Studies on a field problem of mixed viral agents IBR and BVD viruses in cattle suffered from respiratory and reproductive disorders.

By

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SUMMARY

A total of 58 serum samples, 25 nasal swabs, 25 vaginal swabs and tissues (lung, liver and spleen) of 5 aborted foeti were collected from diseased animals suffered from respiratory and reproductive disorders from different farms at 3 governorates of Egypt (Behera- Alexandria and Qaliubia) were subjected to different serological and virological studies in order to detect main cause of this field problem.

Serum samples were tested firstly by serum neutralization test (SNT) for detection antibodies of BHV-1 and BVD viruses, all farms found to be contain positive animals for both viruses. The highest percent was recorded in Behera (90%) for BHV-1 and (80%) for BVD while the lowest percentage was recorded in Alexandria (60%) for BHV-1 and (50%) for BVD. The 50 swabs (nasal and vaginal) and 5 tissues from aborted foeti were tested by Capture ELISA for the antigen detection of BVD using commercial ELISA kits. Results of ELISA were highest in the swabs 40 out of 50 as well as 4 out of 5 tissue samples.

The swabs and tissues were subjected to virus isolation on MDBK cell lines and BHV-1 was successively isolated from 15 swabs while BVD was successively isolated from 10 swabs and both viruses were isolated from 3 aborted tissues. Identification finally was obtained by using direct fluorescent antibody technique (FAT).

The respiratory and reproductive disorders were diagnosed as viral causes and the solution suspected to face this field problem in the future is the successful program of vaccination, otherwise the animals will continuously shedding viruses between populations and suffered from diseases combined with decreasing the production.

INTRODUCTION

Bovine Viral Diarrhea (BVD) has a world wide spread and is the most important disease of cattle Nettleton and Entrican (1995). The Flavivirus bovine viral diarrhea (BVD) virus exists in two biotypes, Cytopathic (CP) and Non-Cytopathic (NCP) as defined by the-
Effect on cultured cells (Perler et al., 2000).

The virus is belonging to genus *Pestivirus* which constitutes with genus *Flavivirus* and genus *Hepacivirus* the family *Flaviviridae* (Ramirez et al., 2001).

The initial clinical signs associated BVD virus were anorexia and diarrhea of varying severity and duration were recorded in chronically affected cattle, while persistently infected cattle with BVD are the major source of virus transmission to susceptible animals (Lindberg et al., 2006).

The economic loss in dairy farms associated with bovine viral diarrhea virus (BVDV) is believed to be high in Abortion rates, rates of calving induction, the time from calving to conception, and the number of services per conception increased, whereas milk production decreased with increasing BVDV antibody and a greater incidence of production of disease because of BVD-induced immune suppression (Heuer et al., 2007).

Bovine viral diarrhea virus (BVDV) has a great economic impact in cattle industry. The goal of BVDV control and eventual eradication will be the detection of BVDV infections, particularly targeting persistently infected animals (Driskell and Ridpath, 2006).

Infectious bovine rhinotracheitis (IBR) is caused by BHV-1 which belongs to the *Alphaherpesvirinae* subfamily (Jones, 1998).

Bovine herpesvirus-1 (BHV-1) is one of the major respiratory pathogens in cattle worldwide (Van Drunen Little and Van den Hurk, 2007).

IBR is characterized by clinical signs of upper respiratory tract such as muco-purulent nasal discharge and conjunctivitis, signs of illness are fever, depression, inappetence, abortions and reduced milk yield. Mortality is low (OIE, 2000).

Infections caused by BHV-1 are very common in Europe, but the disease pattern is quite different: the diseases of the genital tract are most common. BHV1 infections can occur simultaneously with bovine virus diarrhea (BVD) and/or parainfluenza-3 (PI 3) virus (Straub, 1991).

A competitive ELISA was developed and compared with SNT. There was 96.7% agreement between the two tests. The relative sensitivity of the ELISA compared to SNT was 95.2% and the relative specificity was 99.4%. Also the ELISA was quicker and cheaper than the serum neutralization test (Horner and Orr, 1993).

ELISA was used for cytopathic and non-cytopathic bovine diarr-
rhea virus strains used in inactivated vaccines. The ELISA enables the detection of BVDV antigens and shows the potential for in vitro testing of inactivated BVD vaccines in place of the currently required host animal testing (Ludermann and Katz, 1994).

Direct immunofluorescence technique was used for examination of nasal swabs obtained from 95 cattle from 11 herds with respiratory disease for BVDV. Positive samples were detected in 7 of 11 herds (Alkan et al., 2000).

Serological diagnosis is very important for the detection of BVDV as an important pathogen related to reproductive failure. Methods normally used for detection of antibodies are serum neutralization (SN) and ELISA. ELISA-BVDV shows good sensitivity, specificity and reliability. It is easy to transfer, economical and easy to perform (Pacheco and Lager, 2003).

An inactivated BHV-1 vaccine can protect against abortion resulting from a substantial challenge infection, with efficacy similar to that of modified-live BHV-1 vaccines (Zimmerman et al., 2007).

The target animals and vaccination regimes for vaccines against the bovine rhinotracheitis (IBR) and the bovine viral diarrhea virus (BVDV) are very similar. The two vaccines can be applied at the same day for the first or second dose of the BVD basic vaccination and then at the booster vaccinations (third dose onwards) Alvarez et al., (2007).

The present work aimed to throw light on the main cause of field problem of animals suffered from respiratory and reproductive disorders and to find suitable solution. Serum samples were collected from the infected cattle from 3 governorates (Behera, Alexandria and Qaliubia) were subjected to SNT for detection antibodies against BHV1 and BVD Viruses. Nasal, vaginal swabs and tissues from 5 aborted foeti were examined by Capture ELISA for BVD virus using commercial ELISA kits. These swabs and tissues were subjected to virus isolation on MDBK cells followed by identification by direct fluorescence antibody technique (FAT).

MATERIALS AND METHODS

1- samples:
A- Serum Samples:
A total of 58 serum samples were collected from infected animals distributed in 3 governorates (Behera, Alexandria and Qaliubia) for the detection of antibodies against BHV-1 and BVD viruses by using SNT table (1). Serum samples were treated by heat at 56°C for 30 min. before tested.
B- Nasal and Vaginal Swabs:
A total of 25 nasal and 25 vaginal swabs were collected from infected animals and subjected to Capture ELISA and virus isolation for BHV-1 and BVD table (1).

The swabs were immersed in Hank's balanced salt solution (SIGMA), the swabs were centrifuged and 0.2 ml of supernatant was used as inoculums for virus isolation.

C-Tissues:
A total of tissue samples (lung, liver and spleen) were collected from 5 aborted faeti and used for Capture ELISA and virus isolation for BHV-1 and BVD table (1).

Table (1): Field samples for serological and virological investigations collected from infected animals.

<table>
<thead>
<tr>
<th>Egyptian Governorates</th>
<th>No. of Serum</th>
<th>No. of Nasal Swabs</th>
<th>No. of Vaginal swabs</th>
<th>No. of Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Behera</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>2- Alexandria</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>3- Qaliubia</td>
<td>18</td>
<td>5</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>25</td>
<td>25</td>
<td>5</td>
</tr>
</tbody>
</table>

2- Cell culture:
Madin Darby Bovine Kidney (MDBK) cell line was used for virus isolation. The cell line was supplemented with Eagle's Minimum Essential Medium MEM (SIGMA) supplemented with 10% new born serum (for growth) or 2% serum (for maintains), 100 µg/ml streptomycin sulphate and 100 IU/ml penicillin G.

3- Detection of BHV-1 and BVD antibodies:
By using Serum Neutralization Test (SNT): It was carried out according to Pierre and Michel (1993) for detection of antibodies against BHV-1 and BVD using reference viruses.

4- Detection of BVD viral antigen:
ELISA test was adopted by using Commercial kit (ELISA Detection of NS-P2-3 and EO of the Bovine Viral Diarrhea/ Mucosal Disease Virus). Principle, procedure, reading and interpretation of results were made according to the instruction procedure supplied by Pourquier Institute. This test was applied for 50 nasal and vaginal swabs as well as tissues collected from 5 aborted foeti.
5- Virus Isolation:
   All 25 nasal, 25 vaginal swabs and tissues (lung, liver, spleen) from 5 aborted faeti were inoculated and propagated for three successive passages on MDBK cell line for BHV-1 according to OIE (2000) and for BVD according to Cortese et al., (1998).

6-Identification of virus isolates:
   Direct Fluorescent Antibody Technique (FAT): It was carried on fixed tissue culture cells infected with suspected samples. The test was carried out according to the method described by Majewska et al. (1984).

RESULTS

1- Table (2): Illustrate the prevalence of BHV-1 and BVD antibodies among infected cattle in 3 governorates by SNT.

<table>
<thead>
<tr>
<th>Governorates</th>
<th>No. of Serum</th>
<th>No.&amp;% of +ve for BHV1</th>
<th>No.&amp;% of +ve for BVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Behera</td>
<td>20</td>
<td>18 (90%)</td>
<td>16 (80%)</td>
</tr>
<tr>
<td>2- Alexandria</td>
<td>20</td>
<td>12 (60%)</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>3- Qaliubia</td>
<td>18</td>
<td>14 (77.7%)</td>
<td>12 (66.6%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>58</strong></td>
<td><strong>44 (75.8%)</strong></td>
<td><strong>38 (65.5%)</strong></td>
</tr>
</tbody>
</table>

2- Table (3): BVDV antigen detection by using commercial ELISA kit.

<table>
<thead>
<tr>
<th>Governorates</th>
<th>No. of Nasal Swabs</th>
<th>No. of Vaginal swabs</th>
<th>No. of Tissues</th>
<th>No. of +ve Nasal&amp; Vaginal swabs</th>
<th>No. of +ve Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Behera</td>
<td>20</td>
<td>20</td>
<td>-</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>2- Alexandria</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>3- Qaliubia</td>
<td>5</td>
<td>5</td>
<td>-</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>25</strong></td>
<td><strong>25</strong></td>
<td><strong>5</strong></td>
<td><strong>20</strong></td>
<td><strong>4</strong></td>
</tr>
</tbody>
</table>
3- Table (4): Incidence of BHV-1 and BVD isolates from swabs and tissues on MDBK cell line.

<table>
<thead>
<tr>
<th>Governorates</th>
<th>No. of Swabs</th>
<th>No. of BHV-1 isolates</th>
<th>No. of BVD isolates</th>
<th>No. of Tissues</th>
<th>No. of BHV-1 isolates</th>
<th>No. of BVD isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Behera</td>
<td>40</td>
<td>12</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2- Alexandria</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>3- Qaliubia</td>
<td>10</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>15</td>
<td>10</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Photo (1): MDBK cells infected with BVD at 3rd day PI showing shrinkage and chromatin condensation.

4- Direct fluorescent antibody technique (FAT):
   FAT applied on slide of infected MDBK cells with suspected samples. The positive samples appear as bright cytoplasmic fluorescence granules as shown in photo (2).
Photo (2): Infected MDBK cells, stained with FITC-conjugated with anti-BVDV showing bright cytoplasmic fluorescent granules.

Table (5): Comparison between ELISA & virus isolation for BVDV.

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>No. of samples</th>
<th>+ve for ELISA</th>
<th>+ve for isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Swabs</td>
<td>50</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>2-Tissues</td>
<td>5</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The world wide distribution of BHV-1, the prevalence of its disease in cattle populations and its identification reveal that it is one of the most important infectious agents affect respiratory tract which require more attention from veterinarian (Castrucci et al., 1997).

BVDV was established as a viral etiology in cattle causing large economic losses in infected herds (Ramirez et al., 2001).

The use of SNT in seroprevalence study coincided with Edwards et al., (1986) as they recorded that the test has been widely used and is the standard by which other techniques have been evaluated.
Also, Pituco et al., (2002) stated that the SNT is a gold standard technique, but it is requires specialized laboratories for the maintenance of cell cultures.

Antigen Capture enzyme linked immuno assays ELISA was developed and proved its specificity (Shannon et al., 1991).

Direct FAT utilized as herd screen test for detection of BVDV (Trevor and John, 1990).

ELISA and SNT were better for detection of antibodies of BVDV in the herd than IFA. ELISA also allowed the differentiation of the vaccinated from naturally infected animals (Pacheco and Lager, 2000).

The prevalence of antibodies to infectious bovine rhinotracheitis virus (IBRV) and bovine viral diarrhea virus (BVDV) in sera of dairy cows on 4 different farms in the Republic of Croatia. A high percentage (60.8%) of cows had various reproductive disorders. Antibodies to both viruses were found in 80.8% of cows with reproductive disorders, indicated a connection between reproductive disorders and simultaneous infections with IBR and BVD viruses in dairy cows (Biuk-Rudan et al., 1999).

Serological diagnosis is very important for the detection of BVDV which is an important pathogen related to reproductive failure. Methods normally used for detection of antibodies are serum neutralization (SN) and ELISA (Pacheco and Lager, 2003).

In the present study 58 serum samples were collected from infected animals at 3 governorates (Behera, Alexandria and Qaliubia) suffered from respiratory and reproductive disorders were examined for the presence of antibodies to BHV-1 and BVD viruses by SNT. The results were animals were positive in percentage 75.8% for BHV-1 while 65.5% for BVD. It is clear that the highest percentage of positive serum to both viruses was found in Behera governorate (90% for BHV-1 and 80% for BVD) followed by Qaliubia governorate (77.7 for BHV-1 and 66.65 for BVD) and the lowest percentage of antibodies were recorded in Alexandria governorate (60% for BHV-1 and 50% for BVD). These results agreed with the results of Youssef (2006) who reported that the highest detection rate was found in diseased calves at Behira Governorate (83.5 %), while the lowest detection rate was found in contact calves at Alexandria Governorate, ELISA technique was highly sensitive and specific test for detection of anti-BVD antibodies in calf's sera.

Viral antigen Capture ELISA was applied for rapid detection of BVD in 25 nasal and 25 vaginal
swabs and tissues (lung-liver and spleen) collected from 5 aborted foeti, the results showed that 40 out of 50 swabs were positive and 4 out of 5 tissues were positive for BVDV table (3). Capture ELISA could detect certain antigen protein (NSP2-3 and EO); this protein was found in both CP and NCP strain of BVD. Diagnosis of BVD by ELISA is depending on its sensitivity, specificity and accuracy (Ferrari et al., 1999).

Our results showed that BHV-1 and BVD viruses were isolated from nasal & vaginal swabs and aborted tissues collected from diseased animals on MDBK cells and produced characteristic cytopathic effect after 3 successive passages. The cytopathic effect (CPE) for BVDV was cell shrinkage and chromatin condensation photo (1). MDBK cell lines considered one of the suitable cells for diagnosis of BVD (Sozan, 2003).

The number of positive samples for virus isolation was 15 out of 50 for BHV-1 and 10 for BVDV while 3 out of 5 tissues for BHV-1 virus. The decrease number of positive for virus isolation than ELISA may be due to inactivation of living virus in some samples, where antigen could be detected by ELISA table (5) and this agreed with Graham et al., (1998).

Identification of the cytopathic viral agents was done by direct fluorescent antibody technique (FAT). The uses of this technique agreed with Lukkjen et al. (1998) who used FAT for detection of BVDV antibodies in nasal epithelial cells from nasal swabs collected 7-9 days after inoculation of NCP-BVDV.

The stained MDBK with FITC-conjugated anti-BVDV serum and anti BHV-1 serum which inoculated with positive isolates showing bright cytoplasmic fluorescent granules photo (2). DFAT showed that virus replicating takes place entirely within the cytoplasm in association with structure formed modified endoplasmic reticulum (Gray and Nettleton, 1987).

On conclusion, the present study can detect the main causes of respiratory and reproductive disorders due to BHV-1 and BVDV mixed infection. The presence of viruses circulating among cattle population need more epidemiological studies on such viruses and establishment control strategies for both viruses by application vaccination and check up animals periodically to avoid continuous shedding viruses. This opinion is agreed with Beer and Wolf (2003) who reported that, prevention of the reproduction failure and the generation of persistently infected animals, prot-
ection of heifers and cows against transplacental infection is the most important aim of BVDV vaccination. In principal, BVD vaccines with replication competent, attenuated BVDV (modified live vaccines) and vaccines with inactivated BVDV preparations (killed vaccines) are used.

REFERENCES


Edwards, S.; Woods, S.B.; Westcott, D.J.; Emmerson, M.; Jones, P.C. and Phillips,


vaccination programs that help control BVD infection."

