room temperature. Following three washes with TBS-T, 100 µl aliquots of bovine sera diluted 1:10 in TBS-T and were incubated for 1 h at room temperature. After three additional washes, mouse monoclonal anti- bovine immunoglobulin antibody linked to Biotin diluted 1:7000 in TBS-T with 1% of non-fat milk was added to each well and incubated 1 h at room temperature. After three washes with TBS-T, alkaline-labeled streptavidin linked to antibody diluted 1:900 with TBS-T using non-fat milk was added to each well and incubated 1 h at room temperature. After three washes with TBS-T, the phosphatase reaction was visualized with Phosphate substrate tablets (Sigma-Aldrich Chemical Co., St. Louis, MO, USA, C. no:047-8203) and the optical densities (OD) were determined at 405 nm. A ratio of sample to positive control (S/P) was calculated based on the positive and negative control sera included in each plate. Samples with S/P ratios greater than 0.1 were considered positive to BIV.

Serology tests to detect Brucella antibodies: In this study, serologic tests for Brucella were conducted by the veterinary organizations in Isfahan and Chaharmahal and Bakhtiari areas. In brief, sera were initially tested using the Rose Bengal plate test (RBPT), as described by Alton et al (Alton et al., 1988), using the antigen supplied by the Razi Institute in Tehran. Positive results were confirmed with the standard tube agglutination test (STAT) and the 2-mercaptoethanol test (2ME). The STAT and 2ME tests were performed according to the method of Alton et al. (Alton et al., 1975) using the antigen supplied by the Razi Institute (Karaj, Iran). For unvaccinated and vaccinated (RB51) animals, 2ME and STAT titers were calculated and interpreted according to the Veterinary Organization of Iran's instructions (Esmaeili et al., 2012).

Nested PCR assay: Nested PCR was performed in order to detect the BIV proviral DNA 27. The first amplification was performed using a pair of outer primers specific to the BIV pol region (p01: 50-ATGCTAATGGATTTAGGGA-30 and p36: 50-CATTTCTTGGGTGTGAGCTC-30) to amplify a 490 bp fragment. The second amplification was performed to amplify a 176 bp fragment, using a pair of inner primers from the pol region (p02: 50-CATCCTTGTGGTAGAACATT-30 and p37: 50- CCTTACCCTCCAGGAATTAA-30). Briefly, PCR was performed as follows: final concentrations in the reaction mixes were 1X Taq polymerase buffer (PromegaCorp., Madison, WI, USA), 3 mM MgCl2, 200 µM dNTPs, 20 pmol of each primer, 1.25U Taq polymerase and 0.5 µg of genomic DNA, in a total volume of 50 µl. The thermal cycling conditions for the first round of amplification were 1 cycle for 2 min at 94 ºC, 15 s at 51 ºC and 2 min at 72 ºC, then 30 cycles of 45 s at 94 ºC, 15 s at 51 ºC and 10 min at 72 ºC, and a final extension step of 10 min at 72 ºC. Two microliter of the first round reaction was used in the second reaction. The thermal cycling conditions for the second round of amplification were 1 cycle for 2 min at 94 ºC, 15 s at 61 ºC and 1 min at 72 ºC, then 30 cycles of 45 s at 94 ºC, 15 s at 61 ºC and 10 min at 72 ºC, with a final extension step of 10 min at 72 ºC. 6 microliter of the amplified products were loaded on a 1.3% agarose gel, and visualized by staining with ethidium bromide. A BIV-DNA positive control (obtained from the Veterinary Laboratories Agency, Surrey, UK) that originated from the PBMC of a calf experimentally infected with the BIV FL-112 strain was included in each analysis. The DNA for BIV-negative control was obtained from BIV-negative animals. Additionally, a water only negative control reaction was included in each reaction.

DNA sequencing: 6 PCR products were sequenced, and for this purpose, additional specific PCR products were generated using a nested amplification of the proviral pol gene by our coworkers in VLA (UK). Reactions were carried out in 50μl volumes, containing: 200μM each dNTP, 1X Promega Taq polymerase buffer (50mM KCl, 10mM Tris-HCl pH 8.8, 2.5mM MgCl2, 0.1% Trtion X-100, 2μg/ml gelatin), 0.75pM of each primer, and 0.25u of Taq DNA polymerase (Promega). The first round cycling conditions were: 94ºC 2 min, 53ºC for 20 s, 72ºC for 2 min, followed by 36 cycles of 94ºC for 45 s, 53ºC for 20 s, and 72ºC for 1 min and a final extension step of 72ºC for 10 min. The outer primer pair sequences were: P3: 5'-GAA-CGG-GAA-GAT-GGA-GGA-TGT-3', and P38: 5'-GTT-AAG-GGG-TAT-AGA-GGG-ATT-TTT-3'. The nested reaction was carried out in 50μl volumes, using 1μl of the first round product as template, and with 200μM each dNTP, 1X Promega Taq polymerase buffer (50mM KCl, 10mM Tris-HCl pH 8.8, 3mM MgCl2, 0.1% Triton X-100, 2μg/ml gelatin), 1pM of each primer, and 0.25u of Taq DNA polymerase (Promega). The second round cycling conditions were: 94ºC 2 min, 51ºC 20 s, 72ºC 2 min, followed by 36 cycles of 94ºC 45 s, 51ºC 15 s, and 72ºC 1 min, with a final extension step of 72ºC 10 min. The inner primer pair sequences used were: P01: 5'-ATG-CTA-ATG-GAT-TTT-AGG-GA-3', and P36: 5'-CAT-TTC-TTG-GGT-GTG-AGC-TC-3'. The specific products from the second round (491 base pairs) were then sequenced in an automated fluorescent dideoxy sequencing system using the ABI Prism sequencing kit (ABI), with both original internal amplification primers. Sequence data were edited and analyzed with SeqMan Pro version 8.0.2 and MegAlign version 8.0.2 software (DNASTAR, Lasergene).

Statistical analysis: The results were analyzed using ANOVA, chi square, Pearson's correlation coefficient, and T-tests using SPSS software v.16.

Results

Lab- ELISA: Totally, 37 (1.61%) samples were positive for BIV antibodies. 22 / 490 (4.49%) of samples (from 84 non-industrial dairy farms) and 15 / 1800 (0.83%) of samples (from an industrial dairy farm) were BIV seropositive.

S/P ratios ranged from 0.70 to 1.86. 17 out of 84 non-industrial farms (20.2%) were BIV-positive. The age distribution of BIV seropositive animals was determined (Table 2), with 1.6% of animals less than two years of age (1-2), 7.5% of animals between 2 and 4 years of age, 5.9% of animals between 4 and 6 years, and 2.6% of animals between 6 and 8 years of age were found positive. The majority of BIV seropositive animals (77.3%) were found to be between 2 and 4 years old. For the Isfahan area, the overall seroprevalence of BIV was 1.12% (3.3% in the non- industrial farms and 0.83% in the industrial dairy farm) while for the Chaharmahal and Bakhtiari area it was 5.7 % in the non-industrial farms (see tables 3 and 4).

For the BIV S/P ratios, a 2-way analysis of variance (ANOVA) with the two factors of location and the Brucella status (negative, low, high) was performed. The Brucella status was not significant (p=0.391) but location was (p=0.034). Esfahan (SD= 1.26, SE= 0.11) was on the average slightly higher than Shahrekord (SD= 0.92, SE = 0.09).

Co-infection and statistics: Of the BIV seronegative animals (n=2253) 0.53% were sero-

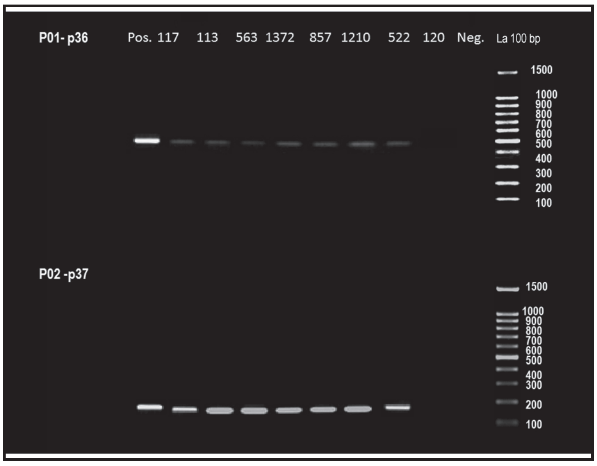

Figure 1. The picture of the agarose gel for 7 BIV-positive in nested PCR. Top row: The first amplification using a pair of outer primers specific to the BIV pol region (p01and p36) to amplify a 490 bp fragment. (Gene ruler is 100 bp). Bottom row: The second amplification to amplify a 176 bp fragment, using a pair of inner primers from the pol region (p02 and p37). (Gene ruler is 100 bp).

positive for Brucella, while 32.43% of the BIV seropositive animals (n=37) were seropositive (see tables 3 and 4). In this study, there was a statistically significant relationship between the BIV status and the Brucella status for all of the samples (p=0.0001, r=0.24), sera from Chaharmahal and Bakhtiari (p=0.044, r=0.13) and samples from industrial farms in Isfahan (p=0.001, r=0.074) using Chi square and Pearson's correlation coefficient test. The highest co- infection rate of BIV_Brucella was 9.1%, in cattle from Chaharmahal and Bakhtiari, but it was not statistically significant. Tables 2 and 3 show the results.

Nested PCR assay: Of the blood samples from BIV seropositive animals (n=37) and BIV seronegative animals (n=8), all samples (1.61%) were positive in the nested PCR test and their PCR products were in the same size of BIV positive control (some of these results have been shown in Figure 1).

DNA sequencing: The 451 nucleotide pol gene fragments sequenced from six animals were compared to the published data for BIV isolates R29 (ac. no: NC001413.1), FL112 (ac. no: L06524.1), FL491 (ac. no: L06525.1), and to each other. The sequences from five (accession numbers: KT281111, KT281112,KT281113, KT281114, KT281115) of the Iranian animals were identical, while that from the sixth (KT281116) varied in three nucleotide positions: 4- A/T, 412- C/T, and 430- C/G. The five Iranian sequences (1, 2, 7, 8, and 9) were 100% similar to the R29 isolate, 99.3% similar to the sixth Iranian sample (6), and 91.1% similar to isolates FL112 and FL491.

The translated amino acid sequences (150 amino acids) were also analyzed. There were 2 substitutions between the sequences from sample 6 and the other five, in the following positions: 1- Phe/Leu and 143- Asp/Glu.

Discussion

Serological survey is an important method to determine the distribution of BIV on livestock, and data on BIV seropositive animals may contribute to the awareness of the worldwide prevalence of the disease.

Currently, there is no gold standard (a completely accurate test) to detect BIV infection (Orr et al., 2003; Suarez et al., 1995; Nash et al., 1995) and one of the difficulties in assessing the role of BIV in predisposing cows to bovine infections or disorders is inconsistency with the methods used to detect infected cattle. Variations in infection prevalence might be influenced by the type of assays used for BIV detection. Substantial misclassification of infection would be expected in epidemiological studies of BIV regardless of which assay was used (Orr et al., 2003).

The culture of the virus is difficult and as expected, the virus could be isolated only within a short time frame. Although the use of PCR for the detection of BIV proved to be more sensitive than either serologic testing or virus isolation, the genetic variation (7-8% nucleotide divergence in the conserved pol segment) of field isolates probably plays a negative role in the results of these diagnostic tests (Meas et

Table 1. BIV seropositive cattle in Isfahan and Chaharmahal and Bakhtiari farms using Lab- ELISA.

	Non-industrial farms		**Industrial farms**		**Total**	
	No.	BIV seropositive	No.	BIV seropositive	No.	BIV seropositive
Isfahan	248	8 (3.3%)	1800	15 (0.83%)	2048	23 (1.12%)
Chaharmahal and Bakhtiari	242	14 (5.7%)	0	0(0%)	242	14 (5.7%)
Total	490	22 (4.5%)	1800	15 (0.83%)	2290	37 (1.61%)

Table 2. A) Co-infection of Brucella and BIV in cattle of different ages in non-industrial farms: (*)Brucella considered positive when serum titre ≥ 80. (**) In this study, the serum samples were randomly collected but some of them were chosen from among the dairy cattle with previous or current Brucella infection.

Age (years)	**BIV+ Brucella+***	**BIV+ Brucella-**	**BIV- Brucella+***	**BIV- Brucella-**	**Total**
1-2	0	2	54	65	121
2-4	7	2	50	61	120
4-6	5	3	57	70	135
6-8	0	3	52	59	114
Total	12	10	213 **	255	490

B) BIV infection in cattle of different ages in non-industrial farms, by area:

	BIV Seropositive			**BIV Seronegative.**
Age (years)	**Isfahan**	**Chaharmahal and Bakhtiari**	**Total**	**Total**
<2	0	2	2 (1.6%)	121
2-4	4	5	9 (7.5%)	120
4-6	4	4	8 (5.9%)	135
6-8	0	3	3 (2.6%)	114
Total	8	14	22 (4.5%)	490

al., 2003). It has been shown that nested PCR is 80% sensitive and 85% specific18, so we confirmed the result of PCR assay by sequencing. Earlier studies such as a study performed by Gonzalez et al. (2001) and Lew et al. (2004) indicated discordance between the serological (ID, ELISA-i, WB, PCR) and the genomic detection of BIV (Lew et al., 2004), with the genomic detection by polymerase chain reaction showing greater sensitivity and specificity. Gonzalez et al. (2001) have provided evidence that their nested PCR has a greater sensitivity than other published methods. In this study, we found a relative descriptive consistency between the serological and the genomic detection of BIV results. However, with only 37 seropositive and 8 seronegative PCR tests, it is not possible to prove any coordination between serological and molecular results definitively and more analysis is needed.

ELISA tests are, in general, relatively accurate and have been considered highly sensitive and specific in detecting specific antibodies. Therefore, Lab-ELISA based on BIV-TM peptide should also be presented as a confirmatory test for BIV because of the high sensitivity of streptavidin to biotin. We propose here that the Lab-ELISA based on recombinant viral antigen or synthetic peptides, as used in this work, should decrease the number of false positive reactions which occur when the serum reacts with non-relevant proteins and could be recommended as a diagnosis test to detect BIV seropositive animals.

BIV Proviral DNA is detectable in PBMC during the early stage of infection (from 4-60 days with peak titers 20 dpi). There is a transient viraemia from 4 to 14 dpi. An antibody

Table 3.Co-infection of Brucella and BIV in Isfahan and Chaharmahal and Bakhtiari areas of Iran. (*) - Brucella seropositive titre defined as >80.

	BIV seronegtive		BIV seropositive		Total	
	.No	Brucella* seropositive	.No	*Brucella seropositive	.No	*Brucella seropositive
Isfahan	2025	113 (5.58%)	23	2(8.7%)	2048	115 (5.61%)
Chaharmahal and Bakhtiari	228	100 (43.85%)	14	10(71.42%)	242	110 (45.45%)
Total	2253	213 (9.45%)	37	12(32.43%)	2290	225 (9.82%)

Table 4. Co-infection of Brucella and BIV in Isfahan industrial and non-industrial farms. (*) - Brucella seropositive titre defined as >80.

	BIV seronegtive samples		BIV seropositive samples		Total	
	No.	Brucella seropositive*	No.	Brucella seropositive*	No.	Brucella seropositive*
(Non-industrial farms)	240	(47.08%) 113	8	(% 25) 2	248	(46.37%) 115
(Industrial farms)	1785	(0%) 0	15	(0%)0	1800	(0%) 0
Total	2025	(5.58%) 113	23	(8.7%) 2	2048	(5.61%) 115

response to the TM glycoprotein commences 12 dpi with peak titers 10-30 wpi, and its response is detectable until 50 weeks post infection. Thus, it is suitable for the long term monitoring of the infection. An antibody response to the CA protein is detected not until 34 dpi. So a CA-based serological assay would not identify the majority of infected cattle (McNab et al., 2010).

Due to the persistent nature of the anti-TM antibody response in BIV infections (Scobie et al., 1999), the TM glycoprotein may also be a promising linear antigenic target and may, therefore, yield a potential antigen for inclusion in a differential serological assay. The inconsistency between previous serological and molecular assays may be because of the nature of antigen or antibody included in each serological assay or the time that blood sampling and tests have been done. In this study, we detected BIV-positive cows by Lab-Elisa and nested PCR within one month. Therefore, the consistency between the results of these two tests isn't unlikely. Of course, according to just 45 PCR results for 37 seropositive samples and 8 seronegative samples, it cannot definitively be concluded that both serological and molecular tests have consistency.

If there are PCR results for seronegative samples, they should be clarified. Previously, the presence of BIV infection in the dairy cattle of industrial farms in Iran was reported by Nikbakht Borujeni et al. (Nikbakht Borujeniet al., 2010) and Tajbakhsh et al. (Tajbakhsh et al., 2010) and the BIV prevalence in these studies was 20.3% and 60% respectively, which are far larger than the world average (4-6%), but their BIV prevalence results are in the range. Sero-prevalence rates of BIV which have been reported worldwide are between 1.4% and 64%, but mostly in the range of 4% to 6%. In total, BIV prevalence varies widely worldwide (Belloc et al. 1996; Kurth et al. 2010). As described, BIV Proviral DNA is detectable in a short time during the infection, so it isn't possible to detect such a high prevalence with using only PCR. The prevalence rate varied in different dairy herds and the higher prevalence in some dairy cattle may be the result of herd management practices and of the extended productive life. In the previous studies on the BIV prevalence in Iran, the state of farms has not been well described. Therefore, large-scale serological and molecular studies with detailed long-term epidemiological observation of BIV incidences will be necessary to confirm these findings (McNab et al., 2010).

In this study, the serum samples were randomly collected, but some of them were chosen from among the dairy cattle with previous or current Brucella infection. The overall prevalence of BIV in west-central Iran in this survey was 1.61%. Out of 490 animals from 84 non-industrial dairy farms, 22 were positive (4.5%). The difference between BIV prevalence in these types of farms is mainly due to the herd management and flock density.

The results presented here give an accurate new estimate of BIV prevalence in west-central Iran. In the present study, a seroepidemiological survey of BIV and Brucellosis was performed to determine a correlation between BIV and Brucellosis infections. It has been hypothesized that infection with BIV, and potential consequent immunosuppression, might predispose cattle to infection by other agents such as Brucella or might affect their response to vaccination (Tajbakhsh et al., 2010). For example, immunosuppression with a delayed IgG response to B. abortus has been described for the closely related Jembrana disease virus in Bali cattle34. The p value for chi square and the Pearson's correlation coefficient test (p=0.0001, r=0.24) provide evidence of association between BIV and Brucellosis. Thus, in this study, statistically significant evidence has been found for the correlation of BIV and Brucella infection rates. However, the probability that BIV infection predispose the animals to infection with Brucellosis needs to be confirmed with further tests such as analysis and monitoring of brucellosis development in experimentally BIV-infected cows and comparing the findings with the results of the same test in an equal BIV-negative control group. Co-infection may simply represent prior exposure to both microorganisms and not in itself indicate any biological association and/or probable synergisms.

Seroepidemiological studies of BIV infections in cattle have been reported in many countries (Nikbakht Borujeniet al., 2010). Despite the worldwide distribution of BIV infection, whether the presence of BIV in a host leads to primarily pathologic changes or can cause secondary bacterial and/or viral infections as a predisposition factor has not been fully elucidated. In the natural infection with BIV, the host is affected differently compared to what has been observed in experimental infection. The presence of BIV combined with the stresses associated with parturition and a modern dairy production system was considered causal for the development of secondary diseases in immunocompromised cattle. In the previous studies, in BIV-infected animals the secondary disease processes and their incidence were reported (Yilmaz et al., 2008).

In conclusion, the seroprevalence of BIV in industrial and non-industrial dairy cattle herds in Iran was 0.83% and 4.5%, respectively. In this study, the overall BIV seroprevalence in Iran was 1.61% and we found that there is a relatively significant relationship between BIV and Brucellosis. Further studies should be designed to investigate the pathogenic and biological properties of local field isolate strains of the virus, and these strains should be included in the assays chosen to detect BIV antibodies.

Studies such as the present work have some limitations. First, it is difficult to know whether the disorders observed were due to BIV infection alone, because of the fact that BIV-positive cattle were not further analyzed for other infectious agents like viruses or bacteria that may play a role in that kind of clinical disorder. Second, it is difficult to select uniform patient and control populations in animal studies. Therefore, control animals were selected from among BIV-serone B) BIV infection in cattle of different ages in non-industrial farms, by area: gative cattle from the same herds including BIV-positive animals, because of the fact that some factors, i.e. climate, magnitude of farm, and management, are well known to affect the health status of dairy cattle. Third, the number of lactations could have been recorded in the present study. The fact that there was no significant difference in age between the groups may have minimized the effects of lactation on differences between them (Yilmaz et al., 2008; Nikbakht Borujeniet al., 2010; Tajbakhsh et al., 2010). These findings suggest that the presence of BIV infections should be considered a health risk for cattle populations, and may have a role in predisposing cattle to infections with other pathogenic microorganisms. Further studies in a larger patient population are required to verify these observations.

Our study adds to the available data on BIV in Iran. Further studies are needed to determine the epidemiology of infections in Iran, and local farmers need to be informed of the health risks these infections pose to their animals. In addition, it cannot be said that the prevalence of Brucella infection is only due to BIV, because there are certainly numerous risk factors of Brucellosis, and our study has proposed BIV as a potential new risk factor of Brucella infection.

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مجله طب دامی ایران، ۱۳۹۵، دوره ۱۰، شماره ۱، ۶۴-۵۳

اولین مطالعه عفونت توأم ویروس نقصان ایمنی گاو و بروسلوز در نواحی مرکزی و غربی ایران

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چکیده

زمینه مطالعه: ویروس نقصان ایمنی گاو یکی از اجرام بیماریزای شناخته شده به عنوان یک عامل سرکوب کننده سیستم ایمنی گاو است اما روند بیماریزایی آن به خوبی مشخص نشده است. در سال‌های اخیر، این فرضیه مطرح شده است که عفونت با BIV گاو را به آلوده شدن به سایر عوامل بیماریزا مستعد می‌نماید. **هدف:** هدف از این مطالعه جستجوی عفونت توأم BIV و بروسلا است تا در آینده مطالعات بیشتری در مورد مستعد شدن گاو به ابتلا به سایر میکروارگانیسم‌ها نظیر بروسلا پس از عفونت با BIV انجام شود. **روش کار:** تعداد ۲۲۹۰ نمونه خون گاو از فارم‌های غیر صنعتی (n=۴۹۰) و صنعتی (n=۱۸۰۰) پرورش گاو شیری واقع در استان‌های چهار محال و بختیاری و اصفهان، ایران، اخذ گردید. حیوانات آلوده به BIV با آزمون‌های Lab- ELISA و nested PCR شناسایی شدند. **نتایج:** میزان کلی شیوع BIV، ۱/۶۱٪ محاسبه شد (۵/۷٪ و ۰/۸۳٪ به ترتیب در فارم‌های غیرصنعتی و صنعتی). **نتیجه‌گیری نهایی:** با استفاده از آزمون‌های آماری مربع کای و ضریب همبستگی پیرسون ارتباط معنی‌داری بین وضعیت آلودگی به BIV و بروسلوز در مورد کل نمونه‌ها (r=۰/۲۴، p=۰/۰۰۰۱) و نمونه‌های مربوط به چهارمحال و بختیاری (p=۰/۱۳، p=۰/۰۴۴) و در فارم‌های صنعتی اصفهان (p=۰/۰۷۴، p=۰/۰۰۱) دیده شد.

واژه های کلیدی: ویروس نقصان ایمنی گاو، بروسلا، شیوع سرمی

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