A new way of occurrence and serodiagnosis for Infectious Bovine Rhinotrchitis in Iranian cattle herds

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Key words:
Infections bovine rhinotrachitis, ELISA, serum neutralization test, bovine herpes virus- 1 (BoHV-1).

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Received: 13 December 2011
Accepted: 2 May 2012

Abstract:

BACKGROUND: Infectious Bovine Rhinotrachitis (IBR) caused by Bovine Herpes Virus-1 (BoHV-1) was first, observed in one of the Tehran Industrial cattle breeders, with importation of cattle in 1965. Later, the isolation of bovine herpes virus approved the existence of the disease in the most of the provinces of the Iran. **OBJECTIVES:** Presence of antibodies against BoHV-1 was evaluated in young bovines of north, south and central provinces of Iran. **METHODS:** Bovines with age 1 to 4 years old, non-vaccinated and without clinical symptoms were included in the study. 558 serum samples were collected equivalent to 10% of the population in each farm. The sera were tested by ELISA and micro serum neutralization (MSN) in monolayers of MDBK cells using BoHV-1 reference strain. **RESULTS:** The results of ELISA showed that 56% (314/558) were positive and 44% (244/558) were negative for BoHV-1 specific antibody. Whoever MSN assay showed that 48% (269/558) were negative and 52% (289/558) were positive. These positive sera demonstrated that 54% had serum neutralization titer between 4-16, 35% on 32 to 64 and 11% on 64. **CONCLUSIONS:** Results suggest that the virus is actively circulating and transmitting in young herds and is a constant source of infection in the herd.

Introduction

Bovine herpes virus (BoHV-1) is member of the alpha herpesvirinae subfamily. Its genome consists of a linear double stranded DNA molecular of about 140 Kb which codes approximately 75 proteins including several glycoproteins identified as glycoproteins B, C, D, E, I and H (Marshall et al., 1986; Roizmann et al., 1992). This virus is economically important pathogen for cattle causing infectious bovine rhinotracheitis (IBR) and infectious pustular vulvo-vaginitis (IPV). The virus is associated with respiratory and genital infection, conjunctivitis, encephalitis, abortion and fatal multi-systemic infections (Gibbs et al., 1977; Yates, 1982). BoHV-1 may cause latent infection in the sensory nervous ganglia, which may be followed by recurrence of the disease. This fact serves as a constant source of infection during viral reactivation and re-excretion periods (Kuttish et al., 1990; Stevens, 1989). IBR was not seen in the Iran until entrance of imported breeds of cattle. Prevalence of the disease was suspected with incidence of abortion in one of the Tehran industrial cattle breeders in 1965, and serological diagnosis attested the fact. In 1973, IBR was reoccurred with importation of English bovines, and isolation of virus, approved the existence of the disease in the most of the provinces of the country. BoHV -1 was first time isolated in Iran and sero-logical survey showed 34% of tested cattle had precipitating antibodies against BoHV-1 (Afshar and Tadjbakhsh, 1970). However, subsequent studies demonstrated an increase up to 74% of serum positive animals (Afshar et al.,1970). The presence of the latent carriers (Sheffy et al., 1973; Thiry et al., 1987) favors the perpetuation of the virus in the herd and when the percentage of adult infected cows is high,

they will certainly infect the young flock even before their reproductive life. Since spreading of the virus is considered as an important epidemiological factor for cattle herds so, this study aimed to report serological status of BoHV-1 infection in bovines from years 2000 to 2010, by two sensitive and specific methods.

Material and Method

Cells and virus: Madin Darby Bovine Kidney (MDBK) cells were cultured in minimal essential medium - Eagle (MEM-E- Gibco Lab. NYUSA) was supplemented with 5 - 10% fetal calf serum (Gibco Lab). The reference BoHV-1 strain was propagated at a multiplicity of infection (MOI) 0.5- 0.7 per cell in MDBK cells. The culture fluids were harvested 36 h post -infection, clarified by centrifugation for 20 min at 12000 g and 4^{o}C and used for sero- neutralization assay.

Experimental design: Fifteen cattle farms from different provinces in north, center and south regions of Iran were randomly selected in this study. The provinces were Yazd, Khorassan, Fars, Markazi, Eastern Azerbaijan and Qom. The number of serum samples was about 10% of cattle population in each farm, representing 50% of prevalence of BoHV-1 infection and 95% confidence interval (Thrusfield, 1986). Samples consisted of 558 bovine's sera from 1 to 4 years old dairy and beef herds, non-vaccinated or without previous diagnostic of BoHV-1 infection.

Serology: Each serum sample was fractionated, inactivated at 56^{o}C for 30 minutes and tested by micro serum neutralization (MSN) assay, and frozen at -20^{o}C until they were used. Anti-BoHV-1 antibodies were assayed by the indirect ELISA technique, using the Herd Check kit following instructions and interpretation of results according to manufacturer. Alternatively to ELISA, a MSN assay (constant virus- varying serum) was carried out with the reference BoHV-1 strain, in 96-well flat-bottom plates with MDBK cells (House et al., 1971). The sera were assayed in two-fold dilutions (1:4 to 1:64) and incubated with BoHV-1 virus (100 DICT50) at 37^{o}C for 1 hour. Then, the mixture virus-serum was added to micro plates with MDBK cells at 0.1 ml/well and the plates were incubated for 3 days at 37^{o}C. The serum neutralization titer (SNT) was expressed as the reciprocal of the highest dilution of the antibodies that inhibited 50% viral cytophatic effects (CPE) according to the method of Reed and Munch. Titers were obtained on individual samples and classified in three groups 4-16; 32-64 and < 64 according to the SNT.

Results

ELISA: The bovine sera were first tested by ELISA method. The sera were analyzed individually and the results obtained from each farm are shown in Table1.

The trials demonstrated that thirteen of analyzed farms were seropositive. 80-100% of bovines from five seropositive farms (no 2,6,11,12,13) and 40-79% from seven seropositive farms (no. 1,3,5,7,9,10,14) and 8% from one farm (no. 4) were seropositive to BoHV-1.

Microserum neutralization test: The results of bovine sera expressed as SNT were classified in three groups, considering the titer of neutralizing antibodies: 4-16, 32-64 and > 64. As shown in tabl2 2, the results demonstrate that all seropositive farms have at least 50 % of infected animals in the first group SNT 4-16. Furthermore, there were three farms (n. 6, 11, and 13) that had almost the same percentages of animals in two first groups SNT 4-16 and SNT 32-64. In contrast, it was found two seronegative farms (n. 8,15) and two farms (n. 4,5) with 8-10% of seropositive animals to BoHV-1. It is worth to observe the results in farms n. 7, 9 and 12. In spite of the small number of the samples, the results resemble other ones with large numbers involved.

Discussion

BoHV-1 has been detected in Switzerland, Denmark and other European's bovines and eradicated by removal strategies, (Ackerman et al 1990). But, this program obviously cannot be implemented in the Iran that has a high prevalence of BoHV-1 infection. The first report of the virus isolation in Iran was in Razi Institute by Hazrati et al., 1974. Since 1973, no official program was carried out to control the disease. The surveillance of the disease should be through intensive vaccination to lower the incidence of BoHV-1 infection. As our trial demons-trated, BoHV-1 is widely disseminated in the young flock of

Table 1. The results of 558 bovine sera analyzed by Elisa test (Herd-Check-IDDEX) were expressed according to the number and the percentage of seropositive and seronegative bovines in each farm. It is remarkable that only three farms (n. 4, 8, 15) showed 0 and 8 % of seropositive animals, considering that most of farms have, at least, antibodies against BHV-1 in 50% of the herd.

Farm Number	Seropositives		Seronegatives		Total Number of sera
	Nnmber of (+)	%	Number of (-)	%	
1	22	47	25	5 3	47
2	24	85	4	14	28
3	31	77	9	23	40
4	4	8	44	92	48
5	21	41	30	59	51
6	59	88	8	12	67
7	8	53	7	47	15
8	0	0	50	100	50
9	9	69	4	31	13
10	12	46	14	53	26
11	34	92	3	8	37
12	15	100	0	0	15
13	57	96	2	4	59
14	18	51	17	49	35
15	0	0	27	100	27

Table 2. The results of the number of bovine sera (n = 558) with negative or positive neutralizing antibodies against B0HV-1 in each farm.* SNT 4-16 : serumneutralization titer 1:4 - 1:16.

	Sero positive				
Farm Number	SNT* 4-16 Number of (+)	SNT 32-64 Number of (+)	SNT >64 Number of (+)	Sero negatives Number of (-)	Total Number of sera
1	10	7	3	27	47
2	17	5	1	5	28
3	20	5	2	13	40
4	4	0	0	44	48
5	3	3	0	45	51
6	26	21	11	9	67
7	7	1	0	7	15
8	0	0	0	50	50
9	3	3	3	4	13
10	5	5	1	15	26
11	12	17	4	4	37
12	11	4	0	0	15
13	24	27	6	2	59
14	14	4	0	17	35
15	0	0	0	27	27

the farms. According to our results, ELISA method showed 56% of bovine sera were positives and 44% negatives for BoHV-1 specific antibody meanwhile MSN method showed 52% positives and 48% negatives. Although the results obtained by MSN and ELISA techniques were somehow similar (Marshall et al., 1986), some differences were found in sero-positive bovines as it was expected, because the neutralizing and non-neutralizing antibodies were detected in ELISA test, which increase the number of seropositive animals (Afshar and Tadjbakhsh, 1970). On the other hand, the titration of antibody was

performed by SNT assay which demonstrated 54% of tested bovine sera were between 4-16, 35% between 32-64 and at least 11% were 64. Presence of antibodies against BoHV-1 in bovine without vaccination means viral contact (House et al., 1971; Reed et al., 1938). Moreover, the high SNT above 64 probably indicates a recent infection or convalescent period (Thiry et al., 1986) and the high incidence of BoHV-1 virus in the herds is expected.

The results of this study are consistent with previous reports (Baker et al., 1960) where bovines from slaughterhouse showed a prevalence of 50% seropositive to BoHV-1 (Afshar et al., 1970). The latency characteristic of BoHV-1 must be considered in the sero-epidemiological surveys where the age of the cattle is involved on results. The previous surveys in Iran demonstrated the situation of the BoHV-1 infection among young animals (Hazrati et al., 1974). In this study we showed that the BoHV-1 is actively circulating in heifers and young cows that represent a new generation in the farm. If cattle are being infected before the beginning of the reproductive life, this fact could represent renewable sources of viral infection in the herd (Kuttish et al.,1990; Thrusfield, 1986).

Our results suggest that BoHV-1 is widely disseminated in the studied dairy and beef cattle even before the reproductive life and a good vaccination program is recommended in order to control the BoHV-1 infection.

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مجله طب دامی ایران، ۱۳۹۱، دوره ۶، شماره ۲، ۱۰۳-۹۹

نگرشی به سروا پیدمیولوژی و میزان وقوع بیماری ویروسی تورم مخاط نای و بینی گاو در ایران

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(دریافت مقاله: ۲۲ آذر ماه ۱۳۹۰ ، پذیرش نهایی: ۱۳ اردیبهشت ماه ۱۳۹۱)

چکیده

زمینه مطالعه: بیماری ویروسی تورم مخاط بینی و نای گاوان برای نخستین بار در سال ۱۹۶۵ در یکی از نواحی پرورش صنعتی پرورش گاو به علت اهمییت بیماری گزارش گردید و پس از آن جداسازی ویروس مولد بیماری در اغلب استان های ایران مورد تائید قرار گرفت. **هدف:** حضور آنتی بادی بر علیه هرپس ویروس تیپ یک گاوی در گوساله های جوان در استان های شمال جنوب و مرکزی مورد بررسی قرار گرفت. **روش‌کار:** گوساله های یک الی چهار سال غیر واکسینه شده بدون علائم بالینی بیماری مورد مطالعه قرار گرفتند. تعداد ۵۵۸ نمونه سرمی معادل ۱۰٪ از کل جمعییت هر فارم جمع آوری گردید. نمونه های سرمی مورد آزمون الایزا و خنثی سازی سرم به روش میکرو در پلیت بر روی سلول های تک لایه ای کلیه گوساله جوان در میکرو پلیت با استفاده از سویه رفرانس مورد مطالعه قرار گرفتند. **نتایج:** نتایج آزمون الایزا نشان داد که ۵۶٪ نمونه های سرمی (۳۱۴/۵۵۸) مثبت و ۴۴٪ (۳۴۴/۵۵۸) منفی و نتایج آزمون خنثی سازی سرم، مویدآنستکه ۴۸ ٪ موارد سرمی (۲۶۹/۵۵۶) منفی و ۵۲٪ (۲۸۹/۵۵۸) موارد مثبت بود. لازم بذکر است که ۵۴ ٪ از سرم های مثبت دارای عیار آنتی بادی بین ۱۶-۴، ۳۵ ٪ دارای عیار بین ۶۴-۳۲ و ۱۱٪ بدارای عیار ۶۴ بودند. **نتیجه‌گیری نهایی:** نتایج حاصله نمایانگر انست که ویروس به طور فعال و با انتشاری گسترده در گله گوساله های جوان در گردش است و منشأ پایدار از عفونت ویروسی در گله به شمار می آید.

واژه‌های کلیدی: آزمون الایزا، خنثی سازی، IBR-IPV ویروس، هرپس ویروس گاوی تیپ.

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