

Short Communication

**Serological study of bovine herpesvirus type 1 and
parainfluenza type 3 in cow farms of Qazvin province
based on different ages and seasons**

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ABSTRACT

The main objective of this study was to estimate the seroprevalence of Bovine herpes virus type 1 and parainfluenza type 3 in a non-vaccinated population cattle in the livestock region of Qazvin province. Totally 504 sera were randomly collected and tested from 8 industrial dairy farms in Qazvin province during March 2010-March 2011. The result of one way analysis of variance was used for the analysis data. Also Tukey Method was employed to detect pair wise differences among the ages determined that there were a significant differences among the average titer in 1 to 3, 1 to 4, 2 to 3 and 2 to 4 year cows ($P<0.005$, $P<0.001$, $P<0.041$, $P<0.001$). Due to the significance at the different titers, and comparing pair wise among different seasons Tukey,s method had been used and showed significant difference in summer with autumn ($P<0.038$) and winter ($P<0.001$). A chi-square test showed the significant differences among ages ($P=0.001$). The sero- prevalence of BHV-1 was estimated to be 7.1% with 1.2% standard error (SE). The result of PI3 showed the significance at the different titers comparing with the different seasons. Therefore Tukey,s method had been used and showed significant difference among seasons ($P<0.001$). The sero-prevalence of PI3 was estimated to be 95% with 1% SE.

Keywords: bovine herpesvirus type 1, Parainfluenza type 3, cow, serological test, ELISA.

INTRODUCTION

Infectious Bovine Rhinotracheitis (IBR) is a respiratory disease of cattle caused by Bovine Herpes Virus type 1 (BHV1). BHV1 includes three sub types, 1 and 2a which are associated with respiratory disease (IBR), 2b is identified with reproductive disease (Infectious Pustular Vulvo-vaginitis, IPV) and 3 which

is referred to as encephalitis (Wentink *et al* 1993). BHV1 is a DNA virus in the genus Varicella virus in the family Herpetoviridae. BHV1 is readily transmitted and has worldwide distribution BHV1 has been eradicated in Denmark, Finland, Norway, Sweden ,Austria, Germany and some parts of France. Immunity to BHV1 involves in complex interaction of humeral antibody and cell mediate immunity which are both activated following natural BHV1 infection or

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vaccination. Elisa is demonstrated to have superior sensitivity for detecting low levels of antibodies (Payment *et al* 1979). The pathogenesis of the disease includes the trigeminal nerve as a route of centripetal spread of the virus acute infection (Homan & Easterday 1980) and also respiratory mucosa can be another route for infection (Winkler *et al* 2000, Kahrs 2001). All ages and breeds of cattle are susceptible. There is no seasonal variation in incidence. The disease complexes associated with the virus occur most commonly in animals that lack acquired immunity from previous natural infection or vaccination (Kahrs 2001, Radostits *et al* 2007). Bovine Parainfluenza Virus type 3 (BPIV3) is one of the factors that induce "shipping fever virus (SF) because of its frequent concert with Pulmonic Pasteurellosis. However at the present time there are multiple factors influenced (Kahrs 2001). Bovine PIV3 was isolated in the late 1950 from cattle with SF (Reisinger *et al* 1959). It has been reported throughout the world from the cows with enzootic calf pneumonia. BPIV3 is a member of the genus Respirovirus in the family of paramyxoviridae (Radostits *et al* 2007). The human, bovine and ovine strains of PI3 virus are antigenically related but they can be differentiated serologically by serum neutralization and Haemagglutination Inhibition Test (Hirsh & Chung Zee 1999, van Vuuren 2004). The natural route of transmission is via the respiratory tract by infected aerosols. PI3 causes epithelial damage inducing bacterial invasion eg. *Manheimia spp* infections in infected cattle. Epithelial cells of the respiratory tract and the type 2 alveolar cells are the targets of PI3 (Hirsh & Chung Zee 1999). The virus is shed in nasal and ocular secretions for up to 8-10 days after exposure (Woods 1968, Kahrs 2001).

As far as we know there are a few reports of the serological studies on BHV1 and PI3 at the different Iranian provinces for example: in Uromieh (Kargar Moakhar *et al* 2002, Morshedi *et al* 2003) in Kerman (Sakhaee *et al* 2009) in Ahwaz (Bokaie *et al* 2009) and in Chahr Mahal Bakhtiary province (Hematzadeh *et al* 2002). The objective of this study is to determine the

seroprevalence of Bovine Herpesvirus type 1 and Parainfluenza in a population of non-vaccinated dairy cattle.

MATERIALS AND METHODS

Animals and herds. Five hundred twelve sera from cows were randomly collected from unvaccinated 1-4 year dairy cattle originating from 8 industrial dairy farms in Qazvin province from March 2010-March 2011.

Serological tests. BHV-1: Commercial Blocking ELISA kit developed by IDEXX, Switzerland was used to determine the presence of antibodies to BHV1 in bovine serum using IBR gB specific monoclonal antibody. The experiment was carried out according to the kit protocol. OD of samples and controls were measured at 450nm. By using ELISA reader. The result of the samples was calculated by: $(\text{Negative Control Mean} - \text{OD sample}) / \text{Negative Control Mean} \times 100$

Then it is interpreted as:

≤45 %	45- 55%	≥ 55 %
Negative	Suspect	Positive

PI3. The experiment was carried out according to the kit protocol. Commercial Indirect ELISA (Bi couple) kit developed by Institut pourquier in France was used to determine the presence of antibodies to PI3 in bovine serum. The OD of odd columns (coated) was subtracted from even columns (uncoated). Results were determined by calculating sample to positive (S/P) ratio. According to kit's instruction, result was multiplied by coefficient of 0.8 to achieve corrected percentage value (PC). Samples with S/P ratios of 20 or higher were considered positive and samples having S/P values less than 20 were considered negative.

STATISTICS. A one way analysis of variance (ANOVA) was used to compare the mean titer of BHV-1 and PI3 in different seasons and ages. In order to detect the pair wise differences between the titer averages among the ages and seasons, Turkey's method was used. SPSS 19.0 and S-pulse packages were employed to data analysis and a value for P of less than

0.05 was assumed to be significant. Also standard error is in 20% \pm 0.05 and 95% Confidence.

RESULTS AND DISCUSSION

The results of BHV-1 were plotted based on the ELISA-BHV-1 test was determined by using 504 sera. Five herds out of eight had at least one sero-positive animal. In five seropositive herds, two herds had two seropositive animals, one herd had two seropositives, one herd had four and the rest of the herds had twenty three seropositive animals.

Table 1 shows mean of Blocking Percentage against BHV-1 gradually increased from 14.02 in 1 year to 26.22 in 4 year cows which was the significant differences with cut off point (55). With respect to season comparing the standard mean titer indicated reduction trend from 19.35 in spring to 13.60 in summer and then elevated in autumn (20.36) and winter (25.27). As the result of sero- prevalence was estimated to be 7.1% (95%CI: 0.048%-0.094%). The result of PI3 by using indirect ELISA showed that there was elevation of mean antibody comparing with age (74.80% in 1 year to 77.02 in 4 years) which was not the significant differences (table 2). Due to the significance at the different titers, and comparing pair wise among different seasons Turkey's method had been used and showed significant difference among seasons ($P < 0.001$) (table 2). As the result the sero-prevalence was estimated to be 95% (95%CI: 93.2-97.2).

Pathogenesis of bovine respiratory syndrome presents the combination of stress factors that disturb the defense mechanisms of host accompanied with the influence of infectious agents (viruses or bacteria). The viral infection reduces defense mechanism of respiratory organs and enables colonization of lower parts of respiratory system for invading bacteria. Therefore respiratory syndrome is multi-causal and because of that term "bovine respiratory disease complex-BRCD" was accepted (Dyer 1982).

By using ELISA test, the prevalence of BHV-1 seropositive cows was determined and may reflect the proportion of BHV-1 carriers after a primary infection. The virus stays latent in neural ganglions that innervate genital or respiratory mucosa and may be re-excreted upon immuno-suppression stimuli (Payment et al 1976, Graham et al 1999, Winkler et al 2000). Transportation of cattle with latent infection can reactivate and re-excrete the virus (Nandi et al 2009). Infection of cattle with BHV-1 impairs resistance to secondary bacterial infection such as *Mycoplasma haemolytica*, *Pasteurella multocida* and *Histophilus somni* leading to fatality and depression of cell mediated immunity (Leite et al 2002).

Table 1. Mean, standard deviation and standard error for Blocking Percentage of BHV-1 titer in different ages and seasons.

	n	n	Mean	sd	se
Total		504	19.58	20.527	0.914
age(in year)	1	125	14.02	6.837	0.612
	2	127	15.72	13.708	1.216
	3	127	22.38	24.821	2.203
	4	125	26.22	27.324	2.444
Season	spring	127	19.35	22.819	2.025
	summer	128	13.6	19.044	1.683
	autumn	127	20.36	16.946	1.504
	winter	122	25.27	21.415	1.939

Table 2. Mean, standard deviation and standard error for Indirect Percentage of antibody of PI3 in different ages and seasons.

	n	Mean	sd	se	
Total	504	77.02	34.025	1.516	
age(in year)	1	124	74.8	32.301	2.901
	2	127	75.09	35.321	3.134
	3	130	76.1	33.042	2.898
	4	123	82.24	35.229	3.177
seasons	spring	125	97.9	44.935	4.019
	summer	127	71.19	30.057	2.667
	autumn	126	67.44	27.849	2.481
	winter	126	71.76	19.452	1.733

The sero- prevalence in our study in BHV-1 was low (7.1%) whereas in PI3 was high (95%). The latter data was in accordance with Durham and Hassard in 1990. The result of BHV-1 indicated that the mean of Blocking Percentage gradually increased from 14.02 in 1 year to 26.02 in 4 year cows, while standard deviation also increased by the age. Also the standard mean titers indicated reduction trend from 19.35 in spring to 13.60 in summer and then elevated in autumn (20.36) and winter (25.40). The result of one way analysis variance determined that there was significant difference among the average titers in 1 to 3 ($P<0.005$) and 4 ($P<0.001$) and also 2 to 3 ($P<0.041$) and 4 ($P<0.001$) year cows. Due to the significance at the different titers, and comparing pair wise among different seasons Turkey's method showed significant difference in summer with autumn (0.038) and winter (0.001). Using chi-square test showed the significant difference among ages per se ($P=0.001$). As a result the percentage of seropositive cows in spring and winter were elevated. It is concluded that the primary design of this project is likely complied with Solis-Caleron et al in 2003 although they used the Serum Neutralization instead of ELISA.

The result of PI3 showed significant difference among seasons ($P<0.001$). The recent data above PI3 was in accordance with Durham and Hassard 1990, Bryson 1990 & Sakhaee et al 2009). Therefore it is suggested that administration of long term safety vaccine against BHV-1 and PI3 should be applied (Murphy et al 1999) and applying Modified Live Virus (MLV) vaccine is necessary (Nandi et al 2009).

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