

## Serological study of bovine viral respiratory diseases in dairy herds in Kerman province, Iran

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### Summary

Respiratory disorders are major concern for dairy cattle industry. Viruses and bacteria in combination with stress play a key role in triggering acute respiratory infections. The most important viral agents are bovine viral diarrhoea virus (BVDV), bovine herpes virus type 1 (BHV-1), bovine respiratory syncytial virus (BRSV), parainfluenza virus type 3 (PIV-3) and bovine adenovirus (BAV). This cross-sectional study was conducted to evaluate the serological status of BVDV, BHV-1, BRSV, PIV-3 and BAV in dairy herds in Kerman province, Iran. From June to November 2007, 181 serum samples were collected from 1–3-year-old cattle from 15 industrial dairy farms in Kerman province using cluster sampling. The samples were tested by commercial indirect ELISA kits. Antibodies were detected against BVDV, BHV-1, BRSV, PIV-3 and BAV in 77.90, 30.39, 100, 100 and 100% of serum samples, respectively. All farms were positive at least for one of these viruses and antibodies against all the 5 viruses were detected in 4 (26.66%) herds among 15 dairy farms. According to the present study, BVDV, BRSV, PIV-3 and BAV are common viruses in dairy herds in Kerman province.

**Key words:** Bovine viral respiratory diseases, ELISA, Serology, Iran

### Introduction

Respiratory disorders are major concern for bovidae. They occur in all countries that practice intensive livestock farming. Bovine respiratory diseases (BRD) complex is a major cause of economic losses in the dairy cattle industry. Viruses and bacteria in combination with stress play a key role in triggering acute respiratory infections. It is generally accepted that viruses are the first pathogens to intervene, whereas bacteria act as the second invaders to worsen the ill-animal's condition (Valarcher and Hägglund, 2006; Solís-Calderón *et al.*, 2007).

The most important viral agents are bovine viral diarrhoea virus (BVDV), bovine herpes virus type 1 (BHV-1), the causative agent of infectious bovine rhinotracheitis (IBR), bovine respiratory syncytial virus (BRSV), parainfluenza virus type 3 (PIV-3) and bovine adenovirus

(BAV) (Hägglund *et al.*, 2007).

Four viral pathogens, BVDV, BHV-1, BRSV and PIV-3 are mainly associated with bovine respiratory disease. These agents cause severe disruption of the respiratory tract and are associated with shipping fever in growing cattle, as well as weaned and transported calves to feedlots for finishing. Moreover, BHV-1 and BVDV can suppress the immune system of the host and increase the risk of secondary bacterial infections and/or mycoplasmas outbreaks of respiratory diseases (Valarcher and Hägglund, 2006).

Bovine viral diarrhoea virus (BVDV) is one of the most important pathogens of cattle with worldwide distribution (Houe, 1995). Serosurveys of cattle from different countries indicate that the prevalence of antibodies to BVDV may range from 40 to 90% (Houe, 1995; Kirkland, 1996).

Infectious bovine rhinotracheitis (IBR) is caused by BHV-1, and is considered to

have a worldwide distribution. Bovine herpes virus type 1 can become latent in the trigeminal ganglia and tonsils. Latency allows the virus to persist, so that the introduction of a carrier into a non-infected herd is the principle way for the spread of the virus. For these latent infections, positive serology means the animal is potential carrier of the virus (Winkler *et al.*, 2000).

Outbreaks of the respiratory disease involving BRSV have been reported from many countries in Europe, the United States and also from some other parts of the world (Van der Poel *et al.*, 1993). Parainfluenza virus type 3 infections cause less serious disease than BRSV (Verhoeff and van Nieuwstadt, 1984), but are nevertheless significantly correlated with respiratory diseases in cattle (Stott and Thomas, 1980). Several surveys in many countries have revealed that infection with PIV-3 is endemic in both beef and dairy cattle (Obando *et al.*, 1999).

Our objective in this cross-sectional study is detection of antibodies against BVDV, BHV-1, BRSV, PIV-3 and BAV in dairy herds in Kerman province, Iran.

## Materials and Methods

### Animals and herds

One hundred and eighty-one serum samples were collected from 1–3-year-old cattle originating from 15 industrial dairy farms in Kerman province, Iran. There are about 20 large industrial dairy farms in this province and in the present study larger herds were selected. We carried out this cross-sectional study, with a random cluster sampling design, from June to November 2007. Between 10 to 15 samples per herds were collected based on herd population. None of the cattle herds in this study were vaccinated against BVDV, BHV-1, BRSV and PIV-3.

### Sample collection and processing

Blood samples (5 ml) were collected aseptically from coccygeal vein of each animal using anticoagulant free vacutainer tubes and transported on ice to the laboratory. Serum was separated by centrifugation of blood at 3000 g for 10 min

at room temperature, the aliquots were transferred into 1.5 µl sterile microtube (Eppendorf), and were kept at -20°C until analysis. These samples were submitted to Microbiology Laboratory of Veterinary Medicine Faculty, University of Kerman.

### Serological tests

Commercial indirect respiratory ELISA kits (Pentakit) developed by Bio-X Diagnostics, Belgium, were used to determine the presence of antibodies to BVDV, BHV-1, BRSV, PIV-3 and BAV. Microtiter plates coated with the respective viral antigens were used according to the manufacturer's instructions. The optical density (OD) was measured at 450 nm with an ELISA Reader (Anthus labtec instrument).

Serum samples were diluted in PBS (1:100) and 100 µl volumes were dispensed into each well, incubated at 37°C for one h (all samples and controls were tested in duplicate) and then rinsed 3 times in washing buffer. Conjugate solution was diluted (1:50) and 100 µl volumes were dispensed into each well, incubated at 37°C for one h, and then washed 4 times in washing buffer. For each plate, 500 µl of chromogen was added to 9.5 ml of the substrate solution and mixed thoroughly, then 100 µl of this substrate solution were dispensed into each well, incubated in the dark at room temperature for 10 min. The reaction was stopped by addition of 50 µl of stop solution, and the OD produced in each well was measured at 450 nm using an ELISA Reader (Anthus labtec instrument) and recorded using a computer. The OD accepted for each sample was determined by subtracting the negative control from the average results of the duplicate samples.

## Results

Fifty five (30.39%) and 141 sera (77.90%) were positive for BHV-1 and BVDV, respectively and positive results for BRSV, PIV-3 and BAV were 100%.

Antibodies against all of 5 viruses were detected in 45 sera (24.86%) among 181 samples. Positive results for BRSV, PIV-3, BAV and BVDV were 53.04% and

corresponding measure for BRSV, PIV-3, BAV and BHV-1 was 5.52%.

As Table 1 shows, antibodies against all five viruses were detected in 4 (26.66%) out of 15 dairy farms. There was no seronegative farm for all five viruses. Eleven (73.33%) and five (33.33%) herds were seropositive for BVDV and BHV-1, respectively and all the herds (100%) were seropositive for BRSV, PIV-3 and BAV.

**Table 1: Frequency distribution of seropositive herds among 15 large industrial dairy farms in Kerman province**

Virus	Number of seropositive herds (%)
Bovine respiratory syncytial virus	15 (100)
Parainfluenza virus type 3	15 (100)
Bovine adenovirus	15 (100)
Bovine viral diarrhoea virus	11 (73.33)
Bovine herpes virus type 1	5 (33.33)
All 5 viruses	4 (26.66)

## Discussion

The results of the present study demonstrate that the prevalence of antibodies to BVDV, BRSV, BAV and PIV-3 in dairy farms in Kerman province is approximately similar to those reported in some other parts of the world (Welty, 1994).

Recent study in the central area of Chile dairy farms showed that the prevalence of BVDV has been about 71.2 to 83% (Meléndez and Donovan, 2003). The results of a serological survey for BVDV antibodies on a collection of 1295 serum samples of 6–12-month-old cattle from 45 farms in Slovakia showed that the average prevalence of BVDV positive samples was around 70% (Vilcek *et al.*, 2003). The prevalence of BVDV antibodies in 18 selected South African dairy herds varied from 79.85 to 100% (Ferreira *et al.*, 2000). The previous study on BVDV prevalence by the detection of antibodies in bulk-tank milk samples in Kerman province, Iran revealed that 89.24% of dairy farms were positive (Khalili *et al.*, 2007).

Serological studies on BVDV in Iran indicated the seroprevalences of 51.75% in Ahvaz (Haji Hajikolaei and Shapouri, 2007), 23.32% in Shahrekord (Hemmat Zadeh *et al.*, 2001), 11.1% in Mashhad (Talebkhan Garoussi *et al.*, 2007) and 51.58% in Tehran (Karegar *et al.*, 1995).

BVDV is endemic in the most cattle-raising countries, and between 60 to 90% of adult animals are antibody-positive (Baker, 1987). However, great regional difference in prevalence exists. The variation in the cattle-population size and herd management can account for such differences (Kirkland, 1996). Nowadays, successful test-and-slaughter control and eradication of BVDV programs are under way in the Scandinavian countries (Alenius *et al.*, 1996). There is no program for control and eradication of BVDV in dairy herds in Iran. Vaccination may be a useful tool to reduce losses by protecting naive cattle against BVDV infection. For disease control, it is necessary to cull the animals with persistent infection and to prevent BVDV new entry.

The results of studies in the Republic of Croatia showed that the prevalence of antibodies to BHV-1 and BVDV in dairy cows on 4 different farms was 85.8 and 79.2%, respectively, and antibodies to both viruses were found in 80.8% of cows with reproductive disorders but only in 46.8% of cows without reproductive disorders (Biuk-Rudan *et al.*, 1999). A survey performed on dairy cattle from Portuguesa reported a seroprevalence of approximately 53% for BHV-1 (Obando *et al.*, 1983).

A similar survey performed in the USA on American bison (*Bison bison*) bulls for detection of antibodies to BVDV, BHV-1, and BRSV reported that detectable antibodies were found against all viruses; 55.3% against BVDV, 43.8% against BHV-1 and 92% against BRSV. These data indicate that a high percentage of bison sampled are seropositive for BVDV, BHV-1 and BRSV (Sausker and Dyer, 2002).

The prevalence of BHV-1 seropositive cows may reflect the proportion of BHV-1 carriers because after a primary infection, the virus stays latent in neural ganglions that innervate genital or respiratory mucosae and may be re-excreted upon immunosuppressive stimuli, such as corticosteroid injection or stress after shipment, calving and etc. The immunity against BHV-1 has no direct effect on the latency state and it modulates the re-excretion of the virus (Pastoret and Thiry, 1985). For these latent infections, positive serology means that the animal is a potential carrier of the virus

(Winkler *et al.*, 2000).

Recent study for detection of antibodies against BRSV and PIV-3 in beef cattle of Yucantan, Mexico showed that seroprevalences were 90.8 and 85.6% for BRSV and PIV-3, respectively (Solís-Calderón *et al.*, 2007). A serological survey on bovine respiratory syncytial virus in Chahar Mahal Bakhtiary province (Iran) showed the infection rate of 80.98% (Tajbakhsh and Momtaz, 2003).

Viruses such as bovine respiratory syncytial virus and bovine herpesvirus type 1 sometimes cause severe disease as single agents, also they can predispose the animal to bacterial infections of the lung (Valarcher and Hägglund, 2006). The high prevalence indicates that most adult cattle have been exposed to BRSV. It is considered that antigenic variation of the subgroups of BRSV is possible and might affect virulence and immunogenicity. However, a chronic carrier state for BRSV in cattle also has been suggested (Elvander, 1996).

The high seroprevalence of PIV-3 virus found in this study is in agreement with the ubiquitous nature of the virus, with its world-wide distribution (Bryson, 1990). Because no vaccines are used in Iran against these 5 viruses, the presence of antibodies indicates that exposure to these agents is common in the region. This is probably due to the lack of control measures against these infections.

According to the findings of the present study, BVDV, PIV-3, BAV and BRSV are common viruses in large industrial dairy herds in Kerman province, Iran. Larger scale studies which will enable more information to be gathered about these agents are therefore warranted.

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## References

Alenius, S; Lindberg, A and Larsson, B (1996).

A national approach to the control of bovine viral diarrhea virus. In: Edwards, S; Paton, DJ and Wensvoort, G (Eds.), *Proceedings of the third ESVV symposium on pestivirus infections*. Lelystad, Netherlands. PP: 162-169.

Baker, JC (1987). Bovine viral diarrhea virus: a review. *J. Am. Vet. Med. Assoc.*, 190: 1449-1458.

Biuk-Rudan, N; Cvetnić, S; Madić, J and Rudan, D (1999). Prevalence of antibodies to IBR and BVD viruses in dairy cows with reproductive disorders. *Theriogenology*. 51: 875-881.

Bryson, DG (1990). Para influenza 3 virus in cattle. In: Dinter, Z and Morein, B (Eds.), *Virus infection in ruminants*. Vol. 3. Elsevier, Amsterdam. PP: 319-333.

Elvander, M (1996). Severe respiratory disease in dairy cows caused by infection with bovine respiratory syncytial virus. *Vet. Rec.*, 138: 101-105.

Ferreira, GM; Lourens, DC and van Vuuren, M (2000). The prevalence of bovine viral diarrhea antibodies in selected South African dairy herds, and control of the disease. *J. South African Vet. Assoc.*, 71: 10-13.

Hägglund, S; Hjort, M; Graham, DA; Ohagen, P; Tornquist, M and Alenius, S (2007). A six-year study on respiratory viral infections in a bull testing facility. *Vet. J.*, 173: 585-593.

Haji Hajikolaie, MR and Seyfi Abad Shapouri, MR (2007). Serological study of bovine diarrhea virus infection of cattle in Ahvaz. *J. Vet. Res.*, 62: 21-26.

Hemmat Zadeh, F; Kojouri, GH; Karegar, P and Rovhani, M (2001). Survey of bovine viral diarrhea in Chaharmahal province, Iran. *J. Vet. Res.*, 56: 85-92.

Houe, H (1995). Epidemiology of bovine viral diarrhea virus. *Vet. Clin. N. Am.: Food Anim. Pract.*, 11: 521-547.

Karegar, P; Hesami, M; Bazargani, T; Gholami, M; Khedmati, K; Ghabousi, B and Jahangiri, MR (1995). Seroprevalence of BVD/MD in cattle farm in Tehran, Iran. *Pajouhesh & Sazandegi*, 28: 112-116 (In persian).

Kirkland, PD (1996). An overview of pestivirus infections in Australia. In: *Proceedings of the international symposium bovine viral diarrhea virus, a 50-year review*. Cornell, USA, Cornell University. PP: 130-132.

Meléndez, P and Donovan, A (2003). Herd-level ELISA seroprevalence of bovine viral diarrhea antibodies in bulk-tank milk in Chilean dairy herds. *Prev. Vet. Med.*, 60: 237-241.

Obando, RC; Hidaigob, M; Merzad, A; Montoyab, B; Klingeborn, J and Moreno-

- LoÁpeza, M (1999). Seroprevalence to bovine virus diarrhoea virus and other viruses of the bovine respiratory complex in Venezuela (Apure State). *Prev. Vet. Med.*, 41: 271-178.
- Obando, C; Pedrique, C and Hidalgo, M (1983). Serological study on infectious bovine rhinotracheitis in Portuguesa state, Venezuela. *Vet. Trop.*, 8: 5-20 (In spanish).
- Pastoret, PP and Thiry, E (1985). Diagnosis and prophylaxis of infectious bovine rhinotracheitis: the role of virus latency. *Comp. Immunol. Microbiol. Infectious Disease.* 8: 35-42.
- Sausker, EA and Dyer, NW (2002). Seroprevalence of OHV-2, BVDV, BHV-1, and BRSV in ranch-raised bison (*Bison bison*). *J. Vet. Diagn. Invest.*, 14: 68-70.
- Solis-Calderón, JJ; Segura-Correa, JC; Aguilar-Romero, F and Segura-Correa, VM (2007). Detection of antibodies and risk factors for infection with bovine respiratory syncytial virus and parainfluenza virus-3 in beef cattle of Yucatan, Mexico. *Prev. Vet. Med.*, 82: 102-110.
- Stott, EJ and Thomas, LH (1980). A survey of virus infections of the respiratory tract of cattle and their association with disease. *J. Hyg.*, 85: 257-270.
- Tajbakhsh, E and Momtaz, H (2003). A serological survey on bovine respiratory syncytial virus (BRSV) in Chahar Mahal Bakhtiary province, Iran. *Pajouhesh & Sazandegi.* 66: 98-103 (In persian).
- Talebkhan Garoussi, M; Bassami, MR and Afshari, SA (2007). Detection of bovine viral diarrhoea virus using a nested RT-PCR assay in bulk milk samples of dairy cattle herds in suburb of Mashhad, Iran. *Iran. J. Biotechnol.*, 5: 52-55.
- Valarcher, JF and Häggglund, S (2006). Viral respiratory infectious in cattle. In: *Proceedings of the 24th World Buiatrics Congress.* Nice, France.
- Van der Poel, WHM; Kramps, JA; Middel, WGJ; Van Oirschot, JT and Brand, A (1993). Dynamics of bovine respiratory syncytial virus infections: a longitudinal epidemiological study in dairy herds. *Arch. Virol.*, 133: 309-321.
- Verhoeff, J and van Nieuwstadt, AP (1984). BRS virus, PI3 virus and BHV1 infections of young stock on self contained dairy farms: epidemiological and clinical findings. *Vet. Rec.*, 114: 288-293.
- Vilcek, S; Mojzisová, J; Bajová, V; Paulík, S; Strojný, L; Durkovic, B and Hipíková, V (2003). A survey for BVDV antibodies in cattle farms in Slovakia and genetic typing of BVDV isolates from imported animals. *Acta Vet. Hung.*, 51: 229-236.
- Welty, VR (1994). *Animal health yearbook.* Food and Agriculture Organization of the United Nations (FAO), Rome. P: 33.
- Winkler, MT; Doster, A and Jones, C (2000). Persistence and reactivation of bovine herpes virus type 1 in the tonsils of latently infected calves. *J. Virol.*, 74: 5337-5346.