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Serological survey for bovine herpesvirus 1 in cattle from different regions in the state of Bahia, Brazil*

Levantamento sorológico para herpesvírus bovino 1 em bovinos de diferentes regiões do Estado da Bahia, Brasil

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SUMMARY

The presence of antibodies against BHV-1 was evaluated in young bovines from fifteen different counties distributed along North, South and Central regions of the state of Bahia. Bovines with age ranged from 1 to 4 years old, non-vaccinated against BHV-1 and without diagnostic of BHV-1 infection were chosen for blood collection. A total of 558 serum samples were collected equivalent to 10% of the population in each farm. The sera were submitted to ELISA (HerdCheck-IDDEX) and Microserum neutralization (MSN) tests. The MSN was performed in monolayers of MDBK cells in microplates using Los Angeles BHV-1 strain. The results of serum samples by ELISA showed that 56% (314/558) were positive and 44% (244/558) were negative. The sera assayed by MSN showed that 48% (269/558) were negative and 52% (289/558) were positive. These positive sera demonstrated that 54% had serumneutralization titer between 4-16, 35% between 32-64 and 11% ≥ 64. Our results suggest that the virus is circulating actively in herds. Furthermore, the high diffusion of the BHV-1 in young bovines could represent a constant source of viral infection in the herd.

UNITERMS: Bovine herpesvirus 1 (BHV-1); ELISA; Serumneutralization; Diagnosis.

INTRODUCTION

Bovine herpesvirus (BHV-1) is a member of the alphaherpesvirinae subfamily. Its genome consists of a linear double stranded DNA molecular of about 140 Kb which codes for approximately 75 proteins including several glycoproteins identified as glycoproteins B, C, D, E, I and H[9,11].

BHV-1 is an economical important pathogen for cattle causing infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV). The virus is associated with respiratory and genital infection, conjunctivitis, encephalitis, abortion and fatal multi-systemic infections[6,19].

BHV-1 can establish latent infections in the sensory nervous ganglia, which may be followed by recurrence of disease. This fact serves as a constant source of infection during viral reactivation and re-excretion periods[8,15].

The BHV-1 serological surveys carried out in Brazil have showed that this virus is spread in milk and beef cattle in many states of the country. In 1963, the first serological survey made in Bahia[5], showed that 34% of cattle tested had neutralizing antibodies against BHV-1. However, subsequent studies demonstrated an increase up to 74% of serum positive animals[2]. Furthermore, it was in Bahia in 1978, the first BHV-1 isolated in Brazil[3].

The presence of the latent carriers[4,13,17] favours 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. Considering this fact as an important epidemiological factor for diffusion of the virus, this study had the objective to report a serological status for BHV-1 infection in bovines from fifteen different herds.

MATERIAL AND METHOD

Cells and virus

Madin Darby Bovine Kidney (MDBK) cells were cultured in Minimal Essential Medium - Eagle (MEM-E-Gibco Laboratories NY USA) supplement with 10% fetal bovine serum (Gibco Lab). The reference Los Angeles BHV-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 hours post-infection, clarified by centrifugation during 20 minutes to 12,000 g at 4ºC and used for seroneutralization assay.

Experimental design

Farms of fifteen different counties distributed along the North, Center and South regions of the state were randomly chosen for this study. The counties were Ruy Barbosa, Jequié, São Sebastião de Passe, São Franscisco do Conde, Mundo Novo, São Miguel das Matas, Vitória da Conquista, Macajuba, Ibitupa, Itaberaba, Rio Real, Jaguaquara, Feira de Santana, Itamaraju and Igripuna.

The number of serum samples collected was about to 10% of cattle population in each farm, which was statistically representative, considering a 50% prevalence of BHV-1 infection and 95% confidence interval[18].

Samples consisted of 558 bovines from dairy and beef herds, with age ranged from 1 to 4 years old, non-vaccinated or without previous diagnostic of BHV-1 infection.

Serology

Each serum sample was fraccionated, inactivated at 56ºC during 30 minutes for testing in microserum neutralization (MSN) assay, and frozen at -20ºC until they were used.

Anti-BHV-1 antibodies were assayed by the indirect ELISA technique, using the HerdCheck kit supplied by IDDEX-Maine-USA following instructions and interpretation of results according to manufacturer.

Alternatively to ELISA test, a MSN assay (constant virus- varying serum) was carried out with Los Angeles BHV-1 strain, in 96-well flat-bottom plates with MDBK cells[7]. Briefly, the sera were assayed in two-fold dilutions (1:4 to 1:64) and incubated with BHV-1 virus (100 $DICT_{50}$) at 37ºC for 1 hour. Then, the mixture virus-serum was added to microplates with MDBK cells at 0.1 ml/ well and the plates were incubated for 3 days at 37ºC.

The serum neutralization titer (SNT) was expressed as the reciprocal of the highest dilution of the antibodies that inhibited 50% viral citophatic effects, according to the method of Reed and Muench[10]. Titers were obtained on individual samples and classified in three groups 4-16; 32-64 and < 64 according to the SNT.

RESULTS

ELISA test

The bovine sera were first submitted to ELISA test.

The sera were analyzed individually and the results obtained in each farm are shown in Tab. 1. The trials demonstrated that from fifteen farms analyzed, thirteen of them were seropositive. Five seropositive farms (n. 2, 6, 11, 12, 13) showed that 80-100% were seropositive bovines to BHV-1, seven seropositive farms (n. 1, 3, 5, 7, 9, 10, 14) had 40-79% and one farm (n. 4) had 8% seropositive to BHV-1 their flock.

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.

Farm Number	Seropositives Number of (+)	%	Seronegatives Number of (-)	%	Total number of sera
1	22	47	25	53	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

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.

Microserum neutralization test

The results are shown in Tab. 2.

Table 2
The results show the number of bovine sera (n = 558) with negative or positive neutralizing antibodies against BHV-1 in each farm.

Farm Number	Seropositives			Seronegatives	Total number of sera
	SNT * 4-16 Number of (+)	SNT 32-64 Number of (+)	SNT > 64 Number of (+)	Number of (-)	
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

* SNT 4-16 : serumneutralization titer 1:4 - 1:16.

As seen in ELISA test, it was found that thirteen farms had neutralizing antibodies to BHV-1 in the herd.

The results of bovine sera expressed as SNT were classified in three groups, considering the titer of neutralizing antibodies: SNT 4-16; SNT 32-64 and SNT > 64.

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 BHV-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

BHV-1 has been eradicated from Switzerland, Denmark and other European[1] countries by tests and removal strategies, but in our country that has a high prevalence of BHV-1 infection, obviously cannot implement that program. The control must be directed toward easing the impact of the disease through intensive vaccination to lower the incidence of BHV-1 infection.

As our trial demonstrated, BHV-1 is widely disseminated in the young flock of the farms.

The results of 558 sera bovine sera by ELISA test, showed 56% positives, 44% negatives and by MSN showed 52% positives and 48% negatives. In spite of some differences, the results obtained by MSN technique were in agreement with the ELISA test. The differences were found in seropositive bovines as it was expected, because the ELISA test employed in this study detected neutralizing and non-neutralizing antibodies, which increase the number of seropositive animals. On the other hand, the BHV-1 SNT showed 54% between 4-16, 35% between 32-64 and at least 11% ≥ 64. If this population never received a vaccination, the presence of antibodies against BHV-1 means viral contact. Moreover, the high SNT > 64 probably indicates a recent infection or convalescent period.

The high diffusion of BHV-1 virus in the herds was expected, because, as a matter of fact, the first report of the virus isolation in Brazil was in Bahia by Alice[3], and since 1978 no official program were carried out to control the disease.

The results obtained in our study are consistent with the data obtained by Anunciação *et al.*[2] where bovines from slaughterhouse showed a prevalence of 50% seropositive to BHV-1.

The characteristic of BHV-1 to cause latency must be considered in the seroepidemiological surveys when it has to choose the age of the cattle involved on trial. Any of the previous surveys done in bovines in the Bahia state demonstrated the situation of the BHV-1 infection among young animals. In this study we showed that the BHV-1 is actively circulating in heifers and young cows, which is extremely important because they represent a new generation in the farm. If they are being infected before the beginning of the reproductive life, this fact could represent renewable sources of viral infection in the herd.

CONCLUSION

Our results suggest that BHV-1 is widely disseminated in the dairy and beef cattle studied.

The sera collected from herds from different counties of the state showed that the bovines are actively infected even before the reproductive life. The high diffusion of BHV-1 virus in those herds studied needs attention. Although, studies have to be continued to evaluate more serum samples, the results reported here show the importance to establish a good vaccination program that help control the diffusion of the BHV-1 virus infection[12,14,16].

RESUMO

A presença de anticorpos contra Herpesvírus Bovino tipo 1 (HBV-1) foi avaliada em bovinos jovens provenientes de quinze municípios diferentes, distribuídos nas regiões Norte, Sul e Central do estado da Bahia. Foram coletadas amostras de sangue de bovinos de 1 a 4 anos de idade, não vacinados contra HBV-1 e sem prévio diagnóstico de infecção pelo vírus HBV-1. O total de amostras correspondeu a 558 soros, equivalente a 10% da população bovina de cada fazenda. Os 558 soros foram submetidos à técnica de Microssoroneutralização (MSN) em microplacas, utilizando-se monocamadas de células MDBK e a cepa de HBV-1 Los Angeles, e ao teste de ELISA comercialmente adquirido: HerdCheck-IDDEX. Os resultados da amostragem feito pelo teste de ELISA mostraram que 56% (314/558) dos soros foram positivos e 44% (244/558), negativos. A técnica de MSN mostrou que 48% (269/558) dos soros foram negativos e 52% (289/558) foram positivos. Destes soros positivos, 54% tiveram títulos soroneutralizantes entre 4-16, 35% entre 32-64, e 11% ≥ 64. Os resultados sugerem que o HBV-1 esteja circulando ativamente na população bovina jovem. Por outra parte, a alta difusão do vírus em animais jovens representaria uma fonte contínua de infecção viral para o rebanho.

UNITERMOS: HBV-1; ELISA; Soroneutralização; Diagnóstico.

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