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Author(s)	SUZAN, Victor M.; ONUMA, Misao; AGUILAR, Romero E. et al.
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PREVALENCE OF BOVINE HERPESVIRUS-1,
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BOVINE LEUKEMIA VIRUS AND BLUETONGUE
VIRUS ANTIBODIES IN CATTLE IN MEXICO

Victor M. SUZAN¹, Misao ONUMA², Romero E. AGUILAR¹ and Yosuke MURAKAMI³

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Sera were collected from dairy and beef cattle in 19 different states of Mexico. These sera were tested for bovine herpesvirus-1 (BHV-1), parainfluenza-3 virus (PIV-3), bovine rotavirus (BRV), bovine leukemia virus (BLV), bovine adenovirus-7 (BAV-7), bluetongue virus (BTV) and bovine viral diarrhea virus (BVDV). Seropositive rates for each virus for dairy cattle tested were 158/277(57.0%) for BHV-1, 217/286(75.0%) for PIV-3, 541/1498(36.1%) for BLV, 134/144(93.1%) for BRV, 39/90(43.3%) for BTV, 55/235(23.4%) for BAV-7 and 93/132(70.5%) for BVDV. The seropositive rates for each virus for beef cattle tested were 601/1154(52.0%) for BHV-1, 830/1271(69.3%) for PIV-3, 1053/1274(83.0%) for BRV, 482/771(62.5%) for BVDV, 51/1271(4.0%) for BLV, 50/110(45.5%) for BTV and 50/70(71.4%) for BAV-7. The positive rates for BVDV in goats were 16/80(20.0%).

Key words: Bovine virus infections, Respiratory diseases, Alimentary diseases, Mexican cattle

INTRODUCTION

Occurrence of respiratory diseases such as bovine herpesvirus-1 (BHV-1) and parainfluenza-3 virus (PIV-3) infections has been known in cattle in Mexico and BHV-1 has been isolated from cattle showing respiratory symptoms.⁹⁾ Alimentary diseases in cattle such as bovine rotavirus (BRV), bovine viral diarrhea virus (BVDV) and bovine adenovirus-7 (BAV-7) infections are of concern to the producer, clinician and diagnostic laboratory. Previously, Correa and associates performed the serologic-

¹ Direccion General de Sanidad Animal, Dr. Mora 15-9 Piso, Mexico, D. F.

² Department of Epizootiology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan

³ Hokkaido Branch Laboratory, National Institute of Animal Health, Sapporo 061-1, Japan

Address reprint requests to Dr. M. ONUMA

al surveys for antibodies against BHV-1, PIV-3 and BVDV in small numbers of serum samples (total 47 sera) from 2 states in Mexico and suggested the spread of these virus infections.⁴⁾ However, the serologic surveys for nationwide bovine virus infections have never been performed in Mexico and the situation of these virus infections is not well known.

The purpose in the present study is to determine, through testing for viral specific antibodies, the prevalence of infections with BHV-1, PIV-3, BRV, BVDV, bovine leukemia virus (BLV), bluetongue virus (BTV) and bovine adenovirus-7 (BAV) in dairy and beef cattle in Mexico.

MATERIALS AND METHODS

Sera

Serum samples were collected randomly from dairy cattle (mainly from Holstein) in 225 herds in 10 states. Serum samples were also obtained from beef cattle (Zebu, Swiss, Hereford and hybrid) in 227 herds in 11 states of Mexico.

Cell cultures

Primary or secondary cultures used were bovine testicle (BT) and bovine kidney (BK) cells. The established cell lines used were Madine Darby bovine kidney (MDBK), monkey kidney (MA 104), hamster lung (HmLu) and fetal lamb kidney (FLK) cells chronically infected with BLV. All of these culture cells were grown in Eagle's minimum essential medium containing 10% calf serum.

Viruses

Stock viruses included: BHV-1, Colorado strain; PIV-3, BNI-1 strain; BRV, Lincoln strain; BVDV, Singer strain; BAV-7, Fukuroi strain; BLV obtained from FLK cells and BTV, CSIRO-20 strain. These viruses were grown in monolayer cultures as follows: BHV-1 in MDBK or BT cells, PIV-3 in BK cells, BRV in MA 104 cells, BVDV in BT cells, BAV-7 in BT cells, BLV in FLK cells and BTV in HmLu cells.

Serologic tests

Three serologic tests including virus neutralization (VN) test using 96 wells microplate, immunodiffusion (ID) test and hemagglutination inhibition (HI) test were used for the detection of antibodies.

Antibodies against BHV-1 and BVDV were detected by VN test. Two-fold dilutions of inactivated serum (56°C for 30 minutes) in duplicate wells of microplates were mixed with an equal volume of BHV-1 (0.05 ml) containing 200 TCID₅₀/0.05 ml and the microplates were incubated for one hour at 37°C. The monolayer of MDBK cells were dispersed, suspended with the medium containing 10% BHV-1 antibody free calf serum and adjusted to about 3×10^5 cells/0.1 ml. Cells (0.1 ml) were added to the microplate and incubated for three days at 37°C. Part of dairy cattle from Hidalgo state were vaccinated with live BHV-1. Preliminary experiments showed that sera from the vaccinated cattle have neutralizing antibody titer of less than 1:8.

Therefore, in the present experiment, sera which had neutralizing antibody titer of 1 : 16 or more were considered to be positive for BHV-1 infection. For BVDV neutralization test, microplates were planted with 2×10^5 cells/well of BT cells one day before inoculation of serum-virus mixture. Dilution of inactivated serum (0.05 ml) starting 1 : 5 was mixed with an equal volume of virus containing 200 TCID₅₀/0.05 ml and incubated at 37°C for one hour. Culture medium was discarded and serum-virus mixtures (0.1 ml) were inoculated onto microplate. After incubation at 37°C for one hour, the cultures were fed with 0.1 ml of maintenance medium containing 5% of BVDV antibody free serum. Microplates were incubated for 4 to 5 days before reading the results. The results of the VN test for BVDV were expressed as positive when 1 : 5 or more dilution of test serum inhibited the formation of cytopathic effect.

For the detection of antibodies to BAV-7, PIV-3 and BRV, HI test was performed as previously described.^{6,7)} Briefly, all sera tested were inactivated at 56°C for 30 minutes and added 1.5 volume of Veronal buffered saline (VBS) containing 0.1% bovine serum albumin and 0.001% gelatin. Sera were treated with an equal volume of 25% kaolin solution for 30 minutes at room temperature and then centrifuged to remove kaolin. The resulting supernatant fluid was taken as 5-fold dilution of the serum. Four units of hemagglutination antigen (0.025 ml) were mixed with serial 2-fold dilution of the serum (0.025 ml) and incubated overnight at 4°C for BAV-7 and PIV-3, and for one hour at room temperature for BRV. After incubation 0.025 ml of bovine erythrocyte suspension (0.3%) was added. The mixtures were then incubated for 4 hours at 4°C for BAV-7 and PIV-3, and for 1.5 hours at 37°C for BRV before reading the results. Serum which had HI antibody titers of 1 : 20 or more was considered to be positive.

The ID test for detection of antibodies against glycoprotein antigen of BLV was performed as previously described.¹³⁾ BTV was propagated in HmLu cells. The culture fluids were concentrated about 100 times by force dialysis against polyethylene glycol #6000. The procedure for detection of antibody against BTV by ID test was the same as that described in the BLV system.

RESULTS

The prevalence of 7 bovine virus antibodies in dairy and beef cattle in Mexico is shown in tables 1 and 2, respectively. The positive percentages in dairy and beef cattle of BHV-1, PIV-3, BRV, BTV and BVDV are similar to each other. The seropositive dairy and beef cattle to each virus varied from 52–57% to BHV-1, 65–76% to PIV-3, 83–93% to BRV, 43–45% to BTV and 63–71% to BVDV. In contrast, the positive percentages of BLV antibody in dairy and beef cattle were quite different from each other (36.1% for dairy cattle and 4.0% for beef cattle). Although sera tested for antibody against BAV-7 were a small number in a limited area, the reactors in dairy and beef cattle were 23.4% and 71.4%, respectively. Sera from

goats were tested for antibody against BVDV and 16 of 80 sera (20%) were positive (Data is not shown in the table).

The HI titer profiles ranged from 1 : 20 to $\geq 1 : 640$ for PIV-3, BRV and BAV-7 and majority of sera had antibody titer of 1 : 80.

Occurrence of respiratory disease with catarrhal inflammation of the mucous membranes in sheep was reported in the state of Hidalgo and Mexico D.F. in 1982. Seventeen of 21 sera from affected sheep had antibody against BTV.

DISCUSSION

The results of this study strongly indicate that cattle in Mexico have serologic evidence of infection with seven bovine viruses tested. Respiratory diseases such as BHV-1 and PIV-3 infections have been of great concern to diagnostic laboratories in Mexico. The BHV-1 was isolated in nasal secretions of cattle affected with respiratory syndromes in Mexico.⁹⁾ QUEVEDO et al.¹⁴⁾ performed a serological survey for BHV-1 using 259 serum samples from 7 states in Mexico and 62.1% of sera had the neutralizing antibody. A serological survey for PIV-3 using a small number of sera (47 samples) from 2 states in Mexico showed an 86% of positive rate.⁴⁾ Present serological surveys using large quantities of serum samples from 19 different states of Mexico show a widespread virus infection in both dairy and beef cattle.

Although alimentary diseases have not been well studied in cattle in Mexico, one report presented the detection of BRV in feces from new-born cows showing diarrhea.¹⁰⁾ Serological survey for antibodies against BRV and BAV-7 has never been performed in Mexico. In an earlier report 75% of 47 sera from 2 states had antibody against BVDV detected in virus neutralization test.⁴⁾ The present serological survey for antibodies against BRV, BVDV and BAV-7 showed that 83% for BRV, 63% for BVDV and 34% for BAV-7 were positive. The results of the present survey indicate that we should pay more attention to these alimentary diseases.

Bluetongue is an acute arthropod-borne virus disease of sheep and cattle although cattle suffer much milder symptoms and may act as nonclinical carriers of the infection. Previous serosurvey for antibody to BTV in Mexico showed that 8.5% of 187 sheep sera and 3.48% of 267 bovine sera tested had the antibody.¹¹⁾ The positive percentage of bovine sera was at a similar level to that in the present results.

A total of 110 cases of bovine lymphosarcoma was observed in 13 states of Mexico during 1969 to 1974.¹⁾ Preliminary serological survey using 240 serum samples from dairy cattle in one state of Mexico showed a positive percentage of 22.5%.¹⁵⁾ In the present experiment, 36.1% of dairy cattle and 4.0% of beef cattle were positive. Similar differences in prevalence of BLV antibodies between dairy and beef cattle have been noted in other countries,²⁾⁸⁾ but not in Japan where the positive rate of BLV antibody in native Japanese beef cattle was higher than that of dairy cattle.⁵⁾ Previous studies showed no significant difference in prevalence of BLV

TABLE 1 *Prevalence of antibodies to selected viruses in Mexican dairy cattle*

STATES	RATE OF REACTORS AGAINST						
	BHV-1 (%)	PIV-3 (%)	BAV-7 (%)	BTV (%)	BRV (%)	BVDV (%)	BLV (%)
Michoacan	9/15 ^a (60.0)	15/15(100)	- ^b	4/10(40.0)	15/15(100)	-	6/15(40.0)
Baja Cali.	10/29(34.5)	23/29(79.3)	-	9/10(90.0)	15/15(100)	-	13/29(44.8)
Hidalgo	13/39(33.3)	33/39(84.6)	55/235(23.4)	1/10(10.0)	27/33(81.8)	80/109(73.4)	459/1251(36.7)
Jalisco	27/40(67.5)	37/49(75.5)	-	3/10(30.0)	45/48(93.8)	-	17/49(34.7)
Nuevo Leon	21/27(77.8)	23/27(85.2)	-	2/10(20.0)	-	-	6/27(22.2)
Queretaro	19/24(79.2)	20/24(83.3)	-	5/10(50.0)	7/7 (100)	-	8/24(33.3)
Guanajuato	23/51(45.1)	37/51(72.5)	-	4/10(40.0)	25/26(96.2)	-	19/51(37.3)
Durango	22/30(73.3)	17/30(56.7)	-	3/10(30.0)	-	-	4/30(13.3)
Est. Mexico	14/22(63.6)	12/22(54.5)	-	8/10(80.0)	-	-	9/22(40.9)
Morelos	-	-	-	-	-	13/23(56.5)	-
Total	158/277(57.0)	217/286(75.9)	55/235(23.4)	39/90(43.3)	134/144(93.1)	93/132(70.5)	541/1498(36.1)

a: Number positive / Number tested

b: Not tested

TABLE 2 Prevalence of antibodies to selected viruses in Mexican beef cattle

STATES	RATE OF REACTORS AGAINST						
	BHV-1 (%)	PIV-3 (%)	BAV-7 (%)	BTV (%)	BRV (%)	BVDV (%)	BLV (%)
Veracruz	49/63 ^a (77.8)	54/72 (75.0)	- ^b	8/10 (80.0)	71/72 (98.0)	49/65 (75.4)	5/72 (6.9)
Sonora Sur	57/125(45.6)	110/155(71.0)	-	3/10 (30.0)	120/155(77.4)	70/115(60.9)	2/155(1.3)
Sonora Norte	22/91 (24.2)	53/102(52.0)	-	1/10 (10.0)	88/102(86.3)	53/74 (71.6)	0/102(0)
Durango	28/135(20.7)	68/135(50.4)	-	4/10 (40.0)	115/137(83.9)	69/107(64.5)	0/135(0)
Baja Cali.	104/165(63.0)	108/167(64.7)	-	10/10 (100)	153/168(91.1)	59/112(52.7)	0/167(0)
Yucatan	49/76 (64.5)	22/76 (28.9)	12/20(60.0)	3/10 (30.0)	74/76 (97.4)	31/51 (60.8)	9/76 (11.8)
Guerrero	96/170(56.5)	130/194(67.0)	-	7/10 (70.0)	146/194(75.3)	19/30 (63.3)	12/194(6.2)
San Luis Potosi	22/38 (57.9)	31/40 (77.5)	-	3/10 (30.0)	39/40 (97.5)	23/40 (57.5)	4/40 (10.0)
Jalisco	53/88 (60.2)	81/94 (86.2)	-	4/10 (40.0)	76/94 (80.9)	20/32 (62.5)	7/94 (7.4)
Coahuila	31/74 (41.9)	61/76 (80.3)	-	4/10 (40.0)	57/76 (75.0)	28/46 (60.9)	4/76 (5.3)
Chihuahua	90/129(69.8)	112/160(70.0)	-	3/10 (30.0)	119/160(74.4)	61/99 (61.6)	8/160(5.0)
Tamaulipas	-	-	38/50 (76.0)	-	-	-	-
Total	601/1154(52.1)	830/1271(65.3)	50/70 (71.4)	50/110(45.5)	1058/1274(83.0)	482/771(62.5)	51/1271(4.0)

a: Number positive / Number tested

b: Not tested

antibodies by breed,³⁾ the difference of prevalence of BLV antibodies in dairy and beef cattle in the present experiments may be caused by the difference in frequency of contact with infected animals as suggested in the previous observation.¹²⁾

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