Bovine viral diarrhoea, bovine herpesvirus and parainfluenza-3 virus infection in three cattle herds in Egypt in 2000

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Summary
This study reported field outbreaks of bovine viral diarrhoea virus (BVDV) infection, either alone or mixed with bovine herpesvirus-1 (BHV-1) and/or parainfluenza-3 virus (PI-3V) in Egypt during 2000. In Lower Egypt, young calves in three cattle herds in El-Minufiya Province, El-Fayoum Province and in governmental quarantine in El-Behira Province, showed symptoms of enteritis, either alone or accompanied by respiratory manifestations. The affected herds were visited and the diseased animals were clinically examined. Many epidemiological aspects, such as morbidities, mortalities and case fatalities, as well as the abortive rate, were calculated. Ethylenediamine tetra-acetic acid-blood samples, sterile nasal swabs and serum samples were obtained for virological and serological diagnosis. The laboratory investigations revealed that the main cause of calf mortalities in the three herds was infection with BVDV, either alone, as on the El-Minufiya farm, or mixed with PI-3V, as on the El-Fayoum farm, or mixed with both BHV-1 and PI-3V, as in the herd in governmental quarantine in El-Behira Province. A total of nine dead calves from the three herds were submitted for thorough post-mortem examination. Tissue samples from recently dead calves were obtained for immunohistochemical and histopathological studies. The most prominent histopathological findings were massive degeneration, necrosis and erosions of the lining epithelium of the alimentary tract. Most of the lymphoreticular organs were depleted of lymphocytes. In pneumatic cases, bronchopneumonia and atypical interstitial pneumonia were evident. The present study suggested that the immunosuppressive effect of BVDV had predisposed the animals to secondary infection with BHV-1 and PI-3V. This study concluded that concurrent infection with BVDV, BHV-1 and PI-3V should be considered as one of the infectious causes of pneumoenteritis and, subsequently, the high morbidities and mortalities among young calves in Egypt. Preventive and control measures against these infectious agents should therefore be adopted. All animals imported into Egypt should be free from BVDV infection. Control programmes for the detection and removal of BVDV-persistent cattle should be applied in cattle herds all over the country.

Keywords
Introduction

Pneumoenteritis is generally considered to be the main hazard to calf health (60). Bovine viral diarrhoea virus (BVDV) is a major pathogen for cattle and contributes to the genesis of a wide variety of pathology ranging from inapparent or mild infection to the inevitably fatal syndrome of mucosal disease (12). The BVDV belongs to the genus Pestivirus of the family Flaviviridae. Both a non-cytopathic (ncp) and an antigenically related cytopathic (cp) BVDV can be isolated from persistently infected animals suffering from mucosal disease (63).

Diseases associated with BVDV infection have been a controversial subject because of the multiple clinical forms and manifestations of the infection, the ability of the virus to suppress the function of the immune system, and the complex epidemiology of the disease (6). The BVDV causes different diseases, including bovine viral diarrhoea (BVD) infection, which is usually subclinical; mucosal disease, which is usually fatal; and congenital abnormalities in calves, caused by an infection of the foetus in midgestation. The BVDV may also cause reproductive failure and be immunosuppressive (22, 23, 30, 48, 51, 57). The BVDV induces immunosuppression (1, 10, 11), which allows for secondary infection of the respiratory tract, and causes significant losses as a result of its interaction with other pathogens (21, 26, 35).

The bovine herpesvirus-1 (BHV-1) and parainfluenza-3 (PI-3V) viruses are the principal causes of pneumonia in cattle (37). The role of BVDV in the bovine respiratory disease complex is controversial (20). Abo El-Lail (4) described a respiratory disease in cattle where both PI-3V and BVDV were involved. It has also been shown experimentally that initial infection with BVDV impairs the ability of infected calves to eliminate the BHV-1 from the infection site (52).

The bovine herpesvirus-1 is a double-stranded deoxyribonucleic acid virus. It is a member of the genus Varicellovirus within the subfamily Alphaherpesvirinae, which belongs to the family Herpesviridae (58, 64). BHV-1 is an economically important pathogen. Infectious rhinotracheitis is one of the most common clinical symptoms of BHV-1 in cattle (28, 56). The viral glycoproteins, which are located on the surface of the virion, play an important role in pathogenesis and immunity (18, 45, 62). Latency is one of the major problems associated with the infection of cattle by BHV-1 (39). Infectious bovine rhinotracheitis (IBR) is characterised by clinical signs of upper respiratory tract disease, such as a mucopurulent nasal discharge, and by conjunctivitis. Signs of general illness are fever, depression, inappetance, abortions and reduced milk yield. Mortality is low. Many infections run a subclinical course (49).

The parainfluenza-3 virus is a single stranded ribonucleic acid virus. It is a member of the genus Paramyxovirus in the subfamily Paramyxovirinae, which belongs to the family Paramyxoviridae (8). The PI-3V infection is commonly subclinical. Clinical disease may not occur until other pathogens are present or when adverse environmental conditions precipitate clinical disease (55).

The aim of this study was to identify the infectious agents of the calf mortalities in the three investigated cattle herds and to report the epidemiological aspects and clinical manifestations associated with the outbreaks. The study also aimed to identify the pathological alterations as well as the corresponding distribution of the viral antigen in the tissues of the diseased calves by using the avidin biotin complex (ABC) immunoperoxidase technique.

Materials and methods

Animals

A total of 157 (9.2%) of the 1,701 young calves in the three investigated cattle herds, which contained 2,354 animals in total, showed evidence of gastroenteritis with severe diarrhoea, either alone, as in Herd 1, or associated with respiratory manifestations, as in Herds 2 and 3. These herds (Table I) were as follows.

Table I

Data on the three cattle herds included in the study of bovine viral diarrhoea virus outbreaks in Egypt in 2000

<table>
<thead>
<tr>
<th>Descriptions</th>
<th>Herd 1</th>
<th>Herd 2</th>
<th>Herd 3</th>
<th>Total number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of investigation</td>
<td>January 2000</td>
<td>February 2000</td>
<td>October 2000</td>
<td></td>
</tr>
<tr>
<td>Number of animals</td>
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<td>292</td>
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<td>375</td>
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<td>485</td>
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<tr>
<td>Age groups of calves:</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 month</td>
<td>10</td>
<td>12</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>&gt; 1 - &lt; 3 months</td>
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<td>18</td>
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<td>34</td>
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<tr>
<td>&gt; 3 - &lt; 6 months</td>
<td>28</td>
<td>32</td>
<td>0</td>
<td>60</td>
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<tr>
<td>&gt; 6 - &lt; 12 months</td>
<td>15</td>
<td>50</td>
<td>1,520</td>
<td>1,585</td>
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<tr>
<td>Total number of calves</td>
<td>69</td>
<td>112</td>
<td>1,520</td>
<td>1,701</td>
</tr>
</tbody>
</table>

Herd 1

Herd 1 was a dairy farm known as Delta Misr dairy cattle farm, located in El-Minufiya Province, Lower Egypt. When the authors visited the site, in January 2000, there were a total of 542 cattle on this farm, including ninety-eight pregnant cows and sixty nine young calves, from one week to eight months old. Liver, spleen and lung tissue samples were obtained from three aborted foetuses. Ethylenediamine tetra-acetic acid (EDTA)-blood samples and sterile nasal swabs were collected from twelve clinically-diseased calves.
Herd 2
Herd 2 was a Friesian dairy cattle farm in El-Fayoum Province. The cattle in this herd were imported from Germany. This dairy farm was visited in February 2000, when it contained 110 dairy cattle, seventy pregnant cows, twelve calves less than one month old, eighteen calves between one to three months old, thirty-two calves between three to six months old and fifty calves from six to twelve months old. The authors collected tissue samples from the liver, spleen and lung of two aborted foetuses, and EDTA-blood samples and sterile nasal swabs were obtained from sixteen clinically-ill calves.

Herd 3
This was a herd of 1,520 fattened young calves aged from six to twelve months, imported from Romania and quarantined at the governmental animal quarantine in El-Behira Province. This quarantine facility was visited about three weeks after the arrival of the calves, by which time there were increasing numbers of diseased calves due to the rapid spread of an infection. On the day of investigation, forty eight calves were suffering from signs of enteritis mixed with respiratory manifestations. Nineteen sterile nasal swabs and nineteen EDTA-whole blood samples were collected from nineteen diseased calves. In addition, tissue samples from the mesenteric lymph nodes, pulmonary lymph nodes, spleen and lung were obtained from two calves that had recently died.

Clinical examination
All of the diseased cattle in the three different infected herds were clinically examined.

Pathological examinations
Nine of the dead calves (two from Herd 1, three from Herd 2 and four from Herd 3) were submitted for thorough post-mortem examination. Tissue specimens were taken from the trachea, lungs, bronchial and mesenteric lymph nodes, spleen, liver, kidneys, rumen, abomasums and parts of the intestines. These samples were fixed in 10% neutral buffered formalin, processed routinely, and then sectioned 4 µm-5 µm thick. The prepared sections were stained with haematoxylin and eosin for histopathological examination, and by lendrum’s phloxin and tartrazine for demonstration of viral inclusion bodies (14).

Immunohistochemical studies
The ABC immunoperoxidase technique was used as a rapid method of confirming the detection of BVDV, BHV-1 and PI-3V in the different organs and tissues obtained from nine dead calves at necropsy. The technique was applied on paraffin sections, in accordance with the method of Wilchek and Bayer (73). According to the intensity of the positive reaction, the results were classified into four degrees from + to +++

Virological investigations
Cell culture
Madin Darby bovine kidney (MDBK) cell culture was used to isolate BVDV from prepared buffy coats, nasal swabs and tissue samples. The buffy coats and nasal swabs were prepared according to Abd El-Rahim et al. (3). The MDBK was also used to isolate BHV-1 from the collected nasal swabs. The EDTA-blood samples (n 78) were centrifuged at 1,000 x g for 10 min. The plasma was removed and the buffy coat (leukocytic fraction) was aspirated with a sterile Pasteur-pipette into a sterile tube and then 5 ml of minimum essential medium (MEM) with 2% foetal calf serum (FCS), which had been proven to be free from both BVDV and BVD antibodies, were added. The mixtures were left at room temperature for 1 h and then frozen at –70°C until used.

Each of the 78 sterile nasal swabs was transferred into a sterile tube and 5 ml of MEM with 2% FCS and antibiotics (800 µg streptomycin, 800 IU penicillin and 400 IU gentamycin/ml) were added, they were well mixed and left at room temperature for 1 h. The swabs were then kept at –70°C until inoculated onto MDBK cell cultures. Tissue samples from aborted foetuses and the recently dead calves were ground in MEM with 2% FCS and antibiotics (800 µg streptomycin, 800 IU penicillin and 400 IU gentamycin/ml) and centrifuged at 1,000 x g for 10 min. The supernatant fluids were separated and kept at –70°C until used for BVDV isolation. The prepared leukocytic fractions, nasal swabs and tissue suspensions were inoculated onto MDBK cell cultures for isolation of BVDV according to the method described by Clarke et al. (16).

Indirect immunofluorescent technique
The indirect immunofluorescent (IF) technique was used on the inoculated cell cultures with cytopathic effect (CPE) to identify the cpBVDV. It was also used on inoculated cell cultures without CPE to detect ncpBVDV biotype. The IF technique was carried out according to OIE (World organisation for animal health) standards (47).

Three blind passages of the tested samples were done on the inoculated cell cultures. If there was no evidence of any CPE, the third passage was conducted on MDBK tubes. Thus, tube cultures including flying cover slips were used to detect ncpBVDV biotype by indirect IF technique. Both positive and negative control samples were also included. The tested samples were considered positive when there was a clear apple green cytoplasmic fluorescence in the infected cell culture, while the negative control, non-infected cells, showed no fluorescence.

Haemagglutination and haemagglutination inhibition tests
Haemagglutination (HA) and haemagglutination inhibition (HI) tests were applied, according to the method described by...
Lennette and Schmidt (40), to a total of 78 nasal swabs, in order to detect PI-3V antigen.

**Virus neutralisation test**

The virus neutralisation (VN) test was used to identify BHV-1 that had been isolated on MDBK tissue culture. The fixed virus variable serum method described by Gillespie et al. (29) was used.

**Epidemiological studies**

Many epidemiological aspects in the three affected herds were studied, such as the incriminated sources of infection, the mode of transmission, age susceptibility, morbidities, mortalities and the abortive rate.

**Results**

**Clinical symptoms**

Inspection of the affected calves in Herd 1 revealed a rise in body temperature (up to between 40°C and 42°C), anorexia, severe depression, salivation, and profuse watery diarrhoea with dehydration. Multiple erosions usually of 1 mm-5 mm in diameter were present in the mucosa of the muzzle, oral cavity and tongue. Higher infection and severe clinical manifestations were observed among young calves from three to twelve months old. In addition to the previous clinical signs, other manifestations observed in diseased animals in Herds 2 and 3 were: severe respiratory and ocular manifestations, including an increase in respiratory rate; serous or mucoid nasal discharge; respiratory dyspnoea; coughing and sneezing; and conjunctivitis with ocular discharge. Severe conjunctivitis with profuse ocular discharge was noticed among affected calves in Herd 3. In Herds 1 and 2, abortion was observed among pregnant cows. Congenital malformations were noticed in Herds 1 and 2, where some newborn calves were weak and blind, and sometimes had musculoskeletal deformities.

**Post-mortem findings**

Nine dead calves from the three investigated herds were subjected to post-mortem examinations. The most prominent aspect of the post-mortem examinations of two calves from Herd 1 was the presence of multiple erosions of about 1 mm to 5 mm in diameter on the muzzles, oral mucosa, tongue, ruminal pillars, abomasums, and small intestines. Congestion and severe haemorrhages were seen in the mucosal surfaces of the abomasums, small intestines and ileocecal valve. Necrotic foci were observed in the hepatic tissues. The bronchial and mesenteric lymph nodes were extremely congested and haemorrhagic. The spleen was congested, while the kidneys were pale in colour.

Post-mortem examinations of seven calves from Herds 2 and 3 revealed the above-mentioned gross pathological lesions and also linear haemorrhages in the tracheal and bronchial mucosa. Multiple patchy congested areas were seen on the pulmonary lobes.

**Immunohistochemical findings**

Detection of the viral antigens by ABC immunoperoxidase technique in tissues collected at autopsy is shown in Tables II, III and IV and in Figures 1, 2 and 3.

**Table II**

<table>
<thead>
<tr>
<th>Organs</th>
<th>BVD virus</th>
<th>BHV-1</th>
<th>PI-3 virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachea</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lung</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Bronchial lymph nodes</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mesenteric lymph nodes</td>
<td>+ + +</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tongue</td>
<td>+ + +</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Spleen</td>
<td>+ + +</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Rumen</td>
<td>+ +</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Abomasum</td>
<td>+ +</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Intestines</td>
<td>+ + +</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Liver</td>
<td>+ + +</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Kidneys</td>
<td>+ + +</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Heart</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

**Table III**

<table>
<thead>
<tr>
<th>Organs</th>
<th>BVD virus</th>
<th>BHV-1</th>
<th>PI-3 virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachea</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Lung</td>
<td>+</td>
<td>–</td>
<td>+ + +</td>
</tr>
<tr>
<td>Bronchial lymph nodes</td>
<td>–</td>
<td>–</td>
<td>+ +</td>
</tr>
<tr>
<td>Mesenteric lymph nodes</td>
<td>+ +</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tongue</td>
<td>+ + +</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Spleen</td>
<td>+ + +</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Rumen</td>
<td>+ +</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Abomasum</td>
<td>+ + +</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Intestines</td>
<td>+ + +</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Liver</td>
<td>+ + +</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Kidneys</td>
<td>+ + +</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Heart</td>
<td>+ +</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>
The BVDV antigen was detected by immunohistochemical assay in tissue samples from four dead calves from Herd 3 (Table IV), which were also positive for virus isolation by the tissue culture method.

An intense positive reaction against BVDV was seen in the basal layer of the mucosal layer of the tongue, the cytoplasm of the lining epithelium of most of the villi, the intestinal glands of both the small and large intestines (Fig. 1), the cytoplasm of the hepatic and kupfer cells, and within the lymphocytes and macrophages of both the spleen and the mesenteric lymph nodes (Figs 2a and 2b).

Meanwhile intense positive reaction against PI-3 was demonstrated in the lining bronchial and alveolar epithelium (Fig. 3b). A severe positive reaction against BHV-1 viral antigen was observed inside the degenerated mucosal epithelium, the submucosal layer of the trachea (Fig. 3a), the lining bronchial epithelium, the lymphocytes and macrophages of the spleen, the bronchial lymph nodes, the lumen of the blood vessels, the lining endothelial cells of the heart, in between the cardiac muscle fibers and the lining endothelial cells of the renal blood vessels, the glomerular capsule and the tuft capillaries, and in the cytoplasm of the lining tubular epithelium.

The BVDV antigen was detected by immunohistochemical assay in tissue samples from four dead calves from Herd 3 (Table IV), which were also positive for virus isolation by the tissue culture method.
**Histopathological studies**

Most of the histopathological lesions were confined to the alimentary and respiratory tracts, the lymphoreticular tissues and the kidneys. In Herd 1, microscopical examination of the gastrointestinal tract revealed epithelial degeneration and necrosis of the lining of the ruminal pillars. The necrotic cells were sloughed leading to erosions or ulcerations with inflammatory cell aggregations mixed with fibrinous exudates associated with haemorrhage and thrombosis of most of the lymphatics. Examination of the abomasums revealed necrosis of the glandular epithelial cells in addition to haemorrhage and dilatation of the gland lumens. In the intestine, pathological changes were prominent in all parts of the intestines (Figs 4a and 4b), and included severe damage and necrosis of the intestinal villi and sloughing of the lining epithelium. The mucosal glands were cystic. There was severe haemorrhage and congestion of the submucosal blood vessels. The ileocecal valve showed depletion in the Peyer’s patches and the villi appeared atrophied with excessive goblet cells formation.

The hepatic cells showed variable degrees of vacuolar degeneration and multiple focal areas of necrosis associated with round cell infiltrations. Both intranuclear and intracytoplasmic acidophilic inclusion bodies, which were positive with phloxin and tartrazine stain, were seen in most of the examined hepatic tissues (Figs 5a and 5b). There was degeneration of the epithelial lining the bile ducts and fibrosis in the portal areas. The lymphoid follicles of the bronchial and mesenteric lymph nodes were markedly depleted, with an accumulation of a large number of macrophages in the subcapsular zone. The white pulp of the spleen was also depleted. There was vasculitis of the small and medium sized blood vessels.

In Herds 2 and 3, microscopic changes were noticed in the respiratory system and there were changes in the alimentary tract. The trachea showed variable degrees of degeneration, necrosis and epithelial exfoliation. Congestion in the submucosal blood vessels and haemorrhages associated with round cell infiltrations in the lamina propria were detected. The lungs showed proliferative reactions involving the bronchiolar and alveolar epithelia, (epithelization), so that the lesions were considered as atypical interstitial pneumonia. Most of the bronchiolar and alveolar epithelial cells were vacuolated. Intranuclear acidophilic inclusions were detected in the lining alveolar epithelium as shown in Figure 6. Most of the bronchi and alveoli contained a mixture of neutrophils, macrophages and lymphocytes, with detached epithelial cells and serous exudates which had almost completely blocked the lumina of the bronchioles and alveoli with multiple syncytial giant cells. In addition, vasculitis and thrombosis were observed in most of the pulmonary blood vessels. Examination of the kidneys revealed nephritis in the tissue samples collected from all three investigated cattle herds.

**Fig. 4**
Pathological changes in the intestines

The hepatic cells showed variable degrees of vacuolar degeneration and multiple focal areas of necrosis associated with round cell infiltrations. Both intranuclear and intracytoplasmic acidophilic inclusion bodies, which were positive with phloxin and tartrazine stain, were seen in most of the examined hepatic tissues (Figs 5a and 5b). There was degeneration of the epithelial lining the bile ducts and fibrosis in the portal areas. The lymphoid follicles of the bronchial and mesenteric lymph nodes were markedly depleted, with an accumulation of a large number of macrophages in the subcapsular zone. The white pulp of the spleen was also depleted. There was vasculitis of the small and medium sized blood vessels.

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Virological diagnosis
Isolation and identification of bovine viral diarrhoea virus
The results of the isolation and identification of both cpBVDV and ncpBVDV biotypes using the cell culture method and the immunofluorescent technique are represented in Table V. Immunofluorescence positive samples were characterised by specific intracytoplasmic fluorescence.

Detection of parainfluenza-3 virus antigen
Table VI shows the results of HA and HI tests which were used to detect PI-3V antigen in nasal swabs collected from the diseased calves in the three investigated cattle herds.

Demonstration of bovine herpesvirus-1
Both the cell culture method and the VN test were used to demonstrate and identify BHV-1 isolates in the collected nasal swabs. Cytopathic effect was observed in MDBK cell culture after 36 h post-inoculation; this appeared in the form of rounding of the cells, shrinkage of cell walls and an increase in granularity, leading to the formation of a bunch of grapes within 72 h-96 h. In the VN test, the isolated virus was neutralised by reference bovine hyperimmune serum against BHV-1. Virus was only isolated from Herd 3, in which thirty-two out of forty-eight nasal swab samples were positive (Table VII).

Epidemiological studies
In 2000, there were outbreaks of severe acute BVD, either alone, as in Herd 1, or mixed with PI-3V infection, as in Herd 2, or mixed with both PI-3V and BHV-1 infection, as in Herd 3. The current study suggested that the immunosuppressive effect of BVDV predisposed cattle in Herds 2 and 3 to infection with PI-3V or both PI-3V and BHV-1. Herd 3 had been imported from Romania just a few weeks before the appearance of clinical signs, and the stress of this transportation facilitated the concurrent infection with both PI-3V and BHV-1. Also, the study suggested that the young calves of Herd 3 (in governmental quarantine in El-Behira Province) were infected with BVDV before being imported into Egypt. Morbidity, mortality, case fatality and abortive rates in the three affected herds are represented in Table VIII.

Discussion
The BVDV infection in cattle has been reported throughout the world, and the wide spectrum of clinical syndromes associated with this positive-stranded and enveloped RNA virus makes it one of the most important viral pathogens of cattle (17). The BVDV spreads widely among cattle herds in the world (5, 23, 54, 68, 72). The BVDV was isolated for the first time in Egypt in 1972 from a calf suffering from severe enteritis (33). The present study recorded an outbreak of acute BVDV infection in 2000 in three cattle herds in Egypt, with clinical manifestations

Table V
Isolation of both cytopathic (CP) and non-cytopathic (NCP) bovine viral diarrhoea virus (BVDV) biotypes

<table>
<thead>
<tr>
<th>Herds</th>
<th>Nasal swabs</th>
<th>BVDV CP</th>
<th>BVDV NCP</th>
<th>Buffy coats CP</th>
<th>BVDV CP</th>
<th>Buffy coats NCP</th>
<th>Dead calves CP</th>
<th>BVDV CP</th>
<th>Dead calves NCP</th>
<th>Aborted foetuses CP</th>
<th>BVDV CP</th>
<th>NCP</th>
<th>Aborteds foetuses</th>
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<td>16</td>
<td>12</td>
<td>78</td>
<td>21</td>
<td>19</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>–</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a) tissue samples from the same four calves were also positive by the immune staining technique
b) from lung and spleen
c) from spleen

Table VI
Detection of parainfluenza-3 virus antigen in the three investigated herds

<table>
<thead>
<tr>
<th>Herds</th>
<th>Number of examined nasal swabs</th>
<th>Positive %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>– (0)</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>10 (62.5)</td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td>28 (58.3)</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>38 (48.7)</td>
</tr>
</tbody>
</table>

Table VII
Isolation of bovine herpesvirus-1 from nasal swabs

<table>
<thead>
<tr>
<th>Herds</th>
<th>Number of examined nasal swabs</th>
<th>Positive %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>– (0)</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>– (0)</td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td>32 (66.7)</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>32 (41)</td>
</tr>
</tbody>
</table>
of enteritis and congenital malformations. Isolation and identification of the causative viruses, as well as the detection of the viral antigens by immunohistochemistry, indicated that the main cause of calf mortalities in the three herds was severe acute bovine viral diarrhoea. Concurrent infection with BVDV and PI-3V was observed in Herd 2. In Herd 3, mixed infection of BVD, PI-3 and BHV-1 was reported. The BVDV was widespread throughout the country as determined by the detection of neutralising BVD antibodies in 99 (37.6%) out of 263 sheep sera in 24 different locations throughout all the provinces of Egypt (31). In March 1982, an outbreak due to BVDV infection was reported by Ghaffar and Ata (27) in cattle and buffalo in Fayoum and Qina Governorates. The BVDV and BHV-1 are prevalent in the country as indicated by the detection of antibodies in bovine and buffalo serum samples collected from some governmental farms in Lower Egypt (32).

The BVDV seems to be transmitted directly from either acutely or persistently infected cattle to susceptible cattle. Persistently infected cattle play a more significant role in the transmission and maintenance of BVDV than acutely infected cattle (13, 67). For a persistent infection to become established, ncpBVDV must infect the developing embryo or foetus during the first four months of gestation (41). Persistently infected-calves usually have poor viability and suffer early disease and death with or without signs of diarrhoea (54). This study suggested that Herds 1 and 2 were suffering from persistent BVDV infection. Animals from Herd 3 may already have been infected with BVDV before being imported.

Both CP and NCP biotypes of BVDV are capable of affecting foetal and neonatal development. The CP biotypes are frequently associated with embryonic death, abortion, and malformation; NCP biotypes are associated with immunotolerance and persistent infection (46). This study suggested that the malformations observed in newborn calves in the affected herds were a result of intrauterine infection with cpBVD biotype.

Clinically, fevers, anorexia, depression, salivation, erosive stomatitis, abortion, and profuse watery diarrhoea with dehydration were the most observable signs of acute BVD in the affected herds. Young calves aged from three to twelve months were more susceptible to the infection and showed severe clinical signs. The BVDV infection should be considered when investigating severe acute outbreaks of enteritis (19) and abortion in cattle (38). In addition to the above mentioned clinical signs, severe respiratory and ocular manifestations, including rapid respiration, mucopurulent nasal discharge, dyspnoea, coughing and sneezing and conjunctivitis were observed among diseased animals in Herds 2 and 3.

Accurate diagnosis of BVDV infection depends upon isolating the virus from nasal swabs or blood or tissue samples from affected animals in a diagnostic laboratory (2, 6, 21, 32, 34, 61, 71). In this study, laboratory investigations revealed isolation and identification of ncpBVDV biotype from the nasal swabs, buffy coats and tissue samples collected from Herds 1 and 2 using the cell culture method and the immunofluorescent technique, while the CP biotype was detected in the samples obtained from Herd 3. The indirect peroxidase staining technique is a practical test for detecting BVDV in infected herds (43), and has been used previously to diagnose BVDV infection (34) and IBR (74). In the present study, the ABC immunoperoxidase method was used as a rapid way of confirming the demonstration of BVD, BHV-1 and PI-3 viral antigens in the collected tissue samples. This technique reflected the involvement of a wide variety of tissues. The location and intensity of BVD, BHV-1 and PI-3 antigens in the examined organs were similar to the observations of Hosny et al. (36) and Shehab et al. (66). The results of the tissue culture method and the avidin biotin complex-immunoperoxidase technique for detecting BVDV infection in tissue samples obtained from 4 dead calves from Herd 3 were correlated. Histopathological findings of severe acute BVD were observed. There was severe congestion and haemorrhages throughout the gastrointestinal tract as seen previously by Ernst et al. (25), Ruth (61), Mebus et al. (42). Massive degenerative changes were observed in the liver and kidneys as detected by Barlow et al. (7), Omran (50), Tyler and Ramsey (69).

The BVDV infections in congregated and stressed cattle may lead to severe respiratory or enteric disease (9, 59). The current study suggested that the immunosuppressive effect of BVDV

<table>
<thead>
<tr>
<th>Herds</th>
<th>Total number of calves</th>
<th>Number of diseased calves</th>
<th>Morbidity rate (%)</th>
<th>Number of dead calves</th>
<th>Mortality rate (%)</th>
<th>Case fatality rate (%)</th>
<th>Total number of pregnant cows</th>
<th>Number of aborted cows</th>
<th>Abortive rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>69</td>
<td>35</td>
<td>50.7</td>
<td>21</td>
<td>30.4</td>
<td>60</td>
<td>98</td>
<td>26</td>
<td>26.5</td>
</tr>
<tr>
<td>2</td>
<td>112</td>
<td>48</td>
<td>42.9</td>
<td>32</td>
<td>28.6</td>
<td>66.7</td>
<td>70</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>1,520</td>
<td>74</td>
<td>4.9</td>
<td>26</td>
<td>1.7</td>
<td>35.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1,701</td>
<td>157</td>
<td>9.2</td>
<td>79</td>
<td>4.6</td>
<td>50.3</td>
<td>188</td>
<td>40</td>
<td>23.8</td>
</tr>
</tbody>
</table>
facilitated the infection of the diseased calves with other viral agents such as BHV-1 and PI-3V. This suggestion coincides with the findings reported by Potgieter et al. (53). The concurrent infection with the BVDV, BHV-1 and PI-3V worsened the clinical disease and the economic losses in the three affected herds. Field outbreaks of mixed infection by BVDV and other viruses such as malignant catarrhal fever virus (65) and foot and mouth virus (36) have been previously reported.

Respiratory and enteric infections are the most costly and troublesome disease problems in calves in Egypt (32). This study revealed that the direct economic losses of BVDV infection (and the indirect losses due to the immunosuppressive effect of the virus, which facilitates infection with other infectious agents), are considerable. These economic losses were estimated at US$13,800, US$15,600 and US$10,400 in Herds 1, 2 and 3 respectively. The economic losses associated with BVDV infections are due to suboptimal reproductive performance due to infertility and embryonic death, abortion, prenatal growth retardation, stillbirths, congenital defects, postnatal growth retardation, and death from mucosal disease (24).

Bovine PI-3V is a common infection of cattle, 60%-90% of cattle in a herd often having serological evidence of past infection (8). The PI-3V was detected in the nasal swabs, which were collected from Herds 2 and 3 using HA and HI tests. Subclinical viral pneumonia, which is associated with the PI-3V and is uncomplicated by secondary infection, is usually of minor importance (55). However, in the current study, severe respiratory signs were observed among the affected calves of Herds 2 and 3 as a result of complication of BVDV infection with PI-3V, as in Herd 2, or with both PI-3V and BHV-1, as in Herd 3. These findings agree with the experiment of Castrucci et al. (15), who found that simultaneous infection of calves with BVDV and PI-3V induced a clinical response which was considered of moderate intensity. A different situation was observed, however, when simultaneous infection was made with BVDV and BHV-1. In the inoculated calves, BVDV and BHV-1 together induced a severe clinical response characterised by a wide variety of signs which included mucoid nasal discharge, profuse salivation and pronounced diarrhoea.

The BHV-1 was found primarily in large, concentrated cattle populations, such as feedlots or intensively managed dairy operations (44). Both the immunosuppressive effect of the BVDV and PI-3V, and the effect of being transported over long distances, predisposed the imported feedlot calves in Herd 3 to infection with BHV-1. The BHV-1 can be spread through nasal secretions or aerosol droplets containing the virus. Close contact among animals is responsible for the high rate of transmission (70). The present study also suggested that the crowdedness and close contact of the 1,520 animals in Herd 3 (in the governmental quarantine in Behira Province) played an important role in rapidly transmitting BHV-1 among the young imported feedlot calves, which had been imported from Romania just a few weeks before these investigations.

In cattle infected with BHV-1, the initial lesions which developed in the upper respiratory tract usually progressed to broncho and/or lobar pneumonia as a result of secondary infection, either a viral infection, such as PI-3V, or a bacterial infection (49). In the present study, it was evident that BHV-1 has a cellular destructive power on the parenchymatous organs and causes necrosis of hepatic cells, as observed previously by Shehab et al. (66). The BHV-1 is distributed worldwide, but has been eradicated from Denmark and Switzerland and control programmes have started in some other countries (49).

Conclusion

The present study leads us to conclude that:

– concurrent infection with BVDV, BHV-1 and PI-3V should be considered as one of the infectious causes of pneumoenteritis and the subsequent high morbidities and mortalities among young calves in Egypt. Consequently, preventive and control measures against these infectious viral agents should be adopted

– the ABC immunoperoxidase technique is one of the most rapid and accurate laboratory tests for the diagnosis of different types of mixed viral infections, such as concurrent infection with BVDV, BHV-1 and PI-3V

– both BVDV and BHV-1 have an immunosuppressive effect and facilitate secondary infection in diseased calves, which subsequently results in high economic losses

– all animals imported into Egypt must be free from BVDV infection

– control programmes for detecting and removing BVDV-persistent cattle should be applied in cattle herds all over the country.
Cas de diarrhée virale bovine et d’infection à l’herpès-virus bovin et au virus para-influenza 3 dans trois troupeaux de bovins en Égypte en 2000

N.M. Aly, G.G. Shehab & I.H.A. Abd El-Rahim

Résumé
Cette étude rapporte la présence de foyers causés par le virus de la diarrhée virale bovine (BVDV) en Égypte, en 2000. Le virus responsable a été détecté soit seul, soit conjointement avec l’herpès-virus bovin de type 1 (BHV-1) et/ou le virus para-influenza de type 3 (PI-3V). En Basse Égypte, des symptômes d’entérite, parfois accompagnés de manifestations respiratoires, ont été observés chez de jeunes veaux dans trois troupeaux, dans les provinces d’El-Minufiya et d’El-Fayoum, ainsi que dans les installations gouvernementales de quarantaine de la province d’El-Behira. Les troupeaux contaminés ont été inspectés et les animaux atteints soumis à un examen clinique. Un grand nombre d’aspects épidémiologiques (morbidité, mortalité et létalité, par exemple) ont été déterminés, dont le taux d’avortement. Des échantillons de sang sur EDTA (acide éthylène diamine tétra-acétique), des frottis nasaux ainsi que des échantillons sériques ont été prélevés à des fins de diagnostic virologique et sérologique. Les analyses de laboratoire ont révélé que la principale cause de la mortalité observée chez les veaux des trois troupeaux était une infection au seul BVDV (élevage d’El-Minufiya), à la présence simultanée du BVDV et du PI-3V (élevage d’El-Fayoum) ou à la présence conjointe du BVDV, du BHV-1 et du PI-3V (troupeau de la station gouvernementale de quarantaine de la province d’El-Behira). Un examen post-mortem complet a été réalisé sur neuf veaux issus des trois troupeaux. Des prélèvements de tissus ont été effectués chez les veaux morts récemment pour examen immunohistochimique et histopathologique. Les analyses histopathologiques ont principalement mis en évidence une dégénérescence massive, accompagnée de nécrose et d’érosion de la paroi épithéliale du tube digestif. La plupart des organes lymphoréticulaires présentaient un important déficit lymphocytaire. Des signes de broncho-pneumonie et de pneumonie interstitielle atypique étaient manifestes dans les cas pulmonaires. Selon les auteurs de cette étude, l’effet immunodépresseur du BVDV aurait prédisposé les animaux à une infection secondaire au BHV-1 et PI-3V. Cette étude a permis de conclure qu’une infection opportuniste à BVDV, BHV-1 et PI-3V devait être envisagée comme l’une des causes de l’infection de pneumoentérite et des taux élevés de morbidité et de mortalité observés ultérieurement parmi les jeunes veaux d’Égypte. Il convient dès lors de mettre en place des mesures préventives et de contrôle contre ces agents infectieux. Tous les animaux importés en Égypte devraient être indemnes de toute infection au BVDV. Il convient de mettre en place des programmes de contrôle destinés à dépister et à éliminer les bovins porteurs du BVDV au sein des troupeaux sur l’ensemble du territoire.

Mots-clés
Diarrea viral bovina e infección por el herpesvirus bovino y el virus de la parainfluenza 3 en tres rebaños vacunos de Egipto en el año 2000

N.M. Aly, G.G. Shehab & I.H.A. Abd El-Rahim

Resumen
Los autores describen el estudio de una serie de brotes infecciosos que se declararon en 2000 en Egipto, causados por el virus de la diarrea viral bovina (VDVB), ya fuera solo o acompañado del herpesvirus bovino 1 (HVB-1) y/o el virus parainfluenza 3 (VPI-3). En las tierras bajas egipcias, y concretamente en las provincias de El-Minufiya y el El-Fayoum y las instalaciones oficiales de cuarentena de la provincia de El-Behira, se observaron síntomas de enteritis en tres rebaños de jóvenes terneras, que a veces se presentaban solos y otras veces acompañados de disfunción respiratoria. Se efectuaron inspecciones de los rebaños afectados y análisis clínicos de los animales enfermos. Se calcularon numerosos parámetros epidemiológicos, tales como las tasas de morbilidad, mortalidad y letalidad y el índice de abortos. Para proceder al diagnóstico virológico y serológico, se recogieron muestras de sangre en ácido etilendiamino tetracético, muestras de exudado nasal en hisopos estériles y muestras de suero. Los análisis de laboratorio revelaron que, en los tres rebaños, la principal causa de mortalidad era la infección por el VDVB, ya fuera solo (como en la explotación de El-Minufiya) o combinado con el VPI-3 (como en la granja de El-Fayoum) o con éste y el HVB-1 (como en el rebaño de las instalaciones de cuarentena de la provincia de El-Behira). Un total de nueve terneras de los tres rebaños fueron sometidas a inspección post-mortem exhaustiva. Se extrajeron muestras tisulares de terneras muertas poco tiempo antes para someterlas a análisis inmunohistoquímico e histopatológico. Desde el punto de vista histopatológico, las observaciones más destacadas fueron una degeneración muy extendida, zonas de necrosis y erosión del epitelio de revestimiento del tracto digestivo. La mayor parte de los órganos linforreticulares habían perdido los linfocitos. Los animales neumónicos presentaban signos evidentes de bronconeumonía y neumonía intersticial atípica. Del estudio se desprende que los efectos inmunosupresores del VDVB habían predisputo a los animales a contraer infecciones secundarias por el HVB-1 y el VPI-3, lo que a su vez lleva a concluir que es preciso tener en cuenta que las infecciones oportunistas por el VDVB, el HVB-1 o el VPI-3 constituyen una de las causas infecciosas de neumoenteritis y, ulteriormente, de las elevadas tasas de morbilidad y mortalidad entre las terneras jóvenes de Egipto. De ahí la necesidad de adoptar medidas preventivas y de control contra esos agentes infecciosos. Todos los animales que Egipto importe deben estar libres de la infección por el VDVB. Por otra parte, los rebaños bovinos de todo el país deben estar sujetos a programas de control para detectar y eliminar los ejemplares que presenten infección persistente por el VDVB.

Palabras clave
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