Experimental Infection of Pregnant Does with Bovine Virus Diarrhoea Virus (BVDV): Pathological Effects on Newly Born Kids

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Abstract: The present study was carried out on a total number of nine healthy pregnant does free from BVD neutralizing antibodies. The animals were I/V and I/P inoculated with two cytopathic strains of BVD virus (NADL-camel strains) at about 65 days of gestation. In NADL strain group, abortion occurred in 3 out of 5 virus inoculated does. Meanwhile, the remaining two does gave birth to 3 kids, one of them died within 4hrs post kidding. In camel strain group, one of four inoculated goats, aborted one fetus. Meanwhile, the other three does gave birth to 4 clinically healthy kids. The most pronounced histopathological changes in the central nervous system (CNS) of the kids were multiple foci of gliosis, congestion and perivascular lymphocytic cuffing in white matter of cerebrum and necrobiotic changes of purkinje cells of cerebellar cortex. Marked depletion of thymocytes associated with thinning of the thymic cortex was also observed. The precolostral sera of kids were positive for neutralizing antibodies against BVDV and were decreased to the half after the kids suckling the colostrum. Moderate intensity of fluorescent reaction in numerous neurons scattered throughout the cerebrum was seen. There were aggregations of specific moderately positive stained cells in the molecular and granular layer of cerebellum. In the thymus, spleen and mesenteric lymph nodes, positive fluorescent reacting cells were noticed. It could be concluded that BVD virus induced pronounced pathological changes in nervous and lymphatic tissues goats kids. Brain, thymus, spleen and mesenteric LN are the most susceptible tissues for the BVDV antigen detection using immunofluorescent technique which is a valuable and confirmative tool in diagnosis of BVD virus infection.

Key words: Goats - kids - abortion · Bovine viral diarrhea virus · Histopathology · Neutralizing antibodies · immunofluorescence

INTRODUCTION

Bovine viral diarrhea virus (BVDV) is a member of the genus Pestivirus, family Flaviviridae along with classical swine fever virus (CSFV) and border disease virus (BDV) of sheep. Pestiviruses are small enveloped, positive-sense RNA viruses that serologically cross-react and cause a variety of clinical manifestations in their natural hosts [1].

Similar to BVD in cattle, pestivirus infections in small ruminants can cause variety of clinical syndromes including reproductive failure, abortion, still birth, respiratory disease, poor growth rate, diarrhea, nervous signs and muscular tremor. Acute infection in immunocompetent animals usually causes transient mild disease followed by seroconversion, whereas infection of fetus before development of immune system leads to birth of persistently infected (PI) animals which are the main source of transmission [2]. Several experimental studies have shown the susceptibility of goat for pestivirus infection causing reproductive dysfunction [3-5] Recently, natural transmission of BVDV from persistently infected cattle to pregnant goats with subsequent abortion was reported [6].

Intrauterine (Transplacental) infection of ovine fetuses with BVDV has been frequently associated with brain pathology and congenital malformations such as hypomyelination, cerebellar hypoplasia or porencephaly and hydranencephaly [7]. Moreover, infection with BVDV in aborted goat fetuses and stillborn kids can result in necrotizing placentitis, encephalitis, nonsuppurative myocarditis and thymic depletion [8] but other organ systems appear to be relatively unaffected [6].
The effect of experimental infection with BVD on the reproductive function of pregnant does with special reference to hormonal and pathological alterations was previously investigated by Desouky et al. [9]. The present study was carried out to clarify the pathological effect of BVDV either of bovine or camel origin of neonatal goats kids in addition to demonstrating the distribution of BVD viral antigen within the tissues of infected kids using fluorescent antibody technique and evaluation the humoral immune status of these kids.

**MATERIALS AND METHODS**

**Animals:** The present work was carried out on 13 healthy pregnant does which were free from BVD virus neutralizing antibodies, brucella and parasitic infestation. Their ages ranged from 12-24 months old and body weight ranged from 18-20 Kg.

**Virus Inoculum:** Two cytopathic strains of BVD virus were kindly obtained from the Department of Virology, Faculty of Veterinary Medicine, Cairo University. National Animal Disease Laboratory (NADL) strain of BVD virus of bovine origin. The titre of this strain was $10^9$ tissue culture infective dose (TCID$_{50}$) per ml. Camel strain of BVD virus. The titre of this strain was $10^{11}$ tissue culture infective dose (TCID$_{50}$) per ml.

Both strains of BVD virus were propagated and titrated in Madin-Darby Bovine kidney cell line (MDBK).

**Experimental Design:**

The animals were allocated into three groups:

- **Group I** included 5 animals, each doe was inoculated with 10 ml {5ml I/V and 5ml I/P} of tissue culture suspension of NADL strain containing $10^6$ TCID$_{50}$ per ml at about 65 days of gestation.
- **Group II** comprised 4 animals; each doe was inoculated with 10 ml {5ml I/V and 5ml I/P} of tissue culture suspension of camel strain containing $10^{11}$ TCID$_{50}$ per ml at about 65 days of gestation.
- **Group III** consisted of 4 animals which kept non infected along the whole period of experimental work and served as control.

Following inoculation, pregnant does were kept under daily observation throughout experimental period for any clinical manifestations. Also, all animals were penned separately and kept at the same managemental conditions and under complete quarantine and preventive measures.

**Newly Born Examination:** Perinatal kids were examined grossly and autopsied as soon as possible after abortion and/or kidding. Also, the apparently healthy kids were examined clinically at birth and daily until they were killed at 7 days old.

**Collection of Blood Sample:** Precolostral blood samples were aseptically taken from kids and just before their sacrificing at 7 days old. Serum samples were harvested by centrifugation at 3000 rpm for 10 min. and kept frozen at -20°C till used for serum neutralization test.

**Fluorescent Microscopical Method:** The direct immunofluorescent antibody technique was adopted by Goldman [10] as rapid confirmatory method to detect the topographical distribution of BVD virus within the tissues.

**Histopathological Study:** Tissue specimens from cerebrum, cerebellum, thymus, mesenteric lymph nodes, ileum and spleen were taken from kids after slaughtering. These tissues were washed, dehydrated by alcohol, cleared in benzene and embedded in paraffin. Tissues were sectioned at 5u thickness and stained with H&E for histopathological examination.

**RESULTS**

**Clinical Symptoms and Post Mortem Findings:** In NADL strain of BVDV group, abortions occurred in 3 out of 5 does inoculated with virus at 7, 17, 21 days post inoculation (PI) of virus at 65 days of gestation. The fourth doe delivered at full term a twin kids, one was clinically healthy. The another one died within 4 hrs post kidding (perinatal death). This was noticeably weak, unable to stand and undersized. Moreover, the kid showed varying degrees of body tremors and apparently respiratory distress associated with subnormal temperature and cardiac depression. Post mortem examination revealed thymic hypoplasia associated with subnormal temperature and cardiac depression. Post mortem examination revealed thymic hypoplasia associated with presence of petechial hemorrhages. Mesenteric lymph nodes and Peyer's patches were congested and hemorrhagic (Fig. 1). Also; iliac lymph nodes were discolored reddish black by congestion and hemorrhages.
Moreover, petechial hemorrhages were seen on the nasal septum. Gross changes were not found in CNS except for congestion of meninges. On the other hand, no significant gross lesions were seen in clinically healthy kid. The remaining doe from NADL strain of BVDV group, gave birth to full term a clinically healthy kid. The postmortem examination showed hemorrhagic and edematous prescapular lymph node of the right side meanwhile that of the left side appeared oedematus and enlarged in size (Fig. 2). The mesenteric lymph nodes were also edematous and enlarged. Focal hemorrhagic area at the neck of urinary bladder was seen, in addition to congestion and hemorrhage at corticomedullary junction of kidney was observed.

In camel strain of BVDV group, one of four goats that received the virus at 65 days of gestation, aborted one fetus at 65 days (PI) of the virus. Meanwhile, the remaining three does gave birth to 4 clinically healthy kids. The macroscopical examination of these kids showed no consistent gross lesions except for congestion of meninges which was seen in one kid. In control group, 4 does gave birth at full term to 6 kids all of which were normal, viable and clinically healthy.

**Histopathological Findings**

**Newly Born Kids of BVDV NADL strain Group**

**Cerebrum:** Showed diffuse and multiple foci of gliosis in white matter (Fig. 3) in addition to few foci of satellitosis and neuronophagia were seen. Many cerebral blood vessels displayed lymphocytic perivascular cuffing associated with perivascular edema (Fig. 4). The submeningeal blood vessels were dilated and congested.

**Cerebellum:** Cerebellar cortex exhibited necrobiotic changes of purkinje cells associated with edema. There were ectopia and diminution in the population of purkinje cells, in addition to these lesions hypocellularity of granular cell layer was seen.
**Spleen:** Showed involution of the white pulp. The follicular artery showed luminal constriction accompanied with proliferation of endothelial cells lining.

**Mesenteric Lymph Node:** Showed subcapsular edema associated with severe congestion and hemorrhage in the cortex and medulla. The lymphoid tissue of cortex and medulla exhibited severe lymphocytic depletion with absence of lymphoid follicle.

**Thymus:** Revealed marked depletion of thymocytes and the cortical lobules were smaller and reduced in size with relatively widening of the medulla. Hassall's corpuscles appeared prominent and increased in number with its proximity to capsule. Severe congestion of blood capillaries and hemorrhages were seen.

**Ileum:** Revealed multiple lymphocytic cell necrosis in the Peyer's patches associated with edema hemorrhages and congestion of blood vessels in submucosa.

**Newly Born Kids of BVDV Camel Strain Group**

**Cerebrum:** Most of cerebral blood vessels appeared dilated and congested. Perivascular lymphocytic cuffing associated with edema were also seen. There were multiple foci of gliosis, in addition to few foci of satellitosis.

**Cerebellum:** The purkinje cells suffered from necrobiotic changes. Some of cells appeared swollen & pale with chromatolysis and were surrounded by microglial cells showing neuronophagia. While others were atrophied, shrunken and undergo lysis and replaced by microglia cells (Fig. 5). Ectopia and numerical decrease of purkinje cells were also seen.

**Thymus:** Revealed partial involution. There was relatively thinning of the cortex associated with widening of medulla. Hassall's corpuscles were somewhat or relatively prominent and increase in number. The decrease in number of thymocytes was clearly noticed.

**Spleen:** Displayed extensive aggregations of neutrophils in the red pulp. The lymphoid tissue appeared within the normal limit. The follicular artery showed luminal constriction accompanied with orientation or proliferation of endothelial cell lining.

**Mesenteric L.N.:** Exhibited presence of excessive numbers of neutrophils within the medullary sinuses. Mild hyperplasia of lymphoid follicles were seen.

**Result of Immunofluorescent:** The presence and distribution of BVD viral antigen in organs of infected kids with either camel or NADL strain of BVDV were nearly the same in each organ but widely differed in degree of intensity of fluorescence as shown in Table 1. In cerebrum, moderate intensity of fluorescent reaction in numerous neurons aggregated or scattered throughout the cerebrum was seen (Fig. 6). The greatest concentration of fluorescent neurons was in the deeper layer of the cerebral cortex.
Table 1: Results of fluorescent antibody technique of newly born kids

<table>
<thead>
<tr>
<th>Kid No. Examined organ</th>
<th>Camel strain of BVDV Group</th>
<th>NADL strain of BVDV Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kid No. 1</td>
<td>Kid No. 2</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Thymus</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mesenteric L.N.</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Spleen</td>
<td>+</td>
<td>-ve</td>
</tr>
</tbody>
</table>

* Perinatal dead kid

Intensity of fluorescence: ++++ high, ++ moderate, + faint, ± trace

Table 2: Serum neutralizing antibodies titers of newly born kids

<table>
<thead>
<tr>
<th>Kid No. sampling</th>
<th>Camel strain of BVDV Group</th>
<th>NADL strain of BVDV Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. 1</td>
<td>No. 2</td>
</tr>
<tr>
<td>Precolostrum</td>
<td>4</td>
<td>-ve</td>
</tr>
<tr>
<td>Postcolostrum</td>
<td>2</td>
<td>-ve</td>
</tr>
</tbody>
</table>

(ND): not done-die within 24 hrs post kidding (perinatal dead kid)

Fig. 7: Spleen, showing few reacting macrophages in the red pulp. DIF stain X400

Meanwhile, Cerebellum showed aggregations of specific moderately positive stained cells in the molecular and granular layer. In spleen, few specific fluorescent reacting cells were scattered in the red pulp of spleen. These cells were mainly compromising macrophages (Fig. 7). There were faintly positive stained cells scattered throughout the parenchyma of the thymus. Mesenteric lymph nodes showed positive stained scattered population of lymphocytes in the interfollicular space. Specific fluorescent reaction in macrophage and reticular cells located within the germinal centers was observed.

**Result of Serum Neutralization Test:** The results of serum neutralization test of kids born to does inoculated with either camel or NADL strain of BVDV are shown in Table 2. The precolostral sera of kids (1, 3) born to the does inoculated with NADL strain of BVDV were positive for neutralizing antibodies (NAabs) with very low detectable titer ranged from 1/2 - 1/4. In case of kid (No. 3), the titer of NAabs was decreased to the half in the postcolostral collecting serum. Meanwhile, the remaining kid (No. 2) was seronegative. On the other hand, the pre and post colostral sera of kids (No. 2, 4) born to does inoculated with camel strain of BVDV were negative for BVDV neutralizing antibodies. Meanwhile, the remaining kids (No. 1, 3) showed very low precolostral detectable NAabs titers which were decreased to the half after the kids suckling the colostrum for 7 days.

**DISCUSSION**

In the present study, the postmortem findings of kids born to does inoculated with NADL strain of BVDV revealed congestion and hemorrhages of lymph nodes, meninges and kidney. Similar results were reported by Parsonson et al. [11] and Hewicker-Trautwein et al. [12]. Such changes could be due to direct effect of the virus on the blood vessels causing damage of endothelial cells lining. In this respect, BVDV antigens were predominately detected in the brain of experimentally infected ovine fetuses and in vascular wall mostly in endothelial cells of capillary and small blood vessels of cerebral, cerebellar cortices and meninges of stillbirth and newborn lambs [1]. Thrombocytopenia has been reported as feature of acute BVD virus infection which occurred as a result of BVD induced myelosuppression or bone marrow necrosis.
Thrombocytopenia resulted in clinical signs of bleeding manifested by bloody diarrhea, epistaxis, petechial and ecchymotic hemorrhages [13]. In this respect, Wilhemmsen et al. [14] added that endothelial damage, defective formation or excessive activation of clotting factors and consumption of platelets were possible explanation for bleeding tendency.

The histopathological findings of kid cerebellum of the present work revealed necrobiotic changes of purkinje cells associated with its ectopia and diminution in population in addition to hypocellularity of granular cell layer. Such alterations were without significant gross cerebellar abnormalities. These results are confirmed by findings of Loken and Bjerkas [3], Plant et al. [15, 16] and Hewicker-Trautwein et al. [17]. In this respect, Trautwein et al. [18] stated that stage of gestation of dam at the time of exposure to BVDV will obviously determine whether cerebellar defects will occur. Brown et al. [19] reported that a paucity of small neurons in the granular layer and ectopia of purkinje cells were sequential changes which have been observed following BVDV attack upon a mitotically active external germinal layer and developing cerebellar vascular system. Hewicker-Trautwein et al. [17] indicated that BVDV destroyed radial glial fibers leading to failure of neuronal migration with some neurons surviving in ectopic positions which may be termed microdysgenesis.

Regarding the effect of BVDV on the cerebrum of kids, the current work showed congestion of blood vessels associated with perivascular lymphocytic cuffing, interstitial and perivascular edema in addition to foci of multiple gliosis, satellitosis and neuronophagia. These findings are in agreement with findings of Broaddus et al. [6], Cutlip et al. [20], Loken et al. [21]. The degeneration and satellitosis of neurons of cerebrum could be attributed to the direct destructive action of the virus [21].

Studies in ovine fetuses following experimental infection with BVDV [7] have shown that during the early post-infectious phase, the virus destroys the fetal ependyma and the tissue of cerebral hemispheric walls. They added that invasion and destruction of fetal brain by BVDV was followed by marked inflammatory reactions which were predominated by brain macrophages. The occurrence of perivascular lymphocytic cuffing indicating immunological interaction between the host and the virus because the kids had precolostral neutralizing antibodies [5]. On the other hand, fluorescent microscopical results revealed presence of BVD viral antigen within the brain, thymus, spleen and mesenteric lymph nodes. These findings are consistent with the previous studies using immunohistochemistry(IHC) technique by Scherer et al. [1], Gardiner [22] and Hewicker-Trautwein et al. [23] in sheep; Fernandez et al. [24] and Wohrmann et al. [25] in cattle; Wohlsien et al. [5], Broaddus et al. [6] and Lamm et al. [8] in goat. Heart, thymus and brain appear to be the most reliable tissues for detecting BVDV antigen using IHC in organs of aborted goat fetuses with or without histologic lesions [8]. The predilection sites of BVDV antigen were the cerebral cortex and hippocampus and BVDV had a selective tropism for neuronal cells that have specific receptors for BVDV on their cytoplasmic membranes. Persistence of BVDV infection of neurons did not cause obvious morphological alterations or cellular destruction and Primary infection of CNS with BVDV mostly likely occurs across the blood-cerebrospinal fluid barrier [24, 25].

The serological results of the present work revealed that about of 50% of affected kids fail to show detectable precolostral serum neutralizing antibodies titer against BVD virus. These findings were in agreement with the other studies on pestivirus infections in newborn kids [3, 4] who indicated that in goat immune competence against pestivirus is thought to develop between 80-100 days of gestation which is about 20 days later than in sheep. French et al. [26] stated that the low levels of antibodies found in the serum of lambs before they suckled could be the result of placental leakage of antibodies from maternal plasma particularly that in ovine species passively acquired antibody is transferred to the kids, the current work showed congestion of blood antibodies from maternal plasma particularly that in ovine lymphatic tissues goats kids. Brain, thymus, spleen and mesenteric LN are the most susceptible tissues for the BVDV antigen detection using immunofluorescent technique which is a valuable and confirmative tool in diagnosis of BVD virus infection.
REFERENCES


