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Isolation of Bovine Herpes Virus-1 in Sudan

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Abstract: During this study swab samples were tested for demonstration of Infectious Bovine Rhinotracheitis/ Infectious Pustular Vulvovaginitis (IBR/IPV) virus (BHV-1) from different regions of the Sudan. Samples were collected from cases suffering from infertility, nasal discharges, vaginal discharges, lacrimation and abortion. The virus was isolated from five cases with typical signs of the disease including two from Niyala, two from Atbara and one from West Kordofan. Also using SNT, ELISA and PHA testes neutralizing antibodies were found, which confirmed the presence of the disease.

Key words: BHV-1, infectious, isolation sudan, IBR/IPV

INTRODUCTION

Bovine Herpes Virus-1 (BHV-1) infection or Infectious Bovine Rhinotracheitis (IBR) is an acute contagious febrile viral disease of domestic and wild bovids. The disease was firstly recognized in the United States during the 1950s[1] and it had since then recognized from many countries throughout the world. It is a disease of economical importance characterized by intensive inflammation of the upper respiratory tract accompanied by dyspnoea, depression, nasal discharge and loss of condition. BHV-1 may also cause an acute gastroenteritis, conjunctivitis, encephalitis, abortion and mastitis[2,3]. The virus can also infect the genital tract and cause pustular vulvovaginitis in females and balanoposthitis in males. The morbidity is extremely high (more than 80%), while mortality is low (10%), but it is high when co-infection or secondary infections occur like with Bovine Viral Diarrhea (BVD) virus and Pasteurella sp[4] .

In Sudan the first isolation of IBR virus was in 1983 from Khartoum University farm[5]. Hassan and Khalda[6], recorded that the disease in Sudan may be confused clinically with rinderpest. The evidence of the disease was supported by detecting neutralizing antibodies in Sudanese cattle whose prevalence in the same areas reached up to 50% with an overall average of 21%[7,8]. This study carried out to study the excistance of BHV-1 virus among cattle using isolation in tissue culure

MATERIALS AND METHODS

Field samples: Samples were collected from suspected IBR disease cases suffering from fever (temperature ranges from 40–41 °C), diarrhea, nasal discharges respiratory signs and depression, lacrimation and death. Other samples were also collected from cases suffering from infertility with uterine lesion and case history of abortion. The examined samples included; sera for detection of neutralizing antibodies, and swabs for virus isolation.

Sera: The collected blood samples were allowed to clot over night at 4°C and then centrifuged at 2000 rpm for 15 min. The serum was separated into sterile bijou bottles, inactivated in water bath at 56°C for 30 min and stored at -20°C till used.

Preparation of samples for virus isolation: As showm in Table 1 eighty one (81) samples were collected from different localities in Sudan. The swabs were vigorously rubbed against the mucosal surfaces and immediately immersed in tubes with 2 mL transport medium. They were then transported on ice to the laboratory where they were left to soak overnight at 4°C before being extracted and stored at -20°C until required for cell culture inoculation.

A lymph node was collected to detect the presence of IBR virus. It was prepared aseptically, the organ was cut into small pieces by sterile scissors, grounded by mortar and pestle and sterile sand then sterile PBS with antibiotics was added, homogenized into a 20% suspension and centrifugated at 1000 rpm for 10 min. The supernatant was then used for virus isolation in cell culture.

Antigen and antisera

Los Angeles reference strain: Los Angeles reference strain (received in 1990 from Prof. Bartha Adorjan, Hungarian Veterinary Research Institute, Hungary

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Table 1: Samples collected for virus isolation from different localities in Sudan

Location	NS	VS	Total
Khartoum	40	11	51
River Nile	2	7	9
Madani	1	2	3
Elobied	-	13	13
Niyala	-	2	2
Abiai	-	3	3
Total	43	38	81

NS: Nasal Swab, VS: Vaginal Swab

Budapest) was kindly supplied by Department of Viral Vaccine Production, Central Veterinary Research Laboratory (CVRL). The virus was grown in bovine kidney cell and used as Ag in PHA test and SNT.

IBR positive control antiserum: Reference bovine antiserum to BHV-1 was obtained from the Central Veterinary Lab. (CVL), New Haw, Weybridge, UK was used.

Negative control serum: Neonatal calf serum obtained from Biological Diagnostic Supplies LTD (BDSL). Flow Laboratories and Institute for Animal Health (2000), Pirbright, England was used.

Isolation in tissue culture: Primary and secondary Bovine Kidney Cells (BKC) were the main cells used in this study. Bovine kidneys obtained from calves 1-4 weeks old were prepared according to standard technique of FAO [9].

For virus isolation, the swab samples were centrifuged at 1500 rpm for 10 min and 0.2 mL from the supernatant was used to inoculate the confluent monolayer tissue culture tubes. The tubes were subsequently incubated at 37°C for 1 h. The inoculum was then removed and maintenance medium with out serum was added. The cultures were incubated at 37°C and examined daily for CPE, which usually completely covers the sheet within 1-2 days after inoculation. The negative samples were observed daily for 10 days and after 5 blind passages if no CPE appeared then the sample were declared as negative.

Two passages were carried in BKC at the CVRL. Further three passages[3-5] were cultivated in Madin Darby Bovine Kidney (MDBK) at the Viral Disease Research Unit, Faculty of Veterinary Medicine, Cairo University, Egypt and presence of BHV-1 was confirmed by direct Fluorescent Antibody Technique (FAT).

Fluorescent Antibody Technique (FAT): Bovine kidney cells were cultured in 6-well tissue culture plate containing immersed coverslips (22x22 mm) and incubated at 37°C. Freshly prepared inoculum from suspected swab sample was added to coverslips after removing the medium and incubated at 37°C for 1 h. GMEM free from serum was added to culture after discarding the inoculum and washing with PBS, non-infected cell cultures were used as controls. The coverslips were incubated for 3-5 days, removed and stained by direct FAT acording to Peter *et al.*[10]

Infected and control coverslips were removed, washed in PBS and dried. Then they were fixed with acetone solution (9 volumes of acetone and 1 volume of water) for 15 min at room temperature, rinsed in PBS and left to dry. Fluorescein-labelled conjugate (Mouse monoclonal FITC anti BHV-1/IBR) obtained from Bio-X Diagnostics SPRC, Belgium (Europe) was added to the samples and left for 1 hour at room temperature. At the end of this incubation period samples were rinsed in PBS solution, dried and covered by mounting medium prepared by mixing glycerol and PBS 9:1. The prepared coverslips were examined immediately using fluorescence microscope (Zeiss, Axiolab).

Serology: Passive Haemagglutination (PHA) test and Serum Neutralization Test (SNT) was used according to Zyambo *et al.*[11] commertial enzyme linked immunosorbent Assay (ELISA) was used.

RESULTS

Virus isolation: BHV-1 was recovered from Niyala, Atbara and West Kordofan as in (Table 2).

Serology: Sera collected from cases from which the virus isolated were found to have high detectable antibodies to IBR when tested by Serum Neutralization Test (SNT), Enzyme Linked Immunosorbent Assay (ELISA) and Passive Heamagglutination test (PHA).

DISCUSSION

In the present study, five isolates of BHV-1 were recovered from naturally infected cases showing clinical symptoms of the disease. Gross and microscopic lesions were consistent with those reported by many workers for IBR virus[12]. Two of these isolates were from Niyala and one from West Kordofan. The samples were collected in the rainy season from local breeds having typical signs of

Table 2: The combarative between localities where the BHV-1 isolated

Locality	Type of sample	No. of samples	No. of isolates	Main clinical signs
Niyala	Vaginal swab sample	3	2	Infertility and necrotic lesions in the vagina.
Atbara	Vaginal swab sample	6	2	One case aborted and the other suffering from infertility.
West Kordofan	Lymph node	1	1	nasal discharges, corneal obesity followed by blindness, lacrimation and after few days the animal recovered from the disease.

IBR including infertility, history of abortion, congestion of the vagina with vaginal discharges beside gross pathological lesions, lacrimation and nasal discharges. These findings agreed with Hassan and Khalda,[6] as they recorded the disease in Western Sudan during sero-surveillance in 1981/1982. These observations indicated that the disease exists in this area may be due to high density of animal populations. In addition, the presence of carriers like sheep and goats among the natural host (cattle) supported the disease occurrence. In this direction BHV-1 was isolated from nasal swabs of two goats with natural respiratory disease, also ocular swabs of goats with keratitis[13]. The virus was also isolated from Atbara (River Nile state) in the drying season that indicates the possibility of the existence of the disease in this season, so the disease must not be ignored.

The isolation of the virus in the rainy season indicated the important role of animals movement, accumulations and the frequent contact in the reproductive season, which provide more opportunity for virus transmission, resulting in a higher incidence of infection.

The IBR viruses isolated in this study showed CPE after seven blind passages in cell culture, this was because the virus needed to be adapted to the tissue culture or the quantity of the IBR virus in the swab was very low. This as in agreement with Hazarati and Amjadi[14]. that further serial passages in the same cell culture were needed to reproduce CPE more evidently in newly isolated IBR virus.

Serological examination compaired to the samples from which the virus isolated were obtaining detectable neutralizing antibodies when tested by PHA, ELISA and SNT confirming virus isolation.

CONCLUSIONS

IBR is widespread in Sudan and this was supported by the isolation of the virus from suspected cases in three widely separated states during this investigation. The present studies lend support to earlier studies that showed the wide prevalence of IBR antibodies in Sudanese cattle[7,8].

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