Clinico-pathological Studies on Bovine Virus Diarrhoea (BVD) in Eastern Saudi Arabia

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Abstract :

To the best of our knowledge, this is the first study to be conducted on bovine virus diarrhoea (BVD) in the Kingdom of Saudi Arabia. The study involved the Eastern Region of the Kingdom.

Samples were collected from clinically suspected cases which were subjected to clinico-pathological and virological studies.

The results indicated that the disease is widespread in the Eastern Province. The epidemiological situation of the disease was discussed in relation to future control of the disease.

Key words : Bovine viral diarrhoea ; Eastern Saudi Arabia

Introduction

Bovine virus diarrhoea is a highly contagious disease of cattle. Cattle of all age groups are susceptible to infection.

The first description of BVD was made in New York, USA in 1946 (Buxton and Fraser, 1977). The etiological agent was isolated in 1957; which was later classified as pestivirus of the family Flaviviridae (Pringle, 1999).

BVD virus is closely related to the border disease virus of sheep and to the classical swine fever virus. Some of the BVD viruses are cytopathogenic, others are non-cytopathogenic.

Recent molecular biological studies revealed that BVD viruses could be differentiated into two distinct serotypes, which exhibit salient antigenic and biological differences. Each biotype is involved in various clinical forms. For instance, type 2 BVD virus isolates are usually non-cytopathogenic and are linked with the severe form of the disease (Carman *et al* 1998). Both genotypes can cause clinically inapparent infections.

BVD can be manifested in several distinct clinical forms :

1- Acute infection :
This acute form usually occurs in young animals. It may be associated with diarrhoea (Baker, 1995). The virus is immunosuppressent, so the affected animals may suffer from concurrent infections. Sporadic severe outbreaks of the acute form have been reported to be associated with haemorrhagic lesions and thrombocytopenia causing high mortality (Bolin and Ridpath,1995; Baker, 1995). Fever, pneumonia and diarrhoea with haemorrhagic signs were also reported in any age group (Carman, 1998),

2- Congenital infections :
Congenital infections due to BVD are usually associated with non-cytopathogenic viruses. They cause abortions, stillbirths and teratogenic effects on the foetus (Brownlie, 1985; Duffell and Harkness, 1985; Baker, 1995; Moennig and Liess, 1995).
Infection of pregnant cows with the BVD virus at the first trimester usually results in abortion of a conceptus that is small and goes unnoticed by the farmer. The cow would return to service but fails to maintain pregnancy. In this case, early embryonic death is suspected (Baker, 1995; Moennig and Liess, 1995). Another outcome of early BVD virus infection during pregnancy would be the death and absorption of the fluids from the foetus leading to its mummification.
Other congenital effects could be eye defects such as cataracts, dysmyelination, retarded growth and arthrogryposis.
When BVD virus infection occurs after 150 days of pregnancy, the immune system of the foetus will be developed and so, its infection will result in antibody response and it will not be affected.

3- Persistent viraemia:
Calves may be born with persistent viraemia if they were infected before 110 days of gestation, i.e before they are immunocompetent (Stokstad, *et al*, 2003). In these calves, the clinical signs vary from the apparently healthy animal to the weak, unthrifty calf that cannot stand or suckle. These latter calves can show ataxia, muscular tremors and blindness. Such calves often die within few days of birth. Their condition is referred to as the " weak calf syndrome".
The viraemic cattle that survive to sexual maturity will be a source of infection to other cattle; and so must be removed from the herd.
Both male and female cattle can transmit the virus sexually.

4- Mucosal Disaese (MD):

Persistently viraemic animals may succumb to mucosal disease (Brownlie, 1985). MD has been shown to be associated with the cytopathogenic BVD virus biotype (Brownlie *et al* 1984; Bolin, 1993).

MD can have a rapid onset that the first signs are morbid animals. It is invariably fatal. In more common cases, animals become anorexic for few days, become reluctant to move. They then show diarrhoea, mouth erosions along the gingival margin. They also show salivation and lacrimation, lose body condition and die.

Necropsy findings are usually those of erosion of the gut mucosa. The most salient features are those noticed on the lymphoid Peyer's patches in the small intestines and in the colonic tonsils.

The epidemiological situation of BVD in Saudi Arabia is not clear. No studies were previously conducted.

The aim of the present study is to probe the clinico-pathological disease situation so as to have a baseline for future studies.

Materials and Methods

Field investigations:

The field investigations involved the following:

Sampling:

Samples from clinically suspected cases were collected from different slaughter houses and veterinary clinics and used for pathological investigations. Such samples also included aborted or dead neonate calves.

Cattle with clinical symptoms suggestive of BVD infection were examined and samples of whole blood were collected in ethylene diamine tetra acetic acid (EDTA), for virus isolation attempts.

Laboratory investigations:

Pathological examinations:

Aborted foetuses or carcasses, from suspected BVD cases, were subjected to postmortem examination. Tissue samples from thymus, lymph nodes, cerebellum, spleen and different parts of intestines were collected, for histopathological examinations, in 10% neutral formalin, processed by paraffin method and 3-4 um sections were made. These sections were stained with the standard method of Haematoxylin and Eosin (H and E).

Virus detection :

The indirect fluorescent antibody test (FAT) of Fernelius and Lambert (1969) was followed to detect BVD virus antigens in MDBK cell cultures which were inoculated with samples from tissues of all suspected pathological cases of aborted or stillborn calves (Buxton and Fraser, 1977).

Results

Clinical Findings :

No mucosal disease nor other suggestive symptoms of BVD infection were observed in adult cattle. However, abortions and stillbirths were seen.

Table (1) shows the clinical manifestations seen in the affected neonate or aborted calves during the period of study. Of the 29 examined calves, 3 were aborted autolysis, 6 were born dead at full term, 3 developed respiratory stress and died few hours post-nata. Six were born weak, one died after 24 hours and five developed nervous signs and died at 10-25 days of age. Five calves were born normal, but showed severe diarrhoea at day 29 of age.

Table (1)
Clinical signs of the aborted and stillborn calves

No. of affected calves	Clinical observations	Malformations
5	Born normal but showed severe diarrhoea at day 20 post-natal.	-*
3	Aborted autolysis.	-
6	Born dead at full term.	-
3	Developed severe respiratory distress and died within few hours.	-
1	Weak at birth, died after 24 hours post-natal.	-
5	Weak at birth, developed nervous signs and died at 10-25 days of age.	-

*- = no malformations were seen.

No apparent deformities were seen in the aborted nor the stillborn calves in this study.

The calves with Respiratory Distress:

The affected calves could not rise to suckle. Three hours postnatally they began to shiver and developed breathing difficulties that progressed rapidly from tachypnea to open mouth breathing with audible respiratory sounds and died.

Calves with nervous signs:

Marked neurological signs were recorded in some calves although they were born without difficulty and was conscious at birth. With elapse of four hours, the calves were still adopting the same sitting position or squatting with the heads from side to side or up and down.

Astasia (inability of the calf to stand) was marked by several unsuccessful attempts to stand (fig. 1). Most of the time, the animals remained squatting with opisthotonus of the head. The animals showed a sleepy appearance and lateral prostration after little period of activity. There was an impairment in the vision due to congenital cataract. The left caudal superficial cervical lymph node was hypoplastic when compared to the right one. In general the calves suffered from stunting growth, anorexia, occulo-nasal discharge (fig.2) Cataract (fig.3) Keratitis (fig.4) and depression.

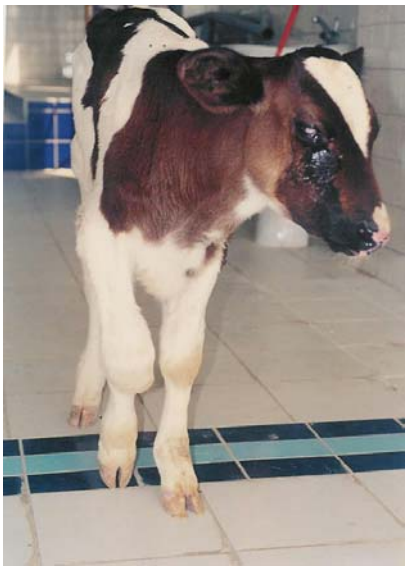

Fig.1: A calf affected with BVD. Note the congenital cataract and swelling of knee joint

Fig 2: A calf affected with BVD. Note occulo-nasal discharge.

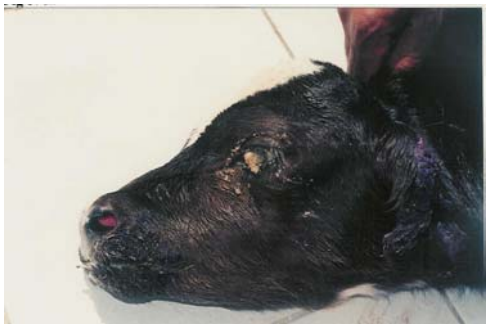

Fig 4: A calf affected with BVD. Note keratitis

Fig 3: A calf affected with BVD. Note the cataract.

Calves with diarrhoea:

In day 20 post-natal, five calves developed severe watery diarrhoea, which soon became bloody. One calf showed severe emaciation and dehydration despite antibiotic treatment, rigorous parenteral feeding and Ringer's fluid rehydration. The other calves had intermittent diarrhoea, anorexia and depression.

Pathological picture:

Stillborn calves

Gross picture

The ventral superficial cervical retropharyngeal and mesenteric lymph nodes were discolored reddish black in cut section due to haemorrhages. The spleen of one calf seemed to be relatively shrunken with irregular subcapsular suffusions. The thymus was studded with multiple petechiae and ecchymoses. The intestinal mucosae of the duodenum, ileum, ileo-cecal junction and proximal part of the colon, as well as, the rectum were congested and oedematous. The placentas revealed no macroscopic abnormalities.

Histopathological results

The lymphoid follicles of the aforementioned lymph node were hypoplastic with multiple haemorrhages in the parenchyma. The spleen showed massive lymphoid depletion (fig. 5) and sub capsular haemorrhage. The interlobular vessels of the thymus were congested and haemorrhages were seen in the parenchyma. Vasculitis was prominent in medium sized vessels with radial orientation of the lining endothelium. There was migration of the intima. Perivascular aggregation of mononuclear cells was also noticed.

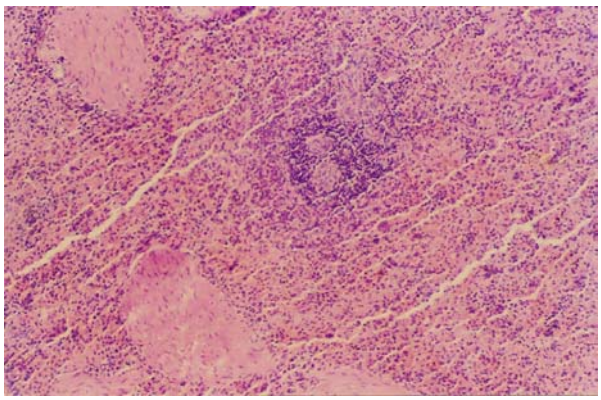

Fig 5: Spleen. Note depletion of Lymphoid follicles. H&E. X10

Calves with Respiratory Stress:
Gross picture

Multiple subpleural haemorrhages and focal atelectatic areas (fig. 6) were embedded one cm. beneath the pleura. The cut section revealed red hepatization of the lung parenchyma and widening of the interlobular septa with edema. The cut section of the brain revealed multiple discrete petechial haemorrhages of both the cerebral hemispheres.

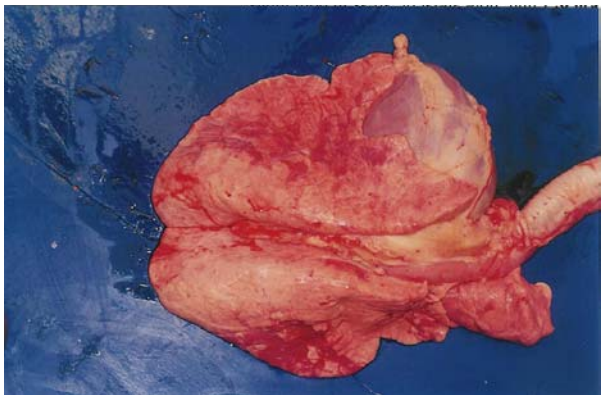

Fig 6: Lung, note atelectatic areas in both lungs.

Histopathological results

Microscopic examination of the lung revealed a variety of pathological changes including pulmonary hyalinization, and bronchopneumonia (fig. 7). Interstitial edema and lymphangiectasia were seen. Smaller air ways were filled with inflammatory cells predominantely neutrophils. Thick layers of eosinophilic fibrillar and/or granular material were lining the alveoli. The interlobular septa were distended with edema, haemorrhages and congested vessels. The lymphatic vessels were dilated and partially occluded with fibrin thrombi. Multiple perivascular cerebral haemorrhages were prominent. In the cerebellum, there were interlacing alterations of ectopia and degeneration of Purkinje cells with a poor population of the molecular layer suggesting cerebellar hypoplasia. The hepatocytes appeared vacuolated with cavitation of the reticular endothelial kupffer cells.

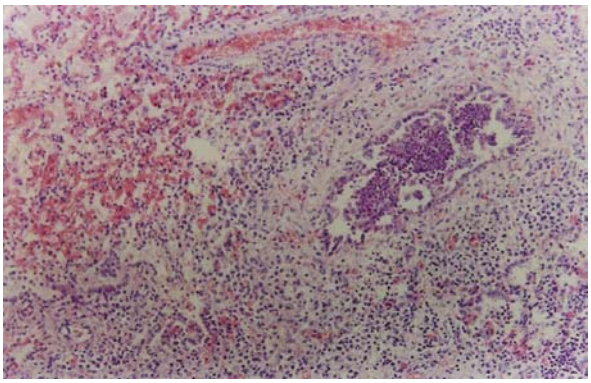

Fig 7: Lung, Bronchopneumonia

Calves with nervous signs:

Gross picture:

The eye showed cataract. There was mild hypoplasia of the cerebellum with narrowing of the folia which appeared with shallower convolutions (fig. 8). Congestion of the ileal mucosa was outstanding with ecchymotic haemorrhages overlaying the peyer's patches. Bronchial and mesenteric nodes were remarkably congested and haemorrhagic.

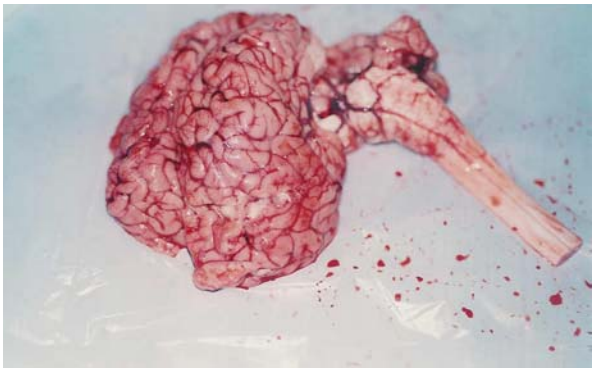

Fig 8: Brain, hypoplasia.

Histopathological results:

There was numerical decrease and ectopia of purkinje cells. Some purkinje axons were swollen, degenerated or necrotic with accompanying neurophagia. Folial edema with cavitation of the white matter was also noted. Non-suppurative leptomeningitis and mononuclear perivasculitis were also present (fig. 9). The folial white matter showed extensive damage to myelinated fibers

with focal gliosis and necrosis of the ganglionic neurons. Thymic follicles were depleted leaving a relative abundance of the thymic epithelial cells with interlobular edema, haemorrhages. Vasculitis was also present in lymph nodes and spleen. The vasculitis was manifest by fibrinoid degeneration of the medullary vessels or follicular arteriole and associated with perivascular edema and mononuclear cell inflitration.

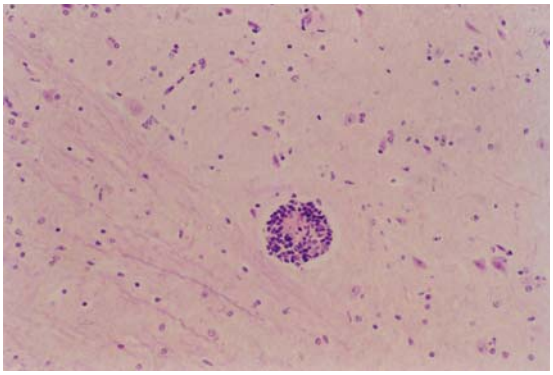

Fig 9: Brain, perivascular cuffing and vasculitits. H&E. X100

Calves with Diarrhoea:

Gross picture:

The buccal mucosa showed erosions and superficial ulceration (fig. 10). there were extensive ulcers in the abomasum towards the pylorus. Severe congestion, edema, haemorrhages and ulcerations/necrosis were observed in the abomasums (fig. 11) and distal part of the ileum and ileo-caecal junction. Congestion and edema of the intestinal mucosal folds gave it the characteristic

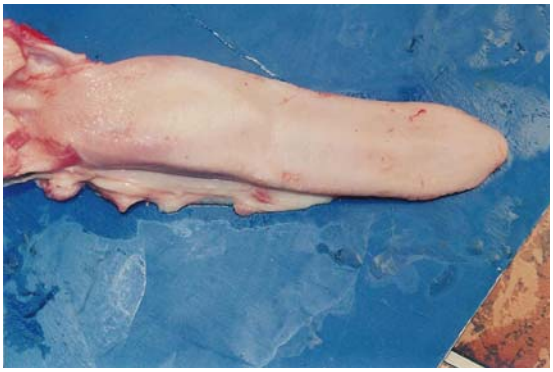

Fig 10: Tongue, erosion and superficial ulcers.

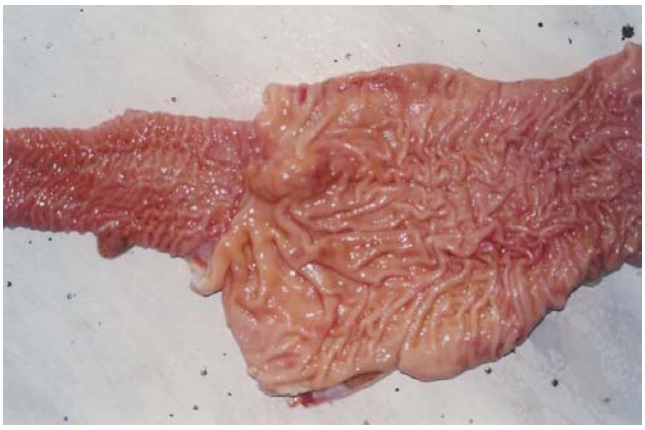

Fig 11: Abomasum and duodenum, note edema and congestion of mucosal folds

"tiger's strips" pattern or zebra markings. The associated lymphoid tissue and mesenteric lymph nodes were severly congested almost black.

Histopathological results:

Severe lymphoid depletion of the lymphoid follicles was the remarkable finding. The mucosal vessels were congested. There was edema in the submucosa with fibrinoid degeneration of the ileal submucosal vessels. There was mild to moderate perivascular aggregation of mononuclear cells. At the ileocecal junction, there was an extensive ulcer with edema of the submucosa (fig.12).

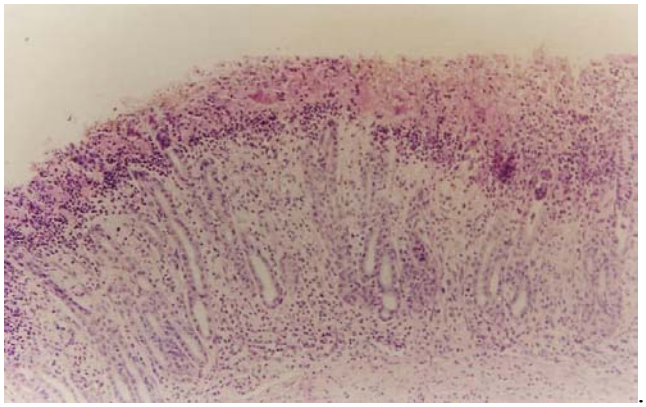

Fig 12: ileum, note mucosal necrosis. H&D. X100

The fluorescent antibody test:

Specific fluorescence was seen in MDBK cell culture inoculated with tissues samples from all (100%) of the examined aborted or stillborn calves. Typical picture is shown in (fig. 13).

No FAT activity was detected in the MDBK cells inoculated in the buffy coats from the adult suspected cattle.

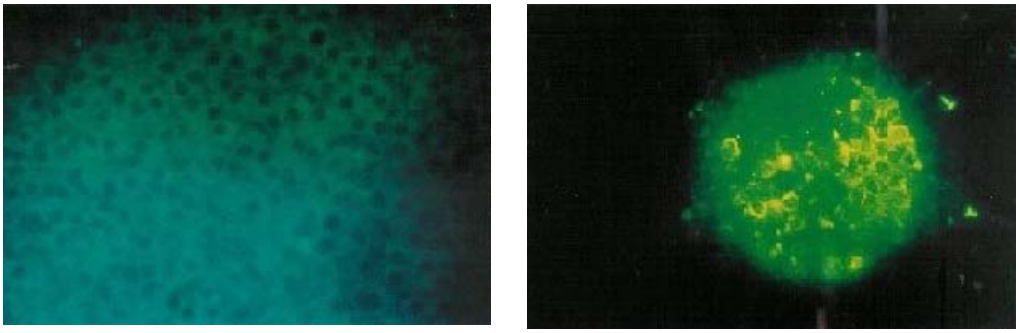

Fig. 13: Specific fluorescence stain for BVD virus in MDBK cell culture inoculated with tissues samples from aborted calf (right). Negative control (left).

Virus Isolation:

Vero cell culture monolayers inoculated with buffy coats from suspected cattle did not show any discernible cytopathic effect even following two blind passages.

Discussion :

Results been described in the present study constitute the first of its kind in the Kingdom of Saudi Arabia.

Findings of this study plainly exposed the BVD epidemiological picture in the Eastern Region of Saudi Arabia.

It is of great interest that typical mucosal disease, which is clinically very similar to rinderpest, was not seen during the survey period. This could be due to high herd immunity in adult cattle that rendered them immune from the disease. However, and since that cattle foetuses do not receive maternal antibodies while *in-utro*, they are not usually protected from the virus especially before 150 days of gestation period when their immune response have not developed yet.

The clinico-pathological picture of the examined aborted and stillborn calves were similar to those reported for BVD virus infection elsewhere (Hegazy, 1995; Barlow *et al* , 1975; Done *et al*, 1980; Whitemore, 1981).

The pathology of infection in the dead calves, in the present study, reflected the involvement of a wide variety of tissues. These tissues could be grouped into three main categories: nervous tissues (brain and optic nerve), gastrointestinal and lymphoid tissues (thymus, spleen and lymph nodes). The common finding in these tissues was the involvement of blood vessels with vasculitis (meningeal vessels, ileal submucosal blood vessels and follicular arteriole and medullary vessels). The vasculitis and the direct effect of the virus replication in the target cells are considered to be the central pathogenesis of BVD virus infection. Jubb *et al*, (1995) supported such a conclusion by stating that vasculitis is a major feature in the pathgenesis of BVD virus infection.

The involvement of the nervous tissues in the present study explains the birth of weak calves with nervous symptoms. The cerebellar hypoplasia could be attributed to the direct effect of the virus on the brain tissues specially the cerebellum. This is evident by poor population of the molecular layer and degeneration and/or necrosis of Purkinje cells. In addition, hypomyelination or defective myelination (dysmyelinogenesis) was clear.

The optic neuritis and the loss of myelin sheath may, in part, explain the birth of blind calves seen in the present study. The same signs were described by Brown *et al,* (1974). Cataract adds to the severity of the lesion which resulted in the loss of eye's sight.

Bovine viral diarrhoea antigen was detected by FAT in all the stillborn calves and those with other affections suspected for BVD virus infection. Such a result indicate that BVD virus infection is widespread in the Eastern Region of Saudi Arabia.

Although vaccination against BVD is routinely practiced in Saudi Arabia, the present study confirmed that clinical BVD is widespread.

Indeed, this situation reflects that a large section of the cattle population can miss vaccination against the disease. On the other hand, the village cattle which are kept at " Al-nakheel" farms are not vaccinated against BVD. So, they can maintain the virus in the environment and this might constitute a continuous threat for dairy farms in the Kingdom.

It is recommended that 'agricultural extension' must be intensified towards 'Al-nakheel' farmers and villagers regarding vaccination of their cattle. Saudi villagers usually keep small number of cows, sometime as low as 4-5 cows per farm.

It would be of interest to examine the ill-effects that BVD vaccines might impose on cattle in Saudi Arabia. This could be done in a future study.

The elucidation of the epidemiology of BVD in Saudi dairy farms is recommended. This is of particular importance as such information is lacking in the literature.

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References:

1. Baker, J.C. (1995). The clinical manifestations of bovine viral diarrhea infection. Vet. Clin. North. Am. Food Animal Practice, 11: 425-445.
2. Barlow,R.M.; Rennie, J.C.; Keir, W.A.; Gardiner, A.C. and Vantist, J.T. (1975). Experiments in border disease. VII. The disease in goats. J. Camp. Pathol., 85: 291-297.
3. Bolin, S.R. (1993). Immunogens of BVD virus. Vet, Microbiol., 37: 263-271.
4. Bolin, S.R. and Ridpath, J.F. (1995). Assessment of protection from systemic infection or disease afforded by low to intermediate titres of passively acquired neutralizing antibody against BVD virus in calves. Am. J. Vet. Res. 56: 755-759.
5. Brown, T.T.: de Lahunta, A. and Bistner, S.I (1974): Pathogenic studies of infection of the bovine fetus with bovine viral diarrhea virus. II. Ocular lesions. Vet. Pathol. 12:394.
6. Brownlie, J. (1985). Clinical aspects of the BVD virus / mucosal disease complex in cattle. In Practice, 7: 195-202.
7. Buxton, A. and Fraser, (1977). In " Animal Microbiology". Vol. 2 "Rickettsia and Viruses" pp. 657-660.
8. Carman, S.; Van Dreumel, T.; Ridpath, J. and Hazlett, M. (1998). Severe acute BVD in Ontario, 1993-1995. J. Vet. Diag. Invest., 10: 27-35.
9. Done, J. T., Terlecki, S. and Richardson. C. (1980). Bovine virus diarrhea-mucosal disease virus: Pathogenecity of the fetal calf following maternal infection. Vet. Rec. 106: 473-479;

10. Duffel, S.J. and Harkness, J. W. (1985). BVD/Mucosal disease infection in cattle. Vet. Rec. 117: 240-245.
11. Fernelius, A.L. and Lambert, G. (1969). Detection of BVD virus and antigen in tissues of experimentally infected calves by cell inoculation and fluorescent antibody techniques. Am. J. Vet. Res.. 30: 1551.
12. Hegazy, A.A.; EI Sanousi, A.A.; Lotfy, M.M.., Hamouda, M.A.; Hassan, H.B.; Hussein, A.H. and Aboellail, T .A. (1995). Pathological and virological studies on calf mortality: B. Mortalities associated with bovine virus diarrhea (BVDY) infection. J. Egyp. Vet. Med. Assoc., 55: 493-503.
13. Jubb, K.V.F., Kennedy, P.C. and Palme, V. (1995). Pathology of domestic animals. Vol. I & II. 4th edition, New York, academic Press, INC.
14. Moennig, V. and Liess, B. (1995). Pathogenesis of intrauterine infections with BVD virus. Vet. Clinic. North. Am., 11: 477-487.
15. Pringle, C.R., (1999). Virus taxonomy. The universal system of virus taxonomy, updated to include the new proposal ratified by the International Committee on taxonomy of viruses during 1998. Arch. Virol. 144: 421-429.
16. Stokstad, M.; Niskanen, R.; Lindberg, A.; Thoren, P.; Belak, S.; Alenius, S. and Loken, T. (2003). Experimental infection of cows with bovine viral diarrhea virus in early pregnancy – findings in serum and foetal fluids.. Journal of Vet. Med.-B, 50: 424-429.
17. Whitemore, H.L.; Zemjanis, R. and Olson, B.S.J (1981). Effects of bovine diarrhea virus on conception in cattle. J. Am. Vet. Med. Assoc. 178: 1065-1067.

دراسات سريرية ومرضية على مرض الإسهال البقري الفيروسي في شرق المملكة العربية السعودية

عادل إبراهيم العفالق وعلي حجازي والطيب ابوالزين وبشر البشر

كلية الطب البيطري والثروة الحيوانية- جامعة الملك فيصل

الأحساء – المملكة العربية السعودية

الملخص

تمثل الدراسة الحالية الأولى من نوعها في المملكة العربية السعودية. انحصرت الدراسة في المنطقة الشرقية حيث جمعت العينات من حالات مشتبه بإصابتها بمرض الإسهال البقري الفيروسي. درست العينات من النواحي السريرية والمرضية والفيروسية. وكذلك سجلت الملاحظات السريرية على البقر والعجول المجهضة أو المولودة حديثا.

أكدت النتائج وجود هذا المرض بشكل واسع بالمنطقة الشرقية من المملكة العربية السعودية. كما شوهدت العديد من حالات الإجهاض والحالات المرضية في العجول حديثة الولادة. والتي ثبت بأنها نتيجة العدوى بفيروس الإسهال البقري الفيروسي . لم تؤكد الدراسة وجود حالات سريرية في البقر البالغة.