STUDIES ON PESTIVIRUS INFECTION IN SHEEP FLOCK (BORDER DISEASE)

***Hamoda, F. K.; Iman, K. A. and Zeidan, S. M.**

Dept. of Anim Med, Fac Vet Med, Zagazig University, Benha Branch, Egypt*

Veterinary serum and vaccine Research Institute, Abbasia, Cairo, Egypt

ABSTRACT

The current investigation was performed on sheep flock of different ages, suffering from problems of a hairy coat, muscular trembles, abortion, stillbirth, weak and frail lambs, barren ewes, nasal discharges, intermittent diarrhea and few deaths.

The clinical criteria suggested that, this field problem attributed to a pestivirus infection (Border disease virus, BDV). The virological and serological analysis proved the detection of BDV and specific neutralizing antibodies. Also a pestivirus (bovine viral diarrhea virus) and specific antibodies were detected in incontact fattening buffalo calves. It is surmised that this problem brought into a flock by new additions that is carriers or when sheep are commingled with buffaloes that are shedding the bovine viral diarrhea virus (BVDV). The detection of the virus at first and second virological examination as well as detection of antibodies in preclostral sera cleared the existences of persistent infection in the investigated flock.

Epidemiological and laboratory investigation in this study clearly indicates that buffalo calves infected with BVD virus constitute a source of infection for sheep, which results in border disease. Also epidemiological data revealed that the morbidity of the disease was higher in newborn lambs (32%) followed by older lambs (28.6%) and adults (5.9%). The bacteriological examination detected salmonella species and E-Coli as intercurrent infection to BDV due to immunosuppressive action of the virus.

INTRODUCTION

Depending on the host origin Pestiviruses called bovine virus diarrhea virus (BVD), hog cholera virus (HCV) and border disease virus (BDV) in cattle, swine and sheep respectively. Cross infection between species can be achieved experimentally and has been demonstrated in field conditions **(Collett et al., 1988; Francki et al., 1991 and Nettleton and Entrican., 1995)**. BVD and BD viruses have a common origin and those changes in the epitope expression might arise

during passage in different speeles **(Carlsson and Belak., 1994)**. BVDV handicaps basic research because it is a frequent contaminant of cultured cells. This contamination most frequently is the result of using fetal bovine serum, derived from BVDV infected animals, as a cell culture supplement **(Nettleton and Entrican., 1995)**.

An assessment of the economic losses due to infertility, abortion, neonatal losses and low carcass weight indicate that an outbreak of border disease can result in a potential reduction of income in excess of 20% **(Sharp and Rawson., 1986)**.

Sheep to sheep eontact is considered to be the principle way in which BDV is spread **(Nettleton., 1988)** but cattle persistently infected with BVD and in close contact with sheep are an important source of strains of virus capable of causing border disease **(Carlsson., 1991)**. The spread of pestiviruses can occur among both cattle and sheep where sheep and cattle are housed together **(Nilsen and Slettbakk., 1995)**.

It is clear that, the great majority of BDV infections are subclinical **(Hungerford., 1990)** and the pathogenesis of BDV is governed by various factors associated with the immunological status of the ewe and the age of the fetus at the time of infection **(Hussin and Waldehiwet., 1994)**. BDV can cross the placenta and establish a persistent infection in the animals infected in utero **(Caffrey et al., 1997)**. Infection of ewe with non cytopathic biotype of BDV during early pregnancy can result in birth of lambs persistently infected with the virus **(Loken., 1995)**. These lambs are apparently healthy and can develop and reach maturity and can be breed and birth of persistently infected lambs and all these persistently infected animals shedding virus in the saliva, urine and feces for the rest of their lives **(Osburn and Castrucci., 1991 and Wolf and Buttner., 1994)**.

It is a well established that, BD is characterized by variable clinical manifestation namely hairy fleece, lower birth weight, neurological dysfunction, reduced growth rate and impaired reproductive performanee **(Radostits et al., 2000 and Pugh., 2002)**. Lambs and kids affected with BDV have reduced body weight eombined with skeletal dysmorphoenesis characterized by short and thin bones, short facial bones and decreased bone density **(Plant et al., 1983)**. Lambs and kids which survive border disease have poor growth rate and are more susceptible to intercurrent diseases especially gastrointestinal worms, lungworms and chlamydia psittaci **(Roeder., 1984)**.

BDV infection before the development of the immune system (60-85 days of gestation) may result in birth of viremic and persistently infected lambs without antibody in precolostral sera **(Barlow et al., 1983)**. Fetuses that survive early intrauterine infection with pestivirus will born virus-positive and antibody negative while those infected in later gestation will be anti-

body—positive and virus negative, these persistently infected animals excrete the virus in nasal secretions and saliva **(Nettleton and Entrican., 1995)**.

BVD virus has been shown to induee profound immunosuppression which may be contribute to the pathogenicity of the virus itself and permit secondary infection to endue. The virus has been implieated in the depression of T-Lymphocyte function, antibody production and the function of mononuclear phagocytes and neutrophils **(Markham and Rammaraire., 1985)**.

Serology is important in monitoring the introduction of BDV in a flock and for the retrospective investigation of new outbreaks of BD in sheep flocks. The presence or absence of antibodies in sera of lambs depends on the immunological status of the ewes and the age of the fetus at the time of exposure to the virus **(Hussin and Woldehiwet., 1994)**. The seropositive sheep to pestivirus occurred due to infection of the fetus after 85 days of gestation or to post-natal infection and these animals is very important in the epidemiology of pestivirus as transmit infection to cattle and exaggeratcd problem of BVD especially there is no vaecination program adopted in Egypt to control of pestivirus infeetion in sheep and goats **(Fouda., 1999)**.

The antigenie relationships and physieal and biological similarities between BVD and BDV had led to the assumption that both viruses belong to one speeies of the genus pestivirus **(Done et al., 1988)** and BDV and BVDV indistinguishable in vitro **(Harkness and Vantsis., 1982)**.

Concurrent infection with salmonella and BVDV would be expected to cause more severe disease than infection with either agent alone owing to the immunosuppressive effects of BVDV infection **(Wray and Roeder., 1987 and Penny et al., 1996)**.

It was emphasized that, in the control of border disease in sheep and bovine diarrhea virus infection in cattle, it is important to minimize the risk of transmission of these related pestiviruses between the two species **(Carlsson and Belak., 1994)**.

Few published literature data eoncerning pestivirus infection or border disease in Egyptian sheep **(Shehata et al., 1989; Baz., 1992; Zaghawa., 1998 and Abd EL-Hakim., 1999; Salem et al., 2000 and Azzam., 2002)**.

The present study aimed to look for a problem of abortion, stillbirth, barren ewes, ill-thrift lambs, respiratory distress and intermittent diarrhea among sheep of different ages in private sheep flock with particular emphasis to declare the possible cause(s) from laboratory investigations, monitoring and description of elinical picture, assessment of some epidemiological data, declaring the incidenee of intercurrent infection and the suggested lines of control of the problem.

MATERIAL AND METHODS

1- Animals and history of the flock : This study was performed on a total of 243 cross breed sheep of different ages (Table 1), they were selected from a private farm at Sharkia governorate. Clinical examination of these revealed that, sporadic cases exhibiting variable clinical signs during winter season as hairy coat, trembles, abortion, stillbirth, weak and frail lambs, barren ewes, nasal discharges and intermittent diarrhea. The flock was clinically examined and sampled for two occasions. History of the flock declared that, clinical cases occurred sporadically at irregular interval. The flock was in contact with 25 apparently healthy fattening buffalo calves at the same locality.

2- Reference virus and antiserum : Reference BVD virus and antisera (strain Oregon C24 virus which is closely related to border disease virus) obtained from Vet.Serum & Vaccine Res. Institute, Abbassia., Cairo. There is no available reference antiserum and antigen for BDV in Egypt, so we used BVD virus and specific antiserum (serologically related to BVDV and often cross neutralize each other).

3- Cell culture : Madin-darby bovine kidney cell culture (MDBK), kindly obtained from Vet.Serum & Vaccine Res. Institute, Abbassia., Cairo, Egypt. It was used in detection of the virus and in serological identification.

4- Sampling : The investigated samples were taken from clinically ill animals (32) and clinically healthy contact animals (55), these animals were sampled for two times with 3weaks intervals. Also contact fattening buffalo calves (25) were sampled.

A- Rectal swabs: Two swabs were taken, the first one on transport media for virus detection and the second one nutrient broth for bacteriological examination.

B- Nasal swabs: It was collected on transport media for virus detection

C- Serum samples: These samples were taken from diseased and clinically normal contact newborns, older lambs and adults sheep (precolostral serum obtained from 3 lambs) to detect pestivirus (BDV) and antibodies. Also serum samples collected from fattening buffalo calves in contact with the investigated sheep flock.

5- Laboratory investigation:

A- Virological examination; The collected materials were prepared for inoculation into MDBK cell cultures and examined for cytopathic effect and the presence of pestivirus was determined and confirmed by indirect immunofluorescence test (IFAT), neutralization and the interference tests according to **Gillespie et al., (1967); EL-Bagoury., (1990) and Thabti et al., (2002).**

B- Serology: Serum neutralization test using reference virus and antiserum was applied **(Rossiter and Jessette., 1982)**.

C- Bacteriological examination: Rectal swabs inoculated into nutrient broth and selenite F broth were incubated for 18-24h at 370c, then a loopful from the growth inoculated to nutrient agar, blood agar, S.S agar and McConky agar plates and all plates incubated at 370c for 24 48h. The isolated bacteria were identified and characterized from colony morphology and biochemical reaction **(Quinn et al., 1994)**.

6- Therapeutic trial : The disease animals were treated symptomatically in addition to fluid therapy while those exhibiting intercurrent infection treated specifically according to the type of secondary invaders **(Radostits et al., 2000)**.

RESULTS AND DISCUSSION

Border disease (BD) could induce economical losses in nat ve flocks alone or in association with other pathogens **(Thabit et al., 2002)**. Originally, pestiviruses were designated as BVDV, HCV or BDV based on the host species from which the virus was isolated. Because these viruses can cross species boundaries this nomenclature was problematic. Later attempts to group pestiviruses have been based on serology and monoclonal antibody binding **(Nettleton and Entrican., 1995 and Martin and Aitken., 2000)**. BD is a congenital virus disease of sheep, characterized by barren ewes, abortion, stillbirths and birth of small weak lambs and a variable percentage of which show tremor, abnormal body confirmation and hairy fleeces **(Martin and Aitken., 2000)**. Sheep can be infected naturally with BVD virus and transmission of the virus from cattle to sheep bas been demonstrated **(Carlsson., 1991)**. In the absence of an effective vaccine, reliable diagnostic techniques are essential to implement effective control measures **(Nettleton and Entrican., 1995)**.

Regarding to virus detection and identification from serum samples and nasal or rectal swabs (Table 2 & 3), the current virologieal studies declared detection of BDV (cytopathic and non-cytopathic biotype) on MDBK cell culture, the presence of BD virus is established and identify by neutralization and interference tests and immunofluorescenec technique. The above results were similar to theses previously reported by **Terpstra., (1981); Roeder et al., (1983) and Nettleton and Entrican., (1995)**.

The detection of the virus (especially non cytopathic biotype) at first and second examination in both clinically ill and clinically normal sheep cleared the existence of persistence infeetion in the herd and these animals were responsible for the recurrence of the infection in this flock.

Such finding was justified by prior work **(Woldehiwet and Nettleton., 1991;Wolf and Buttner., 1994; Loken., 1995; Caffrey et al., 1997 and Abd EL-Hakim., 1999)** and large number of persistently infected (PI) sheep were apparently healthy **(Nettleton et al., 1998)**. Virtually all PI animals are virus positive and antibody negative and a repeat virus positive sample 3-week later will confirm persistent infection and distinguish it from an acute infection **(Nettleton and Entrican., 1995)**.

The detection of pestivirus (BVDV) and specific neutralizing antibodies from contact apparently healthy buffalo calves supported by the study of **Carlsson., (1991)** who diagnosed border disease in sheep caused by transmission of virus from cattle persistently infected with bovine virus diarrhea virus. Also **Radostits et al., (2000)** published that, the major management risk factors in onset of BD are introduction of persistently infected sheep and rarely cattle in a susceptible sheep herd.

Clinical signs described in this study was appeared sporadically in form of less obvious hairy fleece and trembles, beside abortion, stillbirth, weak and frail lambs, barren ewes, nasal discharges and intermittent diarrhea while other animals exhibiting persistent or inapparent infection and death of few lambs. Such observation was similar to that observed by prior work of many workers **(Plant et al., 1983; Barlow et al., 1983; Shehata et al., 1989; Rae., 1994; Nettleton et al., 1998; Abd EL-Hakim., 1999; Martin and Aitken., 2000; Radostits et al., 2000 and Azzam 2002)**. The clinical signs of the disease was marked on lambs than adults **(Sawyer., 1992)** and on animals showing intercurrent infection **(Sanchis and Russo., (1990)**.

Hairy fleece is due to predominance of primary medullated hairs rather than secondary wool **(Osburn and Castrucci., 1991)**. As well, lambs that survive may gradually lose their fleece abnormalities and nervous signs **(Woldehiwet and Nettleton., 1991)**. Also persistent infected adolescent and adult sheep may appear normal as the tremors and fleece abnormalities abate with age **(Swayer., 1992)**.

In Egypt, majority of sheep breeds has coarse wools and the hairiness criterion of the wool was less obvious on infected sheep. This explanation is similar to that of. **Orr and Barlow., (1978)** who reported that, in coarse-fleeeed breeds it may be difficult to asses the hairiness of the birth coats and **Orr., (1982)** who mentioned that, the skin abnormalities are obvious in the very fine and medium fleeced breeds. In this respect **Bonniwell et al., (1987)** reported an unusual outbreak of BD in lambs in which the typical signs of tremors aud abnormalities in fleece were not present and the signs were restricted to abortion and the birth of small weak lambs which were found to be persistently infected with BDV.

The abortion occurred due to that, BDV cross the maternal vascular endothelium of the pla-

centa within 10 days of infection resulting in virus transfer from dam to offspring or a placentitis that result in abortion or the pregnancy may be sustained if the placentitis heal in about 25 days **(Snowdon et al., 1975)**.

Table (4) revealed the morbidity and mortality rates in the investigated sheep flock. it cleared that the morbidity of the disease was 32, 28.6 and 5.9% among newborn lambs, older lambs and adults respectively while the total morbidity within the herd was 13.2%. Mortality rate was 8 and 2.04% in newborns and older lambs while there was no mortality among adults and the total mortality within the flock was 1.23%. The above results were nearly similar to that reported by **Martin and Aitken., (2000)**.

From the epidemiological point of few, the tracing back to a possible origin of the disease, all evidence proved that BDV infection might introduced to the investigated flock due to contact with infected fattening buffalo calves and introduction of inapparent or persistent infected sheep.

As well, clinical evidence proved that these cases appeared sporadically at irregular intervals, such criteria mentioned previously by **Radostits et al., (2000)** who published that, BD may be occur as an outbreak or as a sporadic disease and in most flocks a serious outbreak of the disease is followed by minor disease in subsequent years and the flock developing immunity in the initial outbreak.

Respecting the results of SNT (table 5), the antibodies were detected in 18 animals and the titers range was1/8:1/64. The use of cytopathic strain of BVD virus in performing of SNT was documented previously by the work of **Brockman et al., (1988)** who reported that BVD virus could be used to detect neutralizing antibodies to BDV in sheep sera and it had been in use for this purpose for several years. Neutralizing antibodies were detected in either clinically ill or in inapparently infected animals, such finding nearly similar to that detected previously **(Hafez., 1973; Hassan et al., 1989; Zaghawa., 1998 and Fouda., 1999)**. While few infected animals were have no neutralizing antibodies but have BDV, this contributed to that persistently infected animals are immunocompromised or tolerant and have no antibodies **(Sawyer et al., 1991)**.

The detection of SNT antibodies in precolostral sera of some lambs documented persistence infection of lambs due to in-utero infection with the BD virus. Such finding was in accordance to that presented by **Nettleton and Entrican., (1995)** who found that fetuses that survive early intrauterine infection with pestivirus will born virus positive and antibody negative while those infected in later gestation will be antibody positive and virus negative. In this context **Vantsis et al., (1979)** concluded that after in-utero infection of lambs with BDV, some lambs may be apparently healthy but continue to shed the virus persistently throughout their life. The persistently infected apparently healthy lambs if kept for breeding they will continue to be a source of in-

fection in successive breeding seasons horizontally or vertically **(Woldehiwet and Nettleton., 1991)**.

The incidence of infected animals exhibiting intercurrent infeetion as in table (6) in which E-Coli was detected as 25% in newborns and salmonella sp in 21.4 and 40% of older lambs and adults respectively. The emergence of salmonella or E-Coli infections in border disease infected animals may attributed to a state of immune dysfunction accompanied pestiviruses infection **(Sawyer and Osburn., 1989 and Nettleton and Entrican., 1995)**. Moreover **Lamontagne et al., (1989)** reported that the most important aspect of acute or chronic phases of BVDV or BDV infection was the host's increased susceptibility to secondary bacterial or viral infection due to alteration in the cellular immune responses inducing immunodepression. In this respect **Hajtos et al., (1998)** determined that a predisposing factor of Eperythrozoon ovis infection in sheep was border disease/bovine diarrhea virus infection and **Monies and Simpson., (1997)** diagnosed a concurrent coecidial iufection in lambs infected with border disease.

The results provide substantial evidence that, the specific and symptomatic therapy of cases of salmonellosis or colibacillosis as concurrent infection result in temporary recovery and relapses occur after that. The above results was supported by the view of **Monies and Simpson., (1997)** who suggested that, if lambs with diarrhea fail to respond to treatment, the possibility of persistent pestivirus infection should be considered. There is a prevailing assumption that persistent infected animals tend to succumb to intercurrent infections. We are in need for organized control programs for veterinary services to be effective in terms of animal pestiviruses prevention. Perfect preparation and virological examination of any vaceine produced and used in Egypt against pestiviruses where pestivirus was isolated from live sheep pox vaccine and vaccination with these vaccines caused outbreak of border disease **(Thabti et al., 2002)**.

This study clearly indicates that under Egyptian condition buffalo calves, which are persistently infected with BVDV, constitute a source of infection for sheep which results in border disease. Further investigation should be done to declare the epidemiology and economic impact of border disease in Egyptian sheep and goats and to identify the extent of the relationship between border disease virus and bovine viral diarrhea-mucosal disease virus in Egypt from molecular biology investigation.

In conclusion, the main strategy advocated for the control of BD is identification and culling of the persistent execrator sheep (the most important source of infection), avoid the exposure of susceptible pregnant ewes to BD virus, special considerations adopted in artificial breeding programs and reduce or minimize contact of sheep with infected cattle or other source of infection will play a crucial role in the control of the disease. Also great caution during vaccines prepara-

tion especially living attenuated one which are used in vaccination of sheep because pestivirus are notorious contaminants of laboratory cell cultures and cell culture medium and frequently contaminants of modified live virus vaccines produced on ovine cells. We are indeed to vaccinate sheep and goats against pestivirus using vaccine prepared from local BD virus to control the disease in Egypt.

Table (1): Age and numbers of examined animals.

Animals	age	Numbers
Newborn lambs	Less than 1month	25
Older lambs	More than one month-less than 1 year	49
Adults	1-5 year	169
Total		243

Table (2): Results of virological investigation on first examination of the flock.

Samples	Newborn lambs						Older lambs						Adults					
	T.No		No of +Ve		%		T.No		No of +Ve		%		T.No		No of +Ve		%	
	N	D	N		N	D	N	D	N	D	N	D	N	D	N	D	N	D
R.swabs			0	1	0	12.5			2	2	10	14.3			3	3	12	30
Na swabs	10	8	0	0	0	0	20	14	1	1	5	7.1	25	10	2	0	8	0
Serum			1	1	10	12.5			4	3	20	21.4			6	4	24	40
Total			1	2	10	25			7	6	35	42.9			11	7	44	70

R: Rectal Na: Nasal N: Normal animals D: Diseased animals T:Total No: Number

(3): Results of virological investigation on second examination of the flock (3weeks post first examination).

Samples	Newborn lambs						Older lambs						Adults					
	T.No		No of +Ve		%		T.No		No of +Ve		%		T.No		No of +Ve		%	
	N	D	N		N	D	N	D	N	D	N	D	N	D	N	D	N	D
R.swabs			0	0	0	0			2	1	10	7.7			3	2	12	20
Na.swabs	10	6	0	0	0	0	20	13	1	0	5	0	25	10	1	0	4	0
Serum			1	1	10	16.7			4	2	20	15.4			5	2	20	20
Total			1	1	10	16.7			7	3	35	23.1			9	4	36	40

R: Rectal Na: Nasal N: Normal animals D: Diseased animals T:Total No: Number

Table (4): Morbidity and mortality rates of the border disease in the investigated sheep flock.

Animals	No of examined animals	Morbidity rate		Mortality rate	
		No	%	No	%
Newborn lambs	25	8	32	2	8
Older lambs	49	14	28.6	1	2.04
Adults	169	10	5.9	0.0	0.0
Total	243	32	13.2	3	1.23

Table (5): Results of serum neutralization test.

Animals	First examination						Second examination					
	T.No		No of +Ve		%		T.No		No of +Ve		%	
	N	D	N	D	N	D	N	D	N	D	N	D
Newborns	10	8*	0	2	0	25	10	6	1	1	10	16.7
Older lambs	20	14	1	3	5	21.4	20	13	2	2	10	15.4
Adults	25	10	3	2	12	20	25	10	3	2	12	20
Total	55	32	4	7	7.3	21.9	55	29	6	5	10.9	17.2

The titer of antibodies ranged from 1/8:1/64

*one of three preclostral serum samples gives positive results

Table (6): Incidence of the BD infected animals exhibiting concurrent infection.

Animals	No. of clinically ill animals	Isolated bacteria		
		Type	Number	%
Newborn lambs	8	E-coli	2	25
Older lambs	14	Salmonella sp	3	21.4
Adults	10	Salmonella sp	4	40

Fig (1): BD infected lambs showing hairy fleece (less obvious).

Fig. 1 : BD infected lambs showing hairy fleece (less obvious).

Fig. 2 : BD infected lamb showing diarrhea due to intercurrent infection with E-Coli.

Fig. 3 : BD infected lamb showing diarrhea due to intercurrent infection with Salmonella sp.

REFERENCES

Abd EL-Hakim, U. A. (1999) : Border disease of sheep in Egypt: 1-clinical and diagnostic (indirect enzyme linked immunosorbent assay and reverse-transcription polymerase chain reaction) studies. 5th Sci.Cong. Egyptian Socity for cattle diseases, Assiut., Egypt: 80-85.

Azzam, R. (2002) : Studies on the epizootic process of pestivirus infection in animals. Ph. D. Thesis (Anim. Med. Dept), Fac.Vet.Med, Beni-Suef.Cairo University.

Barlow, R. M.; Gardiner, A. C. and Nettleton, P. E. (1983) : The pathology of spontaneous and experimental mucosal disease-like syndrome in sheep recovered from clinical border disease.J.Comp.Pathol., 93: 451-461.

Baz, T. I. (1992) : Bovine virus diarrhea-mueosal disease and border disease in Egypt. Rev.Sci.Tech., 11: 867-872.

Bonniwell, M. A.; Nettleton, P. F.; Gardiner, A. C.; Barlow, R. M. and Gilmour, J. S. (1987) : Border disease without nervous signs or fleece changes.Vet.Rec., 120:246-249.

Brockman, S. J.; Wood, L.; Edwards, S. and Harkness, J. W. (1988) : Selection of an appropriate pestivirus strain for border disease serodiagnosis .Vet.Rec., 122:586-587

Caffrey, J. K.; Dudgean, A. M.; Donnelly, W. J.; Sheahan, B. J. and Atkins, G. J. (1997) : Morphometric analysis of growth retardation in fetyal lambs following expeimental infection of pregnant ewes with border disease virus.Res.Vet.Sci.,62:245-248.

Carlsson, U. (1991) : Border disease in sheep caused by transmission of virus from cattle persistently infected with bovine virus diarrhea.Vet. Rec., 128(7): 145-147.

Carlsson, U. and Belak, K. (1994) : Border disease virus transmitted to sheep and cattle by a persistently infected ewe: Epidemiology and control. Acta Veterinaria Scandinavica., 35 (1): 79-98.

Collett, M. S.; Anderson, D. K. and Retzel, E. (1988) : Comparisons of the pesti bovine virus with the members of the Flaviviridae.J.General Virol., 69:2637-2643.

Done, J. T.; Terbecki, S.; Ricchardson, C.; Hakness, J.; Sands, J.; Patterson, D. and Duffell, S. (1988) : Bovine virus diarrhea-mucosal disease virus pathogencity for the fetal calf following maternal infection. Vet.Rec., 106:473-479.

El-Bagoury, G. F. (1990) : Evaluation of some recent virological techniques in the diagnosis of bovine viral diarrhea-mucosal disease complex (BVD/MD.Ph. D.Thesis. Fac. Vet. Med. Zagazig University, Benha Braneh.

Fouad, A. E. F. (1999) : Sero-survey on bovine herpesvirus-1, peste des petits ruminants and border disease viruses in sheep.MVSc Thesis (Infectious diseases).Fac.Vet.Med.Alexandria University.

Francki, R. I.; Fanquets, C. M.; Knudson, D. L. and Brown, F. (1991) : Classification and nomenclature of viruses.Arch.Virol., 2:223-233.

Gillespie, J. H.; Bartholomew, P. T.; Thomson, R. G. and Mcentee, K. (1967) : The isolation of noncytopathic bovine virus diarrhea virus from two aborted bovine fetuses.Cornell Vet., 62:564-571.

Hafez, S. M. (1973) : Geographical distribution of bovine viral diarrhea-Mucosal disease (BVD-MD) in Egypt as determined by the detection of neutralizing antibodies in sheep sera. J.Egypt.Vet.Med.Assoc., 35(2): 73 80

Hajtos, I.; Glovits, R.; Erdos, A. and Polfi, V. (1998) : Diagnosis of Eperythrozoon ovis infection in sheep in Hungary. Preliminary communication Magyar Ollatorvosok Lapja., 120 (11): 697-703.

Harkness, J. W. and Vantsis, J. T. (1982) : Virology. In: Border disease of sheep: a virus-induced teratogenic disorder. A collaboratorative review edited by Barlow, R.M. and Patterson, D.S.P Berlin and Hamburg, Paul Parey (advances in veterinary medicine.Fortschritte der Veterinarmedizin., 36:56-64

Hassan, H. B.: EL-Sanousi, A.; EL-Trabili, M. A.; Fathia, M. M.; Ayoub, N. N. K.; EL-Bagoury, G. F. and Moussa, A. M. (1989) : Serological survey for neutralizing antibody to bovine viral diarrhea virus in Egypt. J.Egypt. Vet.Med.Ass., 49(1-2): 381-388.

Hungerford, T. G. (1990) : Diseases of live stock.9th. Ed.McGraw-Hill Book Co., Sydney.

Hussin, A. A. and Waldehiwet, Z. (1994) : The effect of experimental infection with border disease virus on lymphocyte subpopulation in the peripheral blood of lambs.Res.Vet.Sci., 56:201-207.

Lamontagne, L.; Lafortune, P. and Fournel, M. (1989) : Modulation of the cellular responses to T-cell dependent and T- cell independent antigens in lambs with induced bovine viral diarrhea virus infection. Am.J.Vet.Res., 50(9): 1604-1608.

Loken, T. (1995) : Border disease in sheep.Vet.Clin.North.Am.Food. Anim.Pract., 11: 579-595.

Markham, R. J. F. and Ramnaraire, M. L. (1985) : Realse of immunosuppresive substance from tissue culture cells infected with bovine viral diarrhea virus.Am.J.Vet.Res., 46:879-883.

Martin, W. B. and Aitken, I. D. (2000) : Diseases of sheep.3rd ED.Blackwell Scientific Publications London., Oxford.

Monies, R. J. and Simpson, V. R. (1997) : Syndrome in sheep resembling mucosal disease in cattle. Vet. Rec., 141(17):451

Nettleton, P. F. (1988) : Border disease update. Vet.Rec.Suppl: In practice., 10: 76.

Nettleton, P. F. and Entrican, G. (1995) : Review: Ruminant pestiviruses. Br.Vet.J., 151 :615-642.

Nettleton, P. F.; Gilary, J. A.; Russo, P. and Dilssi, E. (1998) : Border disease of sheep and goats.Vet.Res., 29:327-340.

Nilsen, A. and Slettbakk, T. (1995) : The connection between the prevalence of antibodies to bovine diarrhoea virus/border disease virus in sheep and the bovine diarrhoea virus status of cattle in combined livestock operations. Norsk Veterinaertidsskrift., 107(7): 765-771.

Orr, M. B. (1982) : Pathology of the skin and fleece.In: Border disease of sheep: a virus-indueed teratogenic disorder. Advanees in Vet.Med., 36: 13-21.

Orr, M. B. and Barlow, R. M. (1978) : Experiments in border diseasc.X. The postnatal skin lesion in sheep and goats.J.Comp.Path., 88:295-302.

Osburn, B. and Castrucci, G. (1991) : Diaplacental infections with pestiviruses.Arch.Virol. Suppl., 3:71-78.

Penny, C. D.; Low, J. C.; Nettleton, P. F.; Scott, P. R.; Sargison, N. D.; Strachan, W. D. and Honeyman, P. C. (1996): Coneurrent bovine viral diarrhea virus and salmonella typhimurum DT104 infection in a group of pregnant dairy heifers. Vet.Rec., 18:485-489.

Plant, J. W.; Acland, H. M.; Gard, G. P. and Walker, R. H. (1983) : Clinical variations of border disease in sheep according to the source of inoculum.Vet.Rec., 113:58-60.

Pugh, D. G. (2002) : Sheep and Goat medicine. W.B. Saundres Company., A Harcourt Health Sciences Company, Philadelphia, USA.

Quinn, P. J.; Carter, M. E.; Markey, B. K. and Carter, G. R. (1994) : Clinical veterinary microbiology.Mosby-Year Book Europe Limited, London.

Radostits, O. M.; Gay, C. C, blood, D. C. and Hinchcliff, K. W. (2000) : Veterinary medicine A textbook of the diseases of cattle, sheep, pigs, goats and horses. 9 th Ed. W.B.Saunders Company Ltd.

Rae, C. A. (1994) : Lymphocytic enteritis and systemic vasculitis in sheep. Cand. Vet.J.,35(10): 622-625.

Roeder, P. L. (1984) : Studies on border disease virus infection in sheep.Ph.D. Thesis. University of London.

Roeder, P. L.; Sweasey, D. and Terlecki, S. (1983) : Border disease virus infection of newborn lamb.Br.Vet.J.,139:126-136.

Rossiter, P. B. and Jessette, D. M. (1982) : Microlitre techniques for the assay of rinderpest virus and neutralizating antibody. Res.Vet. Sci., 32: 253-256.

Salem, S. A.; Gaber, A. S. and Abo-EL-Hassan, D. G. (2000) : Rapid detection of BVD and IBR viruses as easuses of genital infection in sheep and goats. Egypt.J.Agri. Res., 78(1):91-103.

Sanchis, R. and Russo, P. (1990) : Infection experimentale de l agneau par un pestivirus et une pasteurella hemolytica de type T4. Recl.Med. Vet., 166: 143-150.

Sawyer, M. M. (1992) : Border disease of sheep: the disease in the newborn, adolescent and adult sheep. Comp. Immunol. Micrbiol and Infectious diseases., 15: 171-177.

Sawyer, M. M. and Osburn, B. I. (1989) : Immune dysfunction characteristic of border disease.11th International Symposium of the world association of veterinary microbiologists, immunologists and specialists in infectious diseases, Perugia, Mantova, Italy: 277.

Sawyer, M. M. and Osburn, B. I. (1992) : Long term effects of persistent pestivirus infection on thyroid secretions in border disease sheep. Proceedings of the second symposium on pestiviruses:127-130.

Sawyer, M. M.; Schore, C. E. and Osburn, B. I. (1991) : Border disease of sheep. Aspects for diagnostic and epidemiological consideration. Arch. Virol., Suppl., 3: 97-100.

Sharp, M. W. and Rawson, B. C. (1986) : Vet.Rec.,119:128.Cited by Radostits et al., (2000)

Shehata, H.; Bakeer, A. M. and Zeidan, S. (1989) : Experimental pathologieal sudy on bovine viral diarrhea-mucosal disease in sheep. Egypt. J.Comp. Path.Clin. Path., 2(1): 34-46.

Snowdon, W. A.; Parsonson, I. M. and Broun, M. I. (1975) : The reaction of pregnant ewes to inoculation with mucosal disease virus of bovine rigin.J. Comp.Pathol., 85:241-251.

Terpstra, C. (1981) : Border disease: virus persistence, antibody response and transmission studies. Res in Vet.Sci., 30: 185-190.

Thabti, F.; Fronzaroli, L.; Dilssi, E.; Guibert, J. M.; Hammami, S.; Pepin, M. and Russo, P. (2002) : Experimental model of border disease virus infection in lambs: comparative pathogenicity of pestiviruses isolated in France and Tunisia. Vet. Res., 33(1): 35-45.

Vantsis, J. I.; Linklater, K. A.; Rennie, J. C. and Barlow, R. M. (1979) : Experimental challenge infection of ewes following a field outbreak of border disease.J.Comp.Pathol., 85:111-120.

Woldehiwet, I. and Nettleton, P. F. (1991) : Progeny of sheep persistently infected with border disease virus.Arch.Virol., 3:267-271.

Wolf, G. and Buttner, A. (1994) : Clinical aspects and diagnosis of border disease.Tierazti Prax., 22:35-38.

Wray, C and Roeder, P. (1987) : Res.Vet.Sci., 42:213. Cited by Penny, C.D.; Low, J.C.; Nettleton, P.F.; Scott, P.R.; Sargison, N.D.; Strachan, W.D. and Honeyman, P.C. (1996): Concurrent bovine viral diarrhea virus and salmonella typhimurum DT104 infection in a group of pregnant dairy heifers. Vet.Rec., 18:485-489.

Zaghawa, A. (1998) : Prevalence of antibodies to bovine viral diarrhoea virus and/or border disease virus in domestic ruminants. J.Vet.Med., 45(6): 345-351.

الملخص العربى

دراسات عن العدوى بفيروس البستى فى قطيع من الأغنام (مرض البوردر)

فيصل خليل حمودة وإيمان كامل احمد* وسيد محمد زيدان*

قسم طب الحيوان -كليه الطب البيطرى - جامعه الزقازيق - فرع بنها

معهد بحوث اللقاحات والأمصال بالعباسية*

مرض البوردر من الأمراض الفيروسية الخطيرة التي تصيب الأغنام على مستوي العالم إلا أن هذا المرض لم يحظي بالدراسة البحثية وان هناك نقاط بحثيه مثيره للجدل حول الوضع الوبائي لهذا المرض في مصر. لهذه الأسباب السابقة أجريت هذه الدراسة علي قطيع من الأغنام مكون من ٢٤٣ حيوان مختلفة الأعمار خليطه الجنس بمحافظة الشرقية كانت تعاني من مشاكل مرضيه مختلفة ومتكررة الحدوث وكان هذا القطيع مخاطا في التربيه لعدد ٢٥ عجل تسمين جمسي سليمة ظاهريا. هذا وقد تم اخذ عينات عبارة عن مسحات من الأنف والمستقيم وعينات من السيرم وذللك علي فترتين بينهما ثلاث أسابيع من الأغنام المصابة والمخالطة السليمة ظاهريا. هذا وقد تم اخذ عينات من السيرم ومسحات من مستقيم العجول المخالطة وقد تعرضت العينات للفحص المعملي الفيرولوجي والبكتريولوجي.

الفحص الإكلينيكي للاغنام اظهر أن بعض الحيوانات المصابة تعاني من بعض الارتعاشات العضلية الخفيفة و صوف شعري بصوره ليست كاملة الوضوح. وان بعض الحيوانات أظهرت وجود إفرازات مخاطية أنفية وإسهال متكرر وقد حدث نسبه من الإجهاض أو عقم أو حملان تولد ميته أو ضعيفة وحدثت نسبه من الوفيات في بعض من الحملان. الفحص المعملي الفيرولوجي للعينات اظهر وجود فيروس البستي (البوردر) وقد تم التأكد من وجود الفيروس علي خلايا الزرع النسيجي باستخدام الاختبار الفلوروسنتي و الاختبار الفيروسي المتعادل و اختبار التعارض وذلك باستخدام الفيروس المرجعي والأجسام المناعية القياسية المرجعية لفيروس الاسهال البقري الفيروسي الشديد الشبه بفيروس البوردر. هذا وقد تم عزل والتعرف علي فيروس الإسهال البقري في العينات المأخوذة من عجول التسمين الجمسي المخالطة. الدراسات الوبائية في هذا البحث بينت أن النسبة المرضية في الحملان حديثه الولادة و الحملان اليافعة و الأغنام البالغة كانت ٣٢ و ٢٨٫٦ ، ٩٫٥٪ بينما كانت نسبه الوفيات ٨ ، ٢٫٠٤ صفر ٪ على التوالى. الفحص البكتريولوجي لمسحات المستقيم اظهر وجود ميكروبات الايشرشيا كولاي والسالمونيلا بنسب مختلفة. اختبار السيرم المتعادل اظهر وجود أجسام مناعية بعياريه مختلفة في سيرم الأغنام المصابة و المخالطة السيلمه ظاهريا كما تم تحديد أجسام مناعية في سيرم حمل واحد وذلك قبل تغذيتة علي السرسوب. الاختبارات المعملية الفيرولوجيه والسيرلوجية علي العينات المأخوذة علي الفترتين أعطت نتائج إيجابية. هذا وقد تم إعطاء الحيوانات المصابة العلاج العرضي المناسب واعطاء التوصيات اللازمة للسيطرة علي هذه المشكله الحقلية

خلاصه البحث تتمثل فى الأتى : ١- مرض البوردر من الأمراض الفيروسية الخطيرة التي تصيب الأغنام في مصر بأعراض اكلينيكيه مختلفة وان ظاهرة الصوف الشعري ليست مكتملة الوضوح وذلك لان هذه الظاهرة تظهر جليه في سلاله الأغنام ذات الصوف الناعم وان السلالات الموجودة في مصر تعتبر من السلالات ذات الصوف الخشن. ٢- مرض البوردر قد يصيب الأغنام عن طريق المخالطة مع الماشية التي تعاني من الاصابه الاكلينيكيه والتحت إكلينيكيه أو المستمرة بفيروس الإسهال البقرى لذا يجب عدم مخالطه الماشية للاغنام أثناء التربية. ٣-تفيد نتيجة عزل الفيروس علي فترتين بينهما ثلاث أسابيع مع وجود الأجسام المناعية في سيرم حمل قبل ارضاعه السرسوب وجود العدوى المستمرة بالفيروس وهذه النتيجة لها أهميه قصوى فى الدراسة الوبائية. ٤- المرض له مردود سلبي علي الحالة الصحية والمناعية لما قد تحدثه من تأثير مثبط علي مناعة هذه الحيوانات وتجعلها فريسة للميكروبات الانتهازية مثل الايشرشيا كولاي والسالمونيلا. ٥- عزل فيروس الإسهال البقري من عجول التسمين المخالطة يؤكد امكانيه انتقال هذا الفيروس من الأبقار إلي الأغنام وإصابتها بمرض البوردر. ٦- الحيوانات التي تعاني من العدوى المستمرة يجب تشخيصها مبكرا والتخلص منها لأنها تعتبر بؤره للعدوي المستمرة لباقي القطيع لأنها تفرز الفيروس لفترات طويلة. ٧- الاستخدام الحذر لخلايا الزرع النسيجي أو السيرم أثناء تحضير اللقاحات البيطرية المضعفة وذلك لامكانيه حدوث تلوث من فيروس البستي وهذا يؤدى إلي انتشار المرض بعد استخدام هذه اللقاحات. ٧- تحضير لقاح من فيروس البستي الذي يصيب الأغنام في مصر واستخدامه في تحصين الأغنام اصبح ضرورة حتمية لحماية الأغنام.