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SOME VIRAL AGENTS ASSOCIATED WITH NEONATAL CALF DIARRHOEA

(With 3 Tables and One Figure)

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بعض العوامل الفيروسية المرتبطة بالإسهال في العجول حديثة الولادة

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أجريت تلك الدراسة على عينات براز مأخوذة من عدد أربع وخمسين من العجول البقري والجاموسي حديثة الولادة سواء المصابة بالإسهال أو السليمة ظاهرياً من مزرعة كلية الطب البيطري - جامعة قناة السويس لتحديد الفيروسات المرتبطة بالإسهال في تلك العجول. أجري الكشف عن فيروس كورونا باستخدام الميكروسكوب الألكتروني النافذ (طريقة الصبغ السالب) ، إختبار التلزن الدموي ، عزل الفيروس باستخدام خلايا (أتش رت -١٨) كما تم الكشف عن وجود كل من فيروس روتا وفيروس كاليسي باستخدام طريقة الصبغ السالب للميكروسكوب الألكتروني النافذ. أظهر الفحص باستخدام الميكروسكوب الألكتروني وجود فيروس كورونا في عدد ثماني عينات (٧ر١٤٪) كان منهم عدد ست عينات مأخوذة من عجول مصابة بالإسهال والباقي من عجول سليمة ظاهرياً. كما وجدت إنتجينات التلزن الدموي لفيروس كورونا في عدد عينتين (٧, ٣٪) أحدهما من عجل مصاب بالإسهال والأخر سليم ظاهرياً. كما أظهر الفحص بالميكروسكوب الألكتروني النافذ وجود كل من فيروس روتا وفيروس كاليسى كل منهما في عدد عينه واحده. لم يمكن عزل فيروس كورونا على خلايا (أتش رت -١٨) من العينات السالفة.

SUMMARY

During the study, 54 faecal samples from diarrhoeic and apparently healthy bufffalo and cow calves have been examined for detection of viruses associated with neonatal calf diarrhoea in the farm of the Faculty of Veterinary Medicine, Suez Canal University. The presence of Coronaries

has been screened by using negative stain transmission electron microscopy, haemagglutination and virus isolation using Human Rectal Tumor cells (HRT-18 cells). The presence of other viruses (Root and Caliciviruses) has been detected only by electron microscopic examination. The electron microscopic examination revealed the presence of the characteristic morphology of Coronavirues particles in eight samples (14,7%), six of them were obtained from diarrhoeic calves and the remaining two from apparently healthy calves. Two samples revealed the presence of Coronavirus haemagglutinins (3,7%), one of them from a diarrhoeic calf , while the other from an apparently healthy calf. The electron microscopic examination detected also the presence of Rotavirus and Calicivirus, each in one sample. Isolation of Coronavirus particles on (HRT-18 cells) was not successful.

Key words: *Neonatal calf-Viral diarrhoea.*

INTRODUCTION

Enteric infections leading to diarrhoea are the most important causes of calf morbidity and mortality in Egypt. The aetiology of such infections is multifactorial and calf diarrhoea can be associated with other factors (Acres et al., 1977). Among its proposed causes are fungal and chemical toxins, bacteria, viruses, protozoa and environmental factors. Further factors that could be play a role are the efficacy of transfere of maternal immunity to the calf and development of immunological competence (Wood, 1976). The infectious agents capable of causing diarrhoea in the preweaned calves are numerous. Many viruses have been detected in naturally occuring cases of neonatal diarrhoea, and some of them are expermintaly capable of reproducing the disease. Bovine Coronavirus (BCV) is an important cause of diarrhoea in calves of 3-21 days of age (Saif and Heckert, 1990). This virus is known to cause a more severe disease and higher mortalities than those caused by bovine Rotavirus becase it multiplies in both small and large intestines, whereas the Rotavirus infects the small intestine (Torres-Medina et al., 1985).The main route of infection is the faecal- oral and pregnant cows are belived to be important sources of the virus for newborn calves (Crouch et al., 1985). Rotavirus is endemic in almost all cattle farms and is execreted intermittently by a significant proprtion of the normal calf and adult cow populations. These carrier animals are probably the main source of

infection for batches of calves (Saif, 1990; Vermunt, 1994). There is a growing evidence that Breda virus and small viruses resembling calicivirus can be also responsible for this syndrome (Woode et al., 1982; Woode and Bridger, 1978).

This investigation has been undertaken in order to find the variety of viruses which could be associated with field cases of neonatal calf diarrhoea, and also to compare the reliability of some techniques for detection of these viruses.

MATERIALS AND METHODS

The study was conducted on the herd of the Faculty of Veterinary Medicine , Ismaillia. The total cattle and buffalo population were 922, mainly native breeds including (adult, growing , weaned and newborn). The calves under investigation were classified into two groups (apparently healthy and diarrhoeic). Both groups were allowed or assisted to suckle from their dams. Diarrhoeic calves showed clinical signs of fever, diarrhoea, dry rough coat, weakness and severe body loses.

Samples:

Faecal samples were obtained from the rectum of 30 diarrhoeic and 24 apparently healthy buffalo and cow calves (1-17 days old). Faecal samples (5-10gr) were collected by means of sterile probes introduced into the rectum, kept in sterile plastic bottles and stored at-20 c untile examination.

Techniques:

Examination of faecal samples was carried out in the Institut fur Hygiene und Infektionskrankenheiten der Tiere, Giessen, Germany.

1-Detection of virus particles by electron microscope:

The electron microscopic examination of virus particles was carried out according to Krauss and Arens (1981). Faecal samples were prepared as follows: 0.5 gr of each faecal sample was suspended 1:10 in 5% Minimal Eessential Medium (MEM) Eagle's modification in sterile test tubes and homogenized by vortex mixer for 1-2 minutes. Homogentes were clarified by centrifugation at (2000 g X 20 min.). The supernatant was transfered by means of sterile Pasture pippte and subjected to centrifugation at (80.000 g X 1hour). The pellet was resuspended in 0.1-0.5 ml of sterile PBS. A drop of the resuspended pellet was applied to a 200 mesh copper grid and allowed to remain on the grid for 15-20 minutes. Excess fluid was blotted off with a filter paper, then a droplet of

2% phosphotungestic acid solution was placed on the grid and excess was removed again by the filter paper. The grids were examined by transimission electron microscope (EM 10/ CR Zeiss). Figure (1).

2- Detection of Coronavirus by haemagglutination test:

Haemaggltination test for detection of Coronavirus antigen in faecal samples was carried out according to Mayer et al.(1977).

3-Coronavirus isolation:

Faecal samples were suspended 1:2 (v/v) with MEM containing 800 I.U. Penicillin, 800 mg Streptomycin and 400 I.U.Gentamycin and incubated overnight at 4 c°. After centrifugation of the suspension (6000 RPM X 5 min.), the resultant supernatant was inoculated to (HRT-18 cells), which were grown to confluence in microtiter plates and suplemented with 2% fetal calf serum. The plates were incubated at 37 C° for 7 days in an 5% Co2 atmosphere and were examined daily for plaque formation under the inverted microscope (Laporte et al., 1979).

RESULTS

Electron microscopic examination of faecal samples of both diarrhoeic and apparently healthy calves revealed the appearance of the characteristic morphology of bovine Coronavirus (BCV) in 6 (20%) and 2 (8.3%) of faecal samples of diarrhoeic and apparently healthy calves, respectively. BCV are pleomorphic to rounded in shape, varying in diameter from 80-160 nm and with a mean diameter about 120 nm. The virus enveolpe is seen as a distinct pair of electron dense shells from which the spikes radiate to form a fringe of surface projections. The characteristic morphology of Rotavirus and Calicivirus was also detected, each in one of the faecal samples of diarrhoeic calves 1 (3.3%), but none of them isolated from the faecal samples of the apparently healthy calves. Comparing the results of Coronavirus detection by EM, haemagglutination test and virus isolation, the characteristic morphology of Coronavirus particles was observed in 8 out of 54 samples (14.8%), while Coronavirus haemagglutinns were detected in 2 samples (3.7%). On the other hand, isolation of the virus on (HRT-18 cells) was not successful.

DISCUSSION

The detection of BCV and Rotavirus was expected, as these viruses are well documented causes of diarrhoea in many countries (Buerki, 1984; Snodgrass et al., 1986). In Egypt, reports of the occurrence of severe

cases of neonatal calf diarrhoea were attributed to viral agents especially Corona and Rotaviruses (Shalaby et al. 1991; Abou El-Hassan et al. 1995; El- Sawalhy et al. 1995). Six of eight EM positive BCV samples were found in diarrhoeic calves and the other two samples were in apparently healthy calves which reflect the shedding of the virus also by none diarrhoeic calves. Rotavirus was detected only in one sample (3.3%) from a diarrhoeic calf by electron microscopy as well as Calicivirus. Reynolds et al. (1984) and Durham et al. (1989) indicated the value of EM as a broad spectrum tool for detection of viruses, especially wheother techniques fail to provide an answer . Reynolds et al. (1986) and Baljer et al. (1987) reported that in calves less than 5 days old, Rota and Coronaviruses were detected more frequently than in calves of 6-14 days old. Many workers estimated the economic impact of neonatal disease agents and found that while E. Coli was responsible for the most devasting economic losses, Corona and Rotaviruses infections ranked second and third. Kodituwakku and Harbour (1990) and Clark (1993) indicated that clinically normal adult cows which are persistantly infected with Rotavirus and BCV act as a source of infection for susceptible calves. Based on the HA properties of BCV, HA test was carried out and detected the virus haemaggltinins in 2 samples (3.7%), one of them was negative when examined by EM. Additional evidence that these samples were BCV was not obtained because the virus was not recovered in tissue culture isolation. In this respect, Clark (1993) stated that the virus isolation is rarely used as a mean of diagnosis as BCV is difficult to isolate. There are some possible reasons for differences in the results of BCV detection by EM, HA and virus isolation. With regard to the time elapsed from collection of samples untile their examination, the condition of samples may be affected. Kapil et al. (1990) and Clark (1993) stated that BCV is liable, sensitive to environmental conditions and can be brokendown by time, where the virus particles may be not detected. An important factor to consider when comparing electron microscopy with immunoassys is the possibilty of antigenically different viruses with identical morphology (Reynolds et al., 1984). Another possibility for this difference is that intestinal antibodies could neutralize virus particles in immune complexes. Detection of shedding animals may be limited by the sensitivity and specificity of EM evaluation (Reynolds et al., 1984; Collins, 1987). Supporting this notion, Jimenez (1990) decieded that with respect to antibody content in faeces of calves and results of different methods applied for detection of BCV, the

majority of positive results were recieved with samples containing low amount or no BCV antibodies .

REFERENCES

Abou El-Hassan, D.G.; Salem, S.A.H. and Fayed, A.A. (1995): Neonatal calf diarrhoea in Egypt. 3rd Sci. Cong., Egypt. Society for Cattle Diseases, 57-65.

ACRES, S.D.; SAUNDERS, J.R. AND RADOSTITS, O.M. (1977): Acute undifferentiated neonatal diarrhoea of beef calves: The prevalence of enterotoxigenic E.coli, reo-like virus and other enteropathogens in cow herds. Can.Vet. J., 188:113-121.

Baljer, G.; Eichborn, W.; Goebel, E.; Wolf, M. and Bachman, P.A.(1987): Vorkommen und verbreitung wichtiger durchfallererrager bei neuegebornen Kaelber in Sueddeutschland in zeitraum 1984 bis 1986. Tieraerztl. Umschau., 42:56-65.

Buerki, F. (1984): Pathogenesis and immunoprophylaxis of bovine Rotavirus infection. Acta. Microbiologica. Hung., 31:213-221.

Clarki, M.A. (1993): Bovine Coronavirus. Br. Vet. J., 194:51-70.

Collins, J.K.; Riegel, C.A.; Olson, J.D. and Fountain, A. (1987): Shedding of enteric Coronavirus in adult cattle. Am. J. Vet. Res., 48:361-365.

Crouch, C.F.; Bielfeldt, H.O.H.; Watts, T.C. and Babiukk, L.A. (1985).:Chronic shedding of enteric bovine Coronavirus antigen-antibody complexes by clinically normal cows. J.Gen.Virol., 66:1489-1500.

Durham, P.J.K; Hassard, L.E.; Armstrong, K.R. and Naylor, J.M. (1989): Coronavirus-associated diarrhoea (winter dysentery) in adult cattle.Can. Vet.J., 30:825-827.

El-Sawalhy, A.A.; El-Bagoury, S.A.A. and Youssef, M.A.A. (1995): Bovine viral diarrhoea complicated with Rotavirus infection among breed herd in Sharkia Governate. 3 rd Sci. Cong. Egypt. Society for cattle diseases., 66-77.

Jimenez, C. (1990): Vergleichende Labordiagnostischer Untersuchungen Von Kotproben (EM, Virusisolierung, immunologiche Verfahren) Zum nachweis einer bovinen Coronavirus-infekion bei durchfallkranken Kaelbern. Inaug. Disst. Fachbreich Vet. Justus-Liebig Uni. Giessen, Germany.

Kapil, S.; Trent, A.M. and Goyal, S.M. (1990): Excretion and persistance of bovine Coronavirus in neonatal calves. Arch. Virol., 115:127-132.

Kodituwakku, S.N. and Harbour, D.A. (1990): Persistant excretion of Rotavirus by pregnant cows. Vet.Rec., 126:547-549.

Krauss, H. and Arens, M.(1981): Die elektronmikroskopische untersuchung von kot-oder organmaterial als diagnostischer schnellnachweis bei der Hunde. Prakt. Tieraerzt., 62:388-41.

Laporte, J.L.; Haridon, R. and Bobulesco, P. (1979): In vitro culture of bovine enteric Coronavirus. Viral enteritis. INSERM, 90:99-102.

Mayer, A.; Bachmann, P.A.; Bibrack, B. and Wittmann, G. (1977): Virologische Arbeitsmethoden Bd. 2, Gustav Fischer Verl., Sttutgar., 665-670.

Reynolds, D.J.; Chasey, D.; Scott, A.C. and Bridger, J.C. (1984): Evaluation of ELISA and electron microscopy for the detection of Coronavirus and Rotavirus in bovine faeces. Vet. Rec., 114:397-401.

Reynolds, D.J.; Morgan, J.H.; Chanter, N.; Jones, P.W.; Bridger, J.C.; Debney, T.G. and Bunch, K.J. (1986): Microbiology of calf diarrhoea in southern Britain.Vet. Rec., 152:33-35.

Saif, L.J. and Heckert, R.A. (1990): Enteric Coronavirus. In: SAIF, L.J. and THIEL, K.W. (eds) Viral diarrhaes of man and animals. CRS Press, Boca Raton, Fl, 185-252.

Shalaby, M.A.; Abd El-Hamid, R.S.; El-Sanousi, A.; Sami, A.M.; Fadaly, M.A.; El-Bagoury, G. and Reda, I.M. (1991): Some studies on bovine Coronavirus infection among newborn calves in EGYPT. J.Egypt.Vet. Med. Assoc., 51 (1&2): 455-4666.

Snodgrass, D.R.; Terzola, H.R.; Sherwood, D.; Cambell, I.; Menzen, J.D. and Synge, B.A. (1986): Aetiology of diarrhoea in young calves. Vet.Rec., 119:31-34.

Torres-Medina, A.; Schlfer, D.H. and Mebus, C.A. (1985): Rotaviral and Coronaviral diarrhoea. Vet. Clin. North. Am.(Food Animal Pract.), 1:471-493.

Vermunt, J.J. (1994): Rearing and management of diarrhoea in calves to weaning. Aust. Vet. J., 71 (2):33-41.

Woode,, G.N. (1976): Viral diarrhoea in calves. Vet.Annu., 16:30-34.

Woode, G.N. and Bridger, J.C. (1978): Significance of bovine Coronavirus infection. Vet. Rec., 102:15-16.

(1982): Studies with an unclassified virus isolated from diarrhoeic calves. Vet.Microbiol., 7:221-240

Table 1: Prevalence of viral agents in diarrhoeic and apparently healthy buffalo and cow calves as examined by EM.

	No.of d calves	Coronavirus No. %	Rotavirus No. %	Calicivirus No. %
Diarrhoeic calves	30	6 (20)	1 (3.3)	1 (3.3)
Apparently healthy calves	24	2 (8.3)	- (-)	- (-)

Table 2: Results of examined faecal samples from calves for detection of Coronavirus.

Method	No. of samples	Positive No. %	Negative No. %
EM	54	8 (14.8)	46 (85.1)
H.A.	54	2 (3.7)	52 (96.3)
Virus isolation	54	0 (0)	54 (100)

Table 3: Comparison of results of positive Coronavirus samples as examined by EM, H.A. and virus isolation on HRT-18 cells among buffalo and cow calves.

Test	Buffalo calves		Cow calves	
	Diarrhoeic (n=18) No. %	Healthy (n=15) No. %	Diarrhoeic (n=12) No. %	Healthy (n=9) No. %
EM	2 (11.1)	2 (13.3)	4 (33.3)	- -
H.A.	- -	1 (6.7)	1 (8.3)	- -
Virus isolation	- -	- -	- -	- -

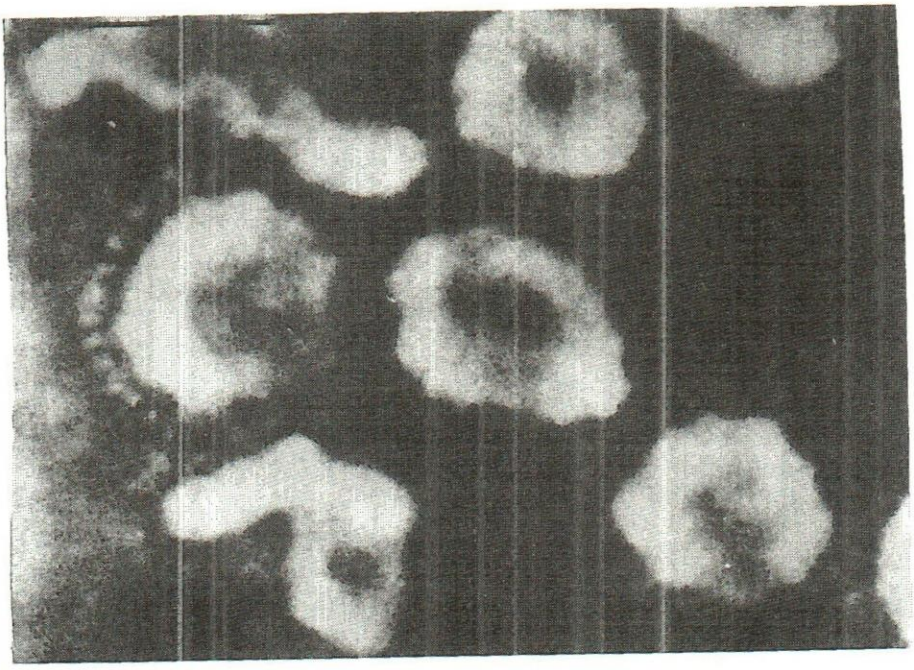

Fig. 1: Electron photomicrograph of Coronavirus particles from faecal material of neonatal calf diarrhoea. Bar =100 nm.