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CLINICAL OBSERVATIONS AND SIGNIFICANCE OF BHV-1 INFECTION IN SMALLHOLDERS OF CATTLE IN AL-AHSA REGION, KSA

(With 4 Tables)

By

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(Received at 15/9/2010)

**ملاحظات اكلينيكية وأهمية العدوي بفيروس الهربس البقري-1 في الأبقار في
تجمعات الأبقار الصغيرة بمنطقة الاحساء بالمملكة العربية السعودية**

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يعتبر فيروس الهربي البقري- 1 المصنف بعائلة فيروسات الهربس من اخطر الأمراض التي تصيب الماشية. تظهر العدوي بهذا الفيروس في صور اكلينيكية متعددة منها: التهاب الأنف والحنجرة- الاجهاض- الالتهاب الحبيبي للاعضاء التناسلية في الذكر والأنثي- العدوي العامة للعجول حديثي الولادة. في هذه الدراسة تم فحص عدد 259 حالة من صغار المربين في منطقة الاحساء بالمملكة العربية السعودية في الفترة من ابريل 2007- ابريل 2010 حيث تمثل هذا العدد ب 159 بقرة و 100 عجل ذكر وعجول رضية. سجلت نسبة الاصابة بالمرض 74 حالة من الاناث (46.6 %) و 24 حالة في الذكور والعجول الرضية 24 حالة (24 %). تم تأكيد العدوي بعزل فيروس الهربس البقري وتصنيفه سيرولوجيا في المعمل. يمكن الخلاصة بان قطاع صغار المربين للماشية في منطقة الاحساء يمثل جزء كبير في انتاج الالبان واللحم. الفخسائر الاقتصادية علي مستوي مربي الماشية الصغار ليست محسوبة رسميا وهي في الواقع كبيرة لذلك فان هذه الدراسة توجب الاهتمام بقطاع مربي الماشية علي كل المستويات الصغيرة ووضع سبل للمقاومة مثل عزل الحيوانات المريضة واتباع سياسة التحصين للمرض كما هو متبع في مزارع الالبان الكبيرة.

كلمات كشاف:

(فيروس الهربس البقري- 1 والأعراض الاكلينيكية وصغار مربيالماشية وعزل وتصنيف
الفيروس)

SUMMARY

Bovine herpesvirus 1 (BoHV-1), classified as an alphaherpesvirus, is a major pathogen of cattle. Primary infection is accompanied by various clinical manifestations such as infectious bovine rhinotracheitis, abortion, infectious pustular vulvovaginitis, and systemic infection in neonates. In this study A total of 259 animal in small holder cattle sector in Al-Ahsa region, KSA were under investigation form April, 2007 till April 2010. The total number of observed cattle was 259 (159 females and 100 males). The clinical signs of the disease was observed in 74 (46.6 %) females and 24 (24 %) males. The clinical symptoms are described in both male and female animals as well as young calves. BHV-1 infection was confirmed by isolation and serological identification of the virus. It can be concluded that the sector of smallholders cattle breeding constitutes an important part for cattle production on both dairy and beef cattle in Kingdom Saudi Arabia. The economic losses in this sector due to infectious diseases are really high but unfortunately not estimated. BHV-1 infection in cattle is one of the problems that threads and persist in small holders cattle causing sever economic losses and constitutes a major source of infection to industrial cattle farms. This is probably due to the absence of application of control program for infectious diseases as for example regular vaccination and isolation of infected animals.

Key words: *BHV-1, clinical observation, small holder, virus isolation*)

INTRODUCTION

Bovine herpesvirus 1 (BoHV-1), classified as an alphaherpesvirus, is a major pathogen of cattle. Primary infection is accompanied by various clinical manifestations such as infectious bovine rhinotracheitis, abortion, infectious pustular vulvovaginitis, and systemic infection in neonates (Muyalkens *et al.*, 2007; Nandi *et al.*, 2009). All BoHV-1 strains isolated hitherto belong to one single viral species, and are classified in three subtypes BoHV-1.1, BoHV-1.2a and BoHV-1.2b. Although most BoHV-1.1 strains have been isolated from respiratory tract diseases or abortive cases and BoHV-1.2 strains from genital organ lesions, the only reliable distinctive criterion is the viral DNA analysis by restriction endonuclease fingerprinting (Edward *et al.*, 1991; Metzler *et al.*, 1985; Miller *et al.*, 1991). Calves infected experimentally by the nasal route with BoHV-1.2 strains showed respiratory clinical signs (Engels *et al.*, 1981; Spilki *et al.*, 2004) and were able to transmit the respiratory infection to control calves (Smith *et al.*, 1980; Edwards *et al.*, 1991). Otherwise, reproductive tract

lesions in heifers were observed after intrauterine inoculation with BoHV-1.1 (Miller and Van der Maaten 1984). Subtypes 1.1 and 1.2a have been associated with severe diseases including infection of the fetus and abortion (Miller *et al.*, 1991). The subtype 1.2b was not associated with abortion (Whetstone and Miller 1989; Edwards *et al.*, 1990; Smith *et al.*, 1995). BoHV-1 is responsible for significant losses incurred by disease and trading restriction in the cattle industry (Bowland and Shewen, 2000). BoHV-1 is one of the eight herpesviruses isolated hitherto from naturally infected cattle (Tab. I). All members of the family Herpesviridae share a common virion morphology based on an icosahedral capsid symmetry, a cell-derived envelope containing virally encoded membrane proteins and a tegument as protein made matrix connecting the capsid and the envelope. BoHV-1 belongs to the extensive subfamily of Alphaherpesvirinae which contains viruses characterized by a relatively large host range, a short replication cycle and the ability to induce latent infection mainly, but not exclusively, in neurons.

Table 1: Herpesviruses isolated from naturally infected cattle after Muyalkens *et al.* (2007).

| Virus species Disease | Herpesvirus subfamily | Disease following primary infection |
|--|-----------------------|---|
| Cattle as natural host | | |
| Bovine herpesvirus 1 (BoHV-1) | Alpha | Infectious bovine rhinotracheitis |
| Bovine herpesvirus 2 (BoHV-2) | Alpha | Bovine mammillitis Pseudo lumpy skin disease |
| Bovine herpesvirus 4 (BoHV-4) | Gamma | Not determined |
| Bovine herpesvirus 5 (BoHV-5) | Alpha | Bovine herpesvirus encephalitis |
| Bovine lymphotropic herpesvirus (BLHV) | Gamma | Not determined |
| Cattle as foreign host | | |
| Alcelaphine herpesvirus 1 (AIHV-1) | Gamma | Malignant catarrhal fever |
| Ovine herpesvirus 2 (OHV-2) | Gamma | |
| Suid herpesvirus 1 (SuHV-1) | Alpha | Aujeszky's disease |

Bovine herpesvirus 5 (BoHV-5) is an alphaherpesvirus responsible for meningoencephalitis in young cattle and is closely antigenically and genetically related to bovine herpesvirus 1 (BoHV-1). Both viruses have common aspects in their pathogenesis: (1) they infect epithelial cells at the

portal of entry and (2) they establish a latent infection in the sensory nerve ganglia, i.e., the trigeminal ganglia (Zajac, *et al.*, 2010). The severity of the disease caused by BoHV-1 is influenced by several factors such as the virulence of the BoHV-1 strain (Kaashoek *et al.*, 1996), resistance factors of the host, especially the age, and potential concurrent bacterial infection. Subclinical BoHV-1 infections are common. Several BoHV-1 strains display a poor ability to induce clinical signs and were classified as weakly virulent strains in a comparative virulence experiment (Kaashoek *et al.*, 1996). Otherwise, these discrete clinical pictures can also be explained by the primary infection of passively immune calves in countries where BoHV-1 is endemic. Indeed, colostral immunity is known to protect efficiently infected animals from clinical signs (Mechor *et al.*, 1987; Lemaire *et al.*, 2000). The latency reactivation cycle of herpesviruses has a deep epidemiological impact since it is responsible for the maintenance of BoHV-1 in a cattle population. BoHV-1 infection of new generation cattle by latent carriers submitted to reactivation stimulus may occur at several occasions as for example at birth (Spilki *et al.*, 2004), mating, during transport (Thiry *et al.*, 1987) or following the introduction of heifers into the group of dairy cows. Therefore the detection of BoHV-1 latent carriers is a concern in the setting up of a BoHV-1 control program.

The sector of smallholders in cattle breeding constitutes an important part for cattle production on both dairy and beef cattle in Kingdom Saudi Arabia. The aim of the present work was planned to fulfill the followings: recording and investigate the clinical observations of BHV-1 in smallholders cattle in Al-Ahsa` region, KSA, collection of information about current control program in this sector, confirmation of clinical cases through virus isolation and identification, and finally recommendation to control the disease in smallholders cattle in KSA.

MATERIALS and METHODS

1- Animals:

The description of the animals is very difficult because they collected from different small system that has irregular management system and there were no confirmed data about any other infection during the period of observation.

A total of 259 animal were under investigation form April, 2007 till April 2010. Details of information are found in the Table (2).

Table 2: Animals under investigation

| Year | Total Animal examined | Sex | Age average |
|-------|-----------------------|---------------------------|--|
| 2007 | 24 | 18 males & 6 female | 3 years 3.5 years |
| 2009 | 70 | 42 males & 28 females | 3 years 3.5 years |
| 2010 | 165 | 99 males & 66 females | 3 years 3.5 years 4 animals under sex months |
| Total | 259 | 159 males & 100 female | 3 years 3.5 years 4 animals under sex months |

2 - Virus isolation and identification:

Buffy coat were collected from feverish animals and inoculated into MDBK cell line in which the monlayer cell culture were grown on Eagle s MEM supplemented with 3 % newborn calf serum. After 48 hours positive samples showed cytopathogenic effect in the cell line that begins with cell rounding that advances to for grape-like structures, beside the formation of synthetial cell (Giant cells).

For confirmation of the specificity of CPE, specific antiserum to BHV-1 (Kindly Supplied by Institute of Virology, Hannover Veterinary School, Germany) was added to the buffy coats and incubated for 30 minutes at 37°C before inoculation to the cell culture. Colorado strain of BHV-1 kindly supplied by Institute of Virology, Hannover Veterinary School, Germany was used as positive control. The CPE were inhibited in both positive samples and the positive control.

3 - Sero-conversion:

Antibodies to BHV-1 were detected in the sera of convalescent animals using a commercial ELISA kits produced by Institute Pourquier, France.

RESULTS and DISCUSSION

Bovine herpesvirus 1 (BoHV-1),is a pathogen of cattle associated with two major syndromes, called infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV), and a variety of clinical signs, such as conjunctivitis, encephalitis and abortions (Pastoret *et al.*, 1982). IBR is a disease of major economic concern in many parts of the world band especially in Europe, both in countries where this infection has been eradicated and in those where the control of IBR is currently or will be

undertaken (Thiry *et al.*, 1999). Table (3) describes the different clinical symptoms of BHV-1 infection observed in smallholder cattle in Al-Ahsa region, KSA. The table summarizes the main items of symptoms with the percentage in the clinical diseased animals. The described symptoms are quite the same that recorded by many authors (Gibbs and Rweyemamu, 1977; Millerand Van der Maaten 1986; Bryan *et al.*, 1994; Hage *et al.*, 1998; Radostitis *et al.*, 2007; Muylkens *et al.*, 2007 and Nandi *et al.*, 2009). Table (4) illustrates the percentage of clinical disease of BHV-1 in small holder cattle in Al-Ahsa region, KSA during 3 years observation (April, 2007- April, 2010). The total number of observed cattle was 259 from which 159 females and 100 males. The clinical disease was observed in 74 (46.6 %) females and 24 (24 %) males. Serological evidence of BHV-1 infection in dairy cattle in Kingdom Saudi Arabia was recorded by Frericks *et al.* (1982) and (1983). The isolation of the BHV-1 from dairy cattle in Kingdom Saudi Arabia was confirmed by Hafez and Chaudry (1985). The emergence of clinical infectious bovine rhinotracheitis in the eastern region of the Kingdom Saudi Arabia was recorded for the first time by Abu-Elzein *et al.* (2008). They suggested that the source of the outbreak could be due to the reactivation of a latent infection. 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. The sector of smallholders in cattle breeding constitutes an important part for cattle production on both dairy and beef cattle in Kingdom Saudi Arabia. The absence of clear regulation in this sector makes difficulty in controlling infectious diseases. On the same time the economic losses (due to mortality in newborn animals, abortion and others) in this sector due to infectious diseases are really high but unfortunately not estimated. BHV-1 infection in cattle is one of the problems that threads and persist in small holder cattle causing sever economic losses and constitutes a major source of infection to industrial cattle farms. This is probably due to the absence of application of control program for infectious diseases as for example regular vaccination and isolation of infected animals.

Table 3: Different clinical symptoms observed in 98 cattle infected with BHV-1 virus.

| Clinical symptom | Number of animal | % in diseased animal |
|---|------------------|----------------------|
| 1) Fever (up to 42°C) with anorexia | 92 | 93.88 % |
| 2) Sever hyperemia of nasal mucous membrane | 83 | 84.69 % |
| 3) Serous nasal discharge from eyes and nose | 66 | 67.35 % |
| 4) Increased salivation | 52 | 53.06 % |
| 5) Grayish foci of necrosis on the mucous membrane of the nasal septum inside the external nares. | 81 | 82.65 % |
| 6) A drastic fall in milk yield | 20 | 20.41 % |
| 7) A short, explosive cough | 16 | 16.33 % |
| 8) Uni-or bilateral conjunctivitis | 25 | 25.51 % |
| 9) Erosions covered with tenacious mucous of soft palate | 14 | 14.29 % |
| 10) Pharyngitis with tenacious mucopurulent exudates | 28 | 28.57 % |
| 11) Bronchopneumonia (loud breath sound, crackles and wheezes) | 18 | 18.37 % |
| 12) Abortion, retention of placenta and Endometritis | 6 | 6.12 % |
| 13) Necrotic lesions on the mucosa of vulva and vagina of cows, penis and prepuce of bulls, they are gray translucent raised foci, erode, ulcerate and become confluent | 34 | 34.69 % |
| 14) Obvious signs of pain (arched back holding the tail out stiffly) and frequent urination | 12 | 12.24 % |

Table 4: Percentage of clinically diseased animals by BHV-1 in smallholder cattle in Al-Ahsa region, KSA.

| Year | Total Animal examined | Sex | Age average | Number of diseased animals | Percentage of clinical disease |
|-------|-----------------------|------------------------|----------------------------|----------------------------|--------------------------------|
| 2007 | 24 | 18 males & 6 female | 3 years | 16 | 88.9 % |
| | | | 3.5 years | 4 | 66.7 % |
| 2009 | 70 | 42 males & 28 females | 3 years | 25 | 59.5 % |
| | | | 3.5 years | 11 | 39.3 % |
| 2010 | 165 | 99 males & 66 females | 3 years | 33 | 33.3 % |
| | | | 3.5 years | 9 | 13.6 % |
| | | | 4 animals under sex months | | |
| Total | 259 | 159 males & 100 female | 3 years | 74 | 46.5 % |
| | | | 3.5 years | 24 | 24 % |
| | | | 4 animals under sex months | | |

ACKNOWLEDGEMENT

We acknowledge the Deanship of Scientific research, King Faisal University for the support to fulfill this research work (Project Number: 5022) for the academic year 1427-1428.

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