Prevalence of BVD infection and associated biochemical changes in buffalo-calves at Kaliobeia Governorate

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Abstract

Determination e of prevalence of Bovine viral diarrhea virus was carried out on 160 buffalo-claves aged from 1- 3 month belonging to several dairy buffalo farms at Kaliobeia Governorate' out of these animals 30 (18.75%) suffered from anorexia with high body temperature, diarrhea with gradual weight loss and varying degree of dehydration. Serum neutralizing of bovine viral diarrhea virus (BVDV) antibodies (Ab) was 7.5% (12/160) by serum neutralization test, and by ELISA, were 10.6% (17/160). BVDV antigen was 8.75 (14/160) % in blood samples by antigen captured ELISA. Biochemically, the examination of serum samples reveled a highly significant decrease of total protein, albumin, globulin and sodium. On the other hand, a significant increase was recorded in serum potassium, urea nitrogen, createnine and liver enzymatic activities of AST, ALT and ALP. The recommendation figured from this study are vaccination of pregnant dairy buffalo according to the Egyptian program to achieve passive immunity in the off springs, as a part of preventive program. Early vaccination of calves born from non vaccinated buffaloes is also required.

Key words: *BVDV, non vaccinated buffaloes , Kaliobeia Governorate*

Introduction

Bovine viral diarrhoea virus (BVDV) continues to be an important pathogen that affects ruminants worldwide, seen among all ages and breeds and have significant economic impact due to substantive damage in infected herds as well as an extensive consequence on animal health, productive and reproductive losses in dairy cattle**,(Houe.1999, Becher and Thiel 2002,** and **Ahmed, 2007).**

It can result in poor reproductive performance, reduced milk yield, ill thrift **(Houe, 1995)**. Clinically, there are three forms of the infection: (1) A persistently infected form (PI) ;(2) An acute transient form, which may be subclinical or may present as fever, diarrhoea and short-term immunosuppression which may complicate other diseases such as pneumonia; and (3) A mucosal disease characterized by severe erosive lesions in the oral and intestinal mucosa, diarrhoea and death (**Kabongo *et al*., 2004).**

In the pregnant animal the virus can be transmitted vertically to the foetus. The timing of transmission is critical to the epidemiology of the disease; infection prior to 100–125 days of gestation may result in foetal death or in birth of a live calf persistently infected with BVDV. Native animals infected with BVDV develop acute infection, clearing the virus from the body within 7–21 days and develop lifelong antibodies **(Nettleton and Entrican, 1995).**On the other hand, PI results as a consequence of foetal infection between 18 and 125days of gestation with the foetus becoming immunotolerant to the virus if born (**Kelling, 2004),** PI animals are BVDV antibody negative and BVDV positive, shedding large amounts of virus, and animals are viremic during their lifetime. In addition, PI animals may develop mucosal disease (MD) if they are super infected with a homologous cytopathic (CP) strain of BVDV, or through a mutation of the infecting ncp BVD virus to the cp form **(Ridpath *et al*., 2006).** The disease is caused by Bovine Virus Diarrhoea Virus (BVDV), which be-

longs to the genus Pestivirus of the family Flaviviridae. The genome of BVDV is a single stranded (+) RNA of about 12.5 kb in length without a poly (A) tail. BVDV isolates comprise 2 different genotypes; BVDV-1 and BVDV-2, both may infect cattle, sheep, also other ruminants and pigs **(Becher *et al*., 1995).** This virus shared antigenicity with border disease and classical swine fever **(Horzinek 1981).** Antibodies (Ab) in the blood serum samples were measured with the BVDV-enzyme linked immunosorbent assay (ELISA) and serum neutralization test (SNT) test, while antigen (Ag) in the leukocyte samples was detected with the BVDV-ELISA (Ag) test.

Each year thousands of neonatal calves are suffering from diarrhea, resulting in economic losses not only by increasing calf fatality but also by decrease in the calf's ability to gain weight, treatment cost, time spent on care as well as subsequent chronic ill thrift and poor growth (**Bazeley, 2003).**

Diarrhea is a well-known clinical sign in neonatal animals. Its etiology is complex involving management, environmental, nutritional, physiological variation and various infectious agents as bacterial, viral and protozoa **(Marcio *et al*., 2000)'** It has been estimated that neonatal calf diarrhea accounts for approximately 75% of mortality of dairy calves under 3 weeks old **(Radostits *et al*., 2002).**

The aim of this study:-

Detection of the prevalence of BVD in dairy buffalo- claves at Kaliobeia Governorate by measuring serum antibodies and viral antigen.

Determination of some biochemical changes associated with BVD in neonatal calves.

Materials and methods

Animals: A total of 160 clinically healthy and diseased neonatal calves aged 1-3 months (showing clinical symptoms of diarrhoea) were used in this study for determination of BVD prevalence in some dairy buffalo farms at Kaliobeia Governorate.

Parasitological examination: Faecal samples and skin scraping were collected from both diarrheic and clinically healthy calves for detection of external and internal parasites as described by **(Shoulsby, 1986).**

Blood samples: Two blood samples (10ml) were collected from jugular vein. The first sample was collected on EDTA as anticoagulant to obtain whole blood, centrifuged at 1500 rpm for 15 minutes and the buffy coat was collected and used for detection of BVD viral antigen in newly born calves. The second was collected without EDTA, kept to clot, centrifuged at 3000 rpm for 20 minutes, and kept at -20°C until analysed. Serum samples were used for detection of antibodies against BVD in newly born calves.

Virus: NADL strain used. which supplied by Veterinary Serum and Vaccine Research Institute.

MDBK cell culture (madin darby bovine cell culture VACSERA, Agoza, Egypt) 96- well tissue culture plate. MDBK cells were added to all the wells of the micro plates (100 µl per well 3 × 105 cells per ml) and observed daily until confluence' Then, 50 µl per well of pooled serum samples sequential 10-fold dilutions were added eight wells per dilution. The plate was freezed and thawed three times and the contained tissue and fluid were centrifuged at 5000 cultured for 3 days at 37°C in a humid atmosphere containing 5% CO2 incubator. The virus was then titrated and used for detection of antibodies against bovine viral diarrhoea virus in serum of newly borne calves by serum neutralization test.

Serum neutralization test: The serum neutralization test Beta procedure (constant virus –variable serum was performed by the use of serial 2-fold dilutions of heat inactivated serum, a 100- to 500-tissue culture infective dose (TCID50) of BVDV-NADL) was used to quantitate serum neutralizing antibodies in newly borne calve serum samples according to **Carbery and Lee (1966).**

Ag-captured ELISA: (Institute Pourquier, Montpellier, France) was used to determine the existence of BVDV Ag blood samples of newly borne calves. The tests were carried out according to the kit procedure.

Determination of biochemical parameters: 10 blood sample were taken from both clinically healthy and diseased neonatal calves aged 1-3 month for colorimetric determination of aspartate amino transferase (AST), alanine amino transferase (ALT) **(Reitman and Frankle 1957)**, alkaline phosphatase (ALP) according to **Szasz, 1976**, total protein and albumin as described by **(Dumas *et al*., 1971**). Determination of blood urea nitrogen (BUN) and blood serum creatinine was carried out according to **(Richterich, 1968 and Giorio, 1974),** respectively**.**

Statistical analysis Data were expressed as means ± standard error (M±SE). The comparison between the three groups was conducted by one way analysis of variance **(SPSS 1993).**

Results and Discussion

Table (1). Antibody titre against bovine viral diarrhoea in tested calves sera by serum neutralization test.

No. of tested serum samples	No. of +ve samples	%of +ve samples	Antibody titer of BVD					
			2		4		8	
160	12	7.5	No.	%	No.	%	No,	%
			6	3.7	4	2.5	2	1.3

Table (2). Detection of antibodies against bovine viral diarrhoea in tested calves serum by ELISA.

No. of tested serum samples	No. of +ve samples	%of +ve samples	OD of +ve serum samples			
			0.200-0.400		0.420-0.600	
160	17	10.6	No.	%	No.	%
			11	6.9	6	3.7

Mean OD +ve control= 0.198 mean OD-ve control=0.021

Table (3). Detection of bovine viral diarrhoea antigen in tested blood samples of calves by ELISA.

No. of tested serum samples	No. of +ve samples	%of +ve samples	OD of +ve blood samples			
			0.200-0.400		0.420-0.600	
160	14	8.75	No.	%	No.	%
			8	5	6	3.75

Table (4). Means and standard errors of serum biochemical constituents of clinically healthy and diarrheic buffalo- calves.

Parameters	Clinically healthy calves	Diarrheic calves
Total protein (gm/dl)	7.7±0.66	5.10±0.20 **
Albumin (gm/dl)	4.1 ± 0.15	2.98 ± 0.10**
Globulin (gm/dl)	3.6 ± 0.26	2.12 ± 0.11*
Sodium (mEq/l)	136.12 ± 5.2	112.3 ± 8.11 **
Potassium (mEq/l)	6.81 ± 0.11	7.01 ± 0.22 *
AST (IU/L)	60.12 ± 2.2	82.2 ± 3.6 **
ALT (IU/L)	40.00 ± 4.20	72.0 ± 3.8 **
ALP (IU/L)	89.5 ± 4.3	110 ± 6.3 **
Urea nitrogen (mg%)	20.3 ± 2.77	35.0 ± 2.98 **
Createnine (mg%)	0.99 ± 0.06	± 0.15 **

*Significant (P<0.05) ** highly significant (P<0.01)

Bovine viral diarrhoea virus (BVDV) infection of cattle is one of the most significant diseases affecting dairy herds. Animals can have signs that range from subclinical to chronic debilitating disease with high mortality. Foetal infections in uterus may produce immunotolerant persistently infected (PI) calves that continually shed virus. Several methods can be used to identify PI animals, including the enzyme-linked immunosorbent assay antigen-capture ELISA **Renshaw, *et al* (2000).** Foetal infection with BVDV after 125 days of gestation (after the development of a competent immune system) usually leads to the birth of normal calves with precolostral BVDV antibodies. Therefore, detecting foetal infection gives a clear indication that BVDV is circulating within the herd, crossing the placenta, and causing foetal infections **Munoz-Zanzi,*et al* (2003).**

In this study the prevalence of Bovine viral diarrhea antibodies in calves' sera by serum neutralization was at rate 7.5% out of total 160 tested serum samples as shown in **table (1).** This result agreed with the result (6.2%) obtained by **Schefers *et al*.,(2008).**
The prevalence of Bovine viral diarrhea antibodies in calves sera by ELISA was 10.6% out of total 160 samples as shown in **table (2)**,this result coincided with the result of **Ahmed and Kawther (2008)**.

Table(3) indicated that the bovine viral antigen in tested calves blood samples by ELISA was formed to be at rate 8.75 of total 160 samples,this result agreed with the result of **Thompson *et al*., (2006)** which revealed the existence of viral antigen in the persistent infection BVDV (Ag+) at a rate 6.25%. And **Houe *et al*., (1993)** who found PI rates of 6.12% contact with more than 10 herds per day at grazing, owning goats and contact with antelope as statistically significant risk factors. This is consequently with the known epidemiology, and small ruminants and deer that have been highlighted as potential reservoirs in eradication campaigns.
Rufenacht,*et al*.,(2000) reported that a recent study in Switzerland suggests a PI rate of 0.64+ 0.34% as low prevalence are needed to maintain the virus.

Control of BVD has been achieved in countries by detection of infected herds and removal of PI animals, supported by vaccination or biosecurity measures**, Hande,*et al*., (2011).**
The biochemical analysis as shown in **table (4)** reveled a highly significant decrease in serum total proteins, albumin and globulins values in diarrheic buffalo – calves when compared with clinically healthy ones.The reduction of total proteins,albumin and globulins may be attributed to the general unthriftines which may affect worsely the hepatic paranchyma resulting in the failure of the liver for protein synthesis (**Mebu *et al*., 2003; El-Dessouky, *et al*., 2004 and Tawfik *et al*., 2004).** Regarding serum sodium level there was a highly significant decrease in diarrheic calves. This decrease may be due to decreased absorption of this element from the inflamed or irritated intestinal tract or may be as a result of the rapid and continuous excretion of this element along with excessive secretion of intestinal juices in cases of enteropathies **(Nasser *et al*., 2000).** The finding of hyperkaleamia in diarrheic buffalo- calves was attributed to the dehydration and peripheral circulatory failure that results in an aerobic oxidation, liberation of lactic acid and occurrence of acidosis that lead to the drawing back of potassium from the cell to the plasma causing hyperkalaemia **(Radostits *et al*., 2002 and Hamdy *et al*., 2005).** Blood urea nitrogen and creatinine in serum of diarrheic buffalo –claves revealed a highly significant increase in comparison with the clinically healthy ones. Such finding attributed to excessive production of urea and creatinine by increase protein catabolism processes in severe toxic, febrile condition,reduce renal function and the beginning of nephropathological changes **(Tawfik *et al*., 2004)**. A significant elevation in serum AST, ALT and ALP activities in diseased calves. These elevation referred to the degenerative changes and necrotic processes which accompanied the for-

mation of intestinal and hepatic lesions due to viral infection and its toxin **(Radostits *et al*., 2002).**

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Hande.Ian G. l1, Kim Willoughby4, Fiona Land1, Bronwyn Koterwas1, Kenton L. Morgan2, Vincent N.Tanya3, Barend M. deC. Bronsvoort*1 Seroepidemiology of Bovine Viral Diarrhea Virus (BVDV)in the Adamawa Region of Cameroon and Use of the SPOT Test to Identify Herds with PI Calves Becher, M., K.D. Paton and H.J. Thiel (2011). Further characterization of border disease virus isolates: evidence for the presence of more than three species within the genus pestivirus. Virol., 209: 200-206.

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نسبة الإصابة والتغيرات البيوكيميائية المصاحبة لمرض الإسهال البقري الفيروسي في عجول الجاموس الحلاب في محافظة القليوبية

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الملخص العربي

تم عمل مسح لعدد 160 عجل جاموسي تتراوح اعمارها ما بين 1-3 شهور في مزارع الجاموس الحلاب في محافظة القليوبية. كان من بين هذه العجول 30 عجل (18.75 %) تعانى من اسهال وارتفاع في درجة الحرارة وضعف عام ونقص تدريجي في الوزن. اوضحت تلك الدراسة ان عدد 12 عجل بها أجسام مناعية لفيروس الاسهال البقري الفيروسي بنسبة 7.5 % بإستخدام اختبار السيرم المتعادل بينما كانت 10.6% (17 حيوان) بإستخدام اختبار الاليزا.

تم اكتشاف انتيجين الفيروس في عدد 14 حيوان بنسبة 8.75% باستخدام اختبار (captured ELIZA).

وقد اوضحت نتائج التحليل البيوكيميائي لمصل الدم من تلك الحيوانات الاجابية لفيروس الاسهال البقري الفيروسي وجود نقص معنوي في البروتين الكلي و الالبيومين والجلوبيولين والصوديوم. بينما كان هناك زياده معنوية في مستوي كل من البوتاسيوم واليوريا والكرياتينين والنشاط الانزيمي لكل من الأسبرتيت امينوترنسفيراز-الالانين امينو ترنسفيراز وانزيم الفوسفاتيز القاعدي.

وبناء علي هذه الدراسة يمكن التوصية بضروه تحصين الأمهات الحوامل طبقا لبرنامج التحصين المصري لاكساب العجول الصغيرة مناعة خلال المرحلة الاولى من العمر.و ضرورة تحصين العجول التى تولد من امهات غير محصنة مبكرا لتفادي الاصابة بمرض الاسهال البقري الفيروسي.