

Emergence of Clinical Infectious Bovine Rhinotracheitis in Eastern Saudi Arabia

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Keywords

Bovine herpesvirus – Antigen antibody reaction – ELISA – Saudi Arabia.

Summary

Dairy industry in Saudi Arabia is one of the largest in the world. Nevertheless, the situation of infectious bovine rhinotracheitis (IBR) virus infection is not known. It is essential to understand the epidemiology of this disease because of its impact on the dairy industry in the country. The present communication reports for the first time the emergence of clinical IBR in the eastern region of Saudi Arabia. Study projects on the epidemiology of the disease in the country are discussed.

■ INTRODUCTION

Infectious bovine rhinotracheitis (IBR), or “red nose”, is a contagious disease of domestic cattle. It is known to cause major economical damages in cattle production, particularly in the dairy industry. It has also been reported in the swine, goat, water buffalo (4) and in many species of wild ruminants (3). However, its distribution has always been associated with domestic cattle.

Historically, IBR was described as a distinct disease in 1955, in feed-lot cattle in Western USA (7). Soon after, the etiologic virus was isolated (6). However, clinical infectious pustular vulvovaginitis (IPVV) has been known in Europe for years before the discovery of IBR in the USA. The virus is distributed worldwide, although it has been eradicated in some countries.

Beside its abortogenic and other ill effects, IBR can also cause latency in the affected cattle (8). The clinical signs of the disease usually follow an incubation period of 2–4 days. There is fever, serous nasal and ocular discharges, salivation, inappetence and depression. In a few days, the nasal discharge becomes mucopurulent. Necrotic lesions may obstruct the upper airways leading to difficulty in breathing. IBR virus infections may involve the genitalia of both male and female cattle, causing balanoposthitis and PVV, respectively. In cases that are complicated by secondary bacterial infection, pneumonia may develop. Mortality is low and infection can be subclinical.

Following infection, the IBR/IPVV virus remains latent in the trigeminal and sacral ganglia. Viral DNA remains in the neurons of the ganglia for the entire life of the animal. When the animal is exposed to stress, the latent infection could be reactivated and consequently the virus may be shed in the environment to

infect new susceptible hosts. IBR/IPV are caused by a bovine herpesvirus 1 (BHV-1) which is a member of the genus *Varicellovirus* of the family *Herpesviridae*.

The dairy industry in Saudi Arabia is highly developed. For instance, a single dairy farm may contain 6000 cows. In spite of this, studies on an important disease of dairy cattle such as IBR are very scarce there. The last published data about the disease date from the 1980s (1, 2, 4). In the present study, we report for the first time clinical IBR infection in a dairy cattle herd of locally-raised Friesian cattle, at Al-Ahsa oasis in Eastern Saudi Arabia.

■ MATERIALS AND METHODS

During April 2003, six adult, locally-bred, sedentary Friesian cows, in a small dairy farm at Al-Ahsa oasis, in the Eastern region of Saudi Arabia, showed inappetence, depression, salivation, lacrimation and serous nasal discharge. The nasal openings were widely dilated and the nasal mucous membranes were drastically hyperemic (the nose was very red). The mean maximum rectal temperature was 40.6°C. Three days later, the nasal discharge became mucopurulent. The eyes were sunken. Necrotic lesions on the nostrils became ulcerative and the animals showed extensive mouth breathing. The vagina was extremely hyperemic. Two cows were recumbent and died ten days after the onset of the disease. The other four cows recovered within fifteen days from the onset of the disease.

Nasal swabs were collected from each of the affected cows and suspended in F-12 cell culture transport medium, containing antibiotics and fetal bovine serum (FBS). All samples were put in an icebox and immediately sent to the laboratory. The swabs were squeezed and the transport medium was clarified by centrifugation at 1500 g for 15 min. The supernatant fluid was collected, antibiotics added and the mix stored at –86°C.

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Serum samples were collected only from cattle with a known history, because animals in this farm were used for teaching purposes. Some of them were brought from different localities and their health records were not available. Only 14 cows, which were born in the farm and not vaccinated against IBR, had no history of IBR disease. All these animals were older than 18 months, an age at which maternal antibodies are expected to have vanished.

The reference positive sera were produced in bovine against the strains ED1 and Oxford of IBRV (BHV-1) (Central Veterinary Laboratory, Weybridge, UK). The reference negative serum was a bovine serum which was negative in the IBR ELISA test.

For virus isolation, baby hamster kidney (BHK-21) and Vero cell culture monolayers were used in 24-well sterile disposable plastic plates. Each well received 0.1 ml of the inoculum. Following an adsorption of one hour, F-12 maintenance medium containing 2% FBS was added. The cell monolayers were observed daily for presence of cytopathogenic effect (CPE). A second passage was made from the cultures which gave CPE. The isolated virus was titrated in the respective cell culture in which it propagated well. It was actually the BHK-21. Ten-fold dilution series were made in microtiter plates, using F-12 medium without serum as diluent. Fifty microliters of the BHK-21 cell suspension (10⁶ cells/mL) were added. Plates were covered, placed in a CO₂ incubator at 37°C and read after five days. The tissue culture infective dose 50 (TCID₅₀/mL) was calculated as described by Reed and Muench (9). The isolated virus was identified by the virus neutralization test (VNT), as described by Berthe (8), using microtiter plates and BHK-21 cells.

Serum samples were collected from cattle during the acute and convalescent phases of the disease. Sera were also collected from 14 apparently healthy non-vaccinated members of the herd over 18 months of age. Test sera were heated at 56°C for 30 min and stored at -20°C until used.

VNT was conducted to examine the acute and convalescent phase sera. In brief, each serum sample was diluted in a two-fold series in F-12 medium without serum. An equal volume (50 µL) of 100 TCID₅₀ virus suspension was then added. The reactants were incubated for 1 h at 37°C and 18 h at 4°C. This was followed by the addition of 50 µL of the BHK-21 cell suspension (10⁶/mL) to each well. The plates were covered and incubated for five days at 37°C and 5% CO₂.

ELISA was used to examine the 14 serum samples, which were collected from the apparently healthy, non-vaccinated cattle in the affected herd. The HerdChek kit (IDEXX labs, USA) was used for the detection of specific antibodies against the IBRg B virus antigen in the cattle sera. The test was performed according to the manufacturer's instructions. Briefly, it was based on a blocking ELISA system whereby the IBRg B viral antigen was immobilized on the microtiter plate wells. Upon incubation of the test serum samples, in the antigen-coated wells, antibodies specific to IBRg B virus antigen were reacted with a conjugate containing specific monoclonal antibody against the IBRg B virus antigen and horseradish peroxidase. Then, the substrate solution was added to each well and the plates were incubated in the dark. Finally, the reaction was stopped and the plates were read at 450 nm. Positive and negative control sera were included in the test.

The blocking percentage (BP) was calculated as follows:

$$BP = \frac{(MNC_{OD} - MTS_{OD})}{MNC_{OD}} \times 100\%$$

where MNC was the mean optical density reading of negative control serum at 450 nm, and MTS was the mean optical density reading of test serum at 450 nm.

Accordingly, a BP lower than 45% was classified as negative for IBR antibodies. BPs between 45 and 55% were considered doubtful and BPs higher than 55% were considered positive.

■ RESULTS

Within three days postinoculation (PI), the BHK-21 cell culture monolayers gave discernible CPE, which was characterized by cell rounding and shrinkage together with the formation of grape-like cell groupings of rounded cells. One week PI, the whole cell culture sheets were destroyed and harvested. The second passage (P/2) in BHK-21 gave complete destruction of the cells within five days. Virus was isolated from the six ailing cattle.

It took nine days for CPE in Vero cell monolayers to destroy less than 50% of the cell monolayer sheet. So, the authors chose to pursue the virus isolation on BHK-21 cells. The titers obtained for the six isolated viruses in the BHK-21 cells ranged between 10⁵ and 10^{6.5} TCID₅₀/ml. The positive control hyperimmune sera neutralized the ability of each of the isolated viruses to cause CPE in the indicator cells. The non-immune serum failed to do this.

In the detection of antibodies by VNT, the titer of each of the acute phase sera was less than 0.3 log₁₀. The four convalescent-phase sera were positive and gave a titer of 1.2 to 1.8 log₁₀, with a mean of 1.5 log₁₀.

Table I shows ELISA results in the 14 sera from the non-vaccinated, apparently healthy cattle from the affected herd. Seven samples (50%) were positive for IBR antibodies, six (43%) were

Table I

ELISA results for presence of IBR antibodies in the sera collected from apparently healthy non-vaccinated cattle in the affected farm

Serum No	BP (%)	Judgment
1	17.3	-
2	90.8	+
3	74.0	+
4	72.7	+
5	5.3	-
6	79.4	+
7	51.0	SUS
8	72.4	+
9	89.3	+
10	95.1	+
11	14.7	-
12	41.3	-
13	18.5	-
14	15.4	-
RPS	95.8	+
RNS	0	-

RPS = reference positive serum
 RNS = reference negative serum
 + = positive
 - = negative

negative and one was suspect (51%). BP values for positive sera ranged from 72.4 to 95.1%. Three of the positive sera had very high BP values (95.1, 90.8 and 89.35%) that were near the BP value of the reference positive serum, which was 95.8%. The remaining four positive sera had BP values between 72.4 and 79.4%.

■ DISCUSSION

Results confirmed that the disease in the present outbreak was due to an IBRV infection. This is the first confirmed record of the disease in the Eastern region of Saudi Arabia. This information is expected to cast light on the disease situation, at least in the Eastern region. At the present time, any information regarding the situation of IBR in the country would be of great value, because of the lack of published data on the subject during the last two decades.

It is difficult to determine the source of the present outbreak. However, it could be due to the reactivation of a latent infection (5). This is highly likely, as there was no recent introduction of new animals in the affected farm. On the other hand, results of the limited serological survey on the non-vaccinated, apparently healthy 14 cattle from the affected herd indicated that a high percentage (50%) were exposed to IBR infection and that some of them gave high BP values which were comparable with the reference positive serum. As no cattle were recently introduced into the farm, this high seroconversion rate could most probably indicate shedding of the IBR virus by carrier cattle in the herd.

As mentioned previously, the dairy industry is a major business in Saudi Arabia. However, studies on the impact of IBR infections on the dairy industry are lacking. On the other hand, some farms practice vaccination against IBR, which will complicate further the epidemiological picture of the disease in the country. Although this study shows the presence of IBR in Saudi Arabia, its epidemiology

has not been studied yet. A nation-wide epidemiological study is therefore urgently needed to help clarify the real disease situation in the country. This is particularly important considering the damages it can cause in the dairy industry.

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Résumé

Abu Elzein E.M.E., Housawi F.M.T., Al-Afaleq A.I., Al-Musa J. Emergence de la rhinotrachéite bovine infectieuse dans l'est de l'Arabie Saoudite

L'industrie laitière de l'Arabie Saoudite est l'une des plus importantes au monde. Malgré cela, la situation actuelle sur les infections par le virus de la rhinotrachéite bovine infectieuse (IBR) n'est pas connue. Il est essentiel de comprendre l'épidémiologie de cette maladie à cause de son impact sur l'industrie laitière dans le pays. Cette communication rapporte pour la première fois l'émergence d'IBR clinique dans la région est de l'Arabie Saoudite. Des projets d'étude concernant l'épidémiologie de la maladie dans le pays sont discutés.

Mots-clés : Herpesvirus bovin – Réaction antigène anticorps – Test Elisa – Arabie Saoudite.

Resumen

Abu Elzein E.M.E., Housawi F.M.T., Al-Afaleq A.I., Al-Musa J. Aparición de la forma clínica de la rinotraqueitis infecciosa bovina en el este de Arabia Saudita

La industria lechera en Arabia Saudita es una de las más grandes del mundo. Sin embargo, se desconoce la situación de la infección viral por rinotraqueitis infecciosa bovina (IBR). Es esencial comprender la epidemiología de esta enfermedad debido a su impacto sobre la industria lechera en el país. La presenta comunicación reporta por la primera vez la aparición de la IBR clínica en la región este de Arabia Saudita. Se discuten proyectos de estudio sobre la epidemiología de la enfermedad en el país.

Palabras clave: Herpes virus bovino – Reacción antígeno anticuerpo – ELISA – Arabia Saudita.