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STUDIES ON ENTERITIS IN NEWLY BORN CALVES

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Abstract

Atotal of 250 diarrhoeic calves were examined virologically, bacteriologically and serologically. Post - mortem and histopathological examination were carried out on dead animals. Virological examination revealed infectious bovine rhinotracheitis and bovine virus diarrhoea form 24%, 10.4% respectively, while bacteriological examination showed that *Salmonella* species were 0.8 and *E. coli* was 4%. Aflatoxicosis was found in ration at 2 farms . Histopathological examination revealed different types of enteritis which were more or less related to the causative agent.

INTRODUCTION

Neonatal calf diarrhoea is responsible for great economic losses in animal industry, therefore, many investigations were done on spontaneous cases of newly-born calves aged from one day to three months. The causes were found to be variable and complex, involving environmental, dietary and multiple infectious agents either alone or mixed with each other (Qviedo *et al* . 1987). Some reported Rotavirus (Voller *et al.* 1979), Coronavirus (Kruiningen *et al* . 1987), Bovine diarrhoea virus (Vallet and Navetal 1988) and infectious bovine rhinotracheitis virus (Marsolais et al . 1978) as the main viral causes of the disease. *Salmonella* spp. (Farid et al. 1987) and *E. Coli* (Nagy et al. 1986) were isolated as bacterial causes of diarrhoea, while aflatoxin was detected as one of the non-infectious causes.

The aim of this report is to determine the predominant causes of enteritis in Egyptian calves and to find out the perfect techniques for their rapid and sure diagnosis in correlation with the histopatholgical examination as a means for reliable diagnosis (Pospischil et al . 1986).

MATERIALS AND METHODS

A total of 250 buffalo-and cattle calves, from which 126 were buffaloes and 124 were cattle, were examined. These animals, were distributed among 1152 found in three governmental and five private farms, and clinically showing symptoms of diarrhoea. Rectal swabs and blood samples were collected from all animals under investigation for virological, bacteriological and serological studies. Postmortem examination was carried out on 37 dead calves died during the course of examination, and tissues from different segments of the intestine and the liver were collected for histopathological examination.

1. Virus detection

a. Enzyme linked immuno-sorbent assay (ELISA) : was carried out as described by Voller *et al.* (1979). This test was used either to detect the virus antigrn in the fecal sample preparaion using direct ELISA or to detect antibodies in the sera of affected animals.

i. Virus : Infectious bovine rhinotracheitis (IBR) and bovine viral diarrhoea (BVD) viruses were brought from Abbassia Serum and Vaccine Production Research Institute, and standard rotavirus brought from Faculty of Vetrerinary Medicine, Cairo University.

ii. Antisera : Hyperimmune sera to both IBR and BVD viruses were brought from Virology Section, Animal Health Res. Institute, Dokki, Cairo, Egypt.

b. Immnofluorescent antibody technique . (IFA) : The technique was done to detect the specific fluorescent of IBR and BVD antigen according to the method described by Majewsha *et al.* (1984).

i. Tissue culture : Primary Embryonic Calf Kindey (ECK) cell cultures were grown in Minimum Essential Medium (MEM) containing 10 % bovine serum , 100 IU penicillin , 100 mg streptomycin and 100 mg kanamycin for propagation , titration of viruses and immunofluorescent antibody technique in which the cells were inocu-

lated with ELISA positive samples.

2. Bacterial isolation:

The fecal samples were cultivated on *Salmonella-Shigella* agar (Difco), nutrient agar (Difco), MacConkey lactose bile salt agar (Difco), then, the suspected colonies were subjected to biochemical characterization using (Indol test, methyl red test, Vogus proskaure test and Christen's urea agar test according to Cruickshank *et al.* (1975), then , Gram's stained films were performed. Serological identification for positive samples was carrid out by polyvalent antiserum for *salmonela* spp. and polyvalent and monovalent antisera for *E. Coli* produced by (Difco Lab.) DETROIT Mitchigan, U.S.A.

3. Food analysis:

Rancidity and aflatoxin estimation in the food samples were carried out after Adamesteanu *et al.* (1974).

4. Immunological studies:

(a) Total protein : Serum total protein : According to Henry *et al.* (1975).

(b) Acrylamide gel disc electrophoresis : Carried out after Davis (1964).

5. Pathological studies:

Thirty- seven calves died during examination , post-mortem examinations were done as soon as possible after death, and the specimens from different segments of small intestines and liver were collected and immediately fixed in 10 % neutral formalin, dehydrated, cleared, embeded in paraffin, sectioned at 2-4 microns and stained with Haematoxylin and Eosin, to be ready for examination (Culling 1963).

RESULTS

The records collected from different localities showed that, the morbidity percentage was 38-84 % , while mortality rate was about 5-47 % . It was found that , the mortlity rate was low in farms with good hygienic condition . Detction of viral agent in the fecal samples was performed by using ELISA and IFA as illustrated in Table 1.

Table 1. Results of both ELISA and IFA tests applied on the diarrhoeic calves serum.

Total	ELISA Positive samples				IFA positive samples			
	IBR	%	BVD	%	IBR	%	BVD	%
250	60	24	26	10.4	13	5.2	2	0.8

From table 1 it could be concluded that IBR infection is the prevalent infection among the examined animals . Moreover, it is clear that, direct detection of antigen by ELISA is more sensitive than that of IFA . All samples were negative for rotavirus detction by ELISA.

Bacterial isolation is showen on Table 2.

Table 2. Results of bacretiological examimation of fecal swabs.

Total	Sal. spp.		E. coli	
	Positive	%	Positive	%
250	2	0.8	10	4.2

The bacteriological results showed a relatively low percentage for both *Salmonella* spp. and *E. Coli.*

Serological examination is shown on Table 3.

Table 3. Antibodies detection in serum samples by ELISA.

Total	IBR		BVD	
	Positive	%	Positive	%
250	79	31.6	95	38.0

Immunological examination is showen on Table 4 .

Table 4. Type of viral isolates from diseased calves .

Total	Total protin g/ dl		Alb	Globulin	
				beta	gamma
20 Mean values	Normal	6.16 ± 0.24	2.12 ± 0.09	1.1 1.3 ± 0.10 0.04	1.64 ± 0.06
	Dirrhoeic	6.60 ± 0.19	2.32 ± 0.19	*** 1.29 ± 2.43 ± 0.08 0.17	*** 0.06 ± 0.07

*** : Highly significant at P< 0.001

- High significant increase in beta globulin in diarrhoeic calves.

- High significant decrease in gamma globulin in diarrhoeic calves.

Results of post - mortem examination:

The dead calves were severaly emaciated and dehydrated with sunken eyes, multiple petechial and / or ecchymotic haemorrhages occasionally seen on serous surfaces. The abdominal cavity contained large amount of peritoneal fluid. The intestines appeared congested and distended with fluid . The bowels contained mixture of mucus and fluid feces, haemorrhages, blood clots and/or mucosal threads. The mesenteric lymph nodes were swollen and haemorrhagic, the liver was enlarged and congested. The lungs were mostly within normal, but occasionally showed variable degrees of bronchopneumonia.

Histopathological findings:

After microscopic examination of the different parts of the small intestines , the pathological changes could be grouped in the following three groups :

a) Mild changes b) Moderate changes c) Severe changes .

a) Mild changes: were seen in 8.1 % of the examined cases. They were negative for both bacterial and viral isolation, but their sera contained antibodies to BVD and / or IBR . Microscopically , the changes were not more than mild degenerative and inflammatory reactions along the three parts of the small intestine, the villi were mostly normal covered by tall columnar epithelium, some villi had cuboidal epithelial lining with increased number of Goblet cells. Some villi showed desquamation of their epithelium particularly at the tips (Fig . 1) The epithelial covering the crypts of Lieberkuhn were not affected . Few mononuclear cells, mainly, lymphocytes , monocytes and plasma cells were seen in the villus corium (Fig. 1) . The lacteals and the blood capillaries were slightly dilated.

The lamina propria was congested, and slightly oedematous , among which few mononuclear inflammatory cells could be traced.

The duodenal gland , Payer's patches, the muscular layer and the adventitia showed negligible changes.

b) Moderate changes: This group represented 18 % . All were positive for IBR virus, except in 2 cases in which BVD was mixed with IBR virus and all of them were negative for bacterial isolation.

In this group of animals, the microscopic changes were more or less severe than in the previous group and of atrophic nature . In all levels of small intestine, the villus epithelium was partially or completely desquamated. The villi varied from moderately short, cylindrical , to short blunt and tongue - shapped , or being just ridges on the mucosa, or might be completely disappeared (Fig. 2) and the crypts open directly on the surface . In less affected cases, the crypts epithelium were not involved, but in moderate or severe cases the crypt epithelium was degenerated and changed and replaced by cuboidal immature epithelium. Along with villus atrophy , there were variable degrees of hypercellularity in th connective tissue core, the individual villi . These cells were mainly reticular cells and lymphocytes, histiocytes and plasma cells.

The lamina propria was congested, contained many dilated blood vessels , lymphatics and cellular infiltration as well as variable degrees of oedema. In the ilium , the Payer's patches were hypertrophied and large number of lymphocytes were diffusely infiltrating, the lamina propria.

C) Severe changes: Severe and advanced changes were seen in 74 % . Nearly all of them were positive for BVD virus isolation alone or mixed with IBR virus, only one case was mixed with *Salmonella* spp.

The microscopic changes in this group varied considerably at different sites of the gut and even within an individual section . The most prevalent and common change was the necrosis of the tips of the whole villi. These were transformed into pinkish , homogenous structureless material , which might be fragmented and detached or being attached to the crypts (Fig . 3). The crypts epithelium might be hyperactive and hypertrophied or became partially degenerated and damaged (Fig . 3). In many cases , the lamina propria was congested and oedematous with heavily cellular inflitration, mainly , lymphocytes, histiocytes, macrophages and plasma cell. Both the circular and longitudinal muscular layer were degenerated with few mononuclear cellular infitration.

Liver:

Unlike the intestines, the pathological changes in the liver could not be grouped neither according to histological changes nor to the isolated organism . So , the histopatholgical findings in the hepatic parenchyma suffered from variable degrees of necrobiotic changes starting from cloudy sweling and hydropic degeneration in most hepatocytes, to variable areas of mecrosis in many hepatic lobules (Fig. 4).Sometimes, the haptic cells were seen degenerated and atrophied with distortion and dissociation of the hepatic cords, which were mainly due to oedema and cellular infiltration . Mononuclear cellular infiltration (mainly lymphocytes, histiocytes, monocytes and plasma cells) was also seen in the portal areas with activation of Kupffer cels (Fig. 4).

Nearly in all cases, there were severe congetion of the hepatic vessels and sinusoids, with irregular areas of haemorrhage between the hepatic cells; some vessels showed swollen and degenerated intema with hyalinization or fibrinoid degeneration of the media.

DISCUSSION

The results emphasize the complexity of diarrhoeic syndrome and indicate some reasons why this syndrome is difficult to be controlled or diagnosed, despite

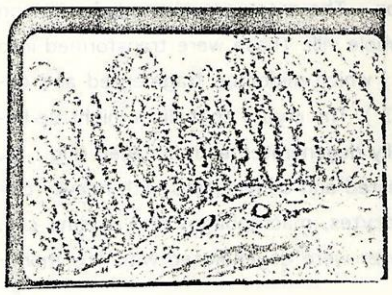

Fig. 1. Showed many villi were denuded of epitenilium , witn cellular infiltration in the lamina propria (H&E X 100)

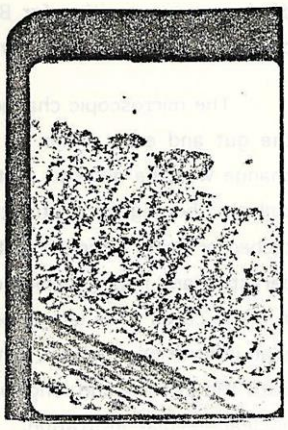

Fig. 2. Short jand atrophied villi or nearly disappeared (H&E X 100)

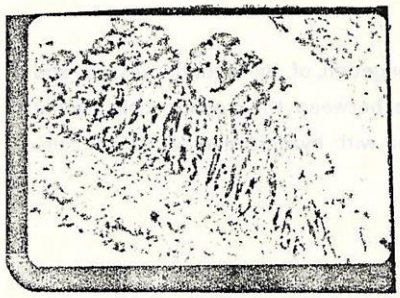

Fig. 3. Fragmented and necrosed villi some of the crypts epithelium were hyperactive, others were damaged (H&E X 100)

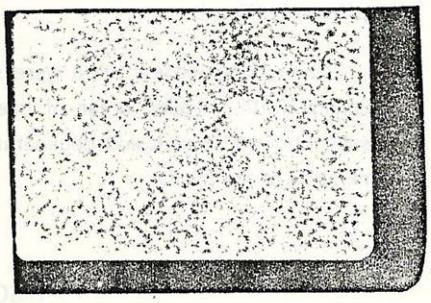

Fig. 4. Multiple areas of necrosis with cellular infiltration (H&E X 100)

of good hygieneic conditions, the periodic antibiotic administration and the careful follow- up of the animals.

It is of imprtance to mention that the high percentage of diarrhoeic animals were seen in winter, which indicated that changes in weather like wet, windy and cold condition were usually predisposed outbreaks of diarrhoea.

In the present study, IBR antigen was detected in faeces of diarrhoeic calves using ELISA, denoting actual virus secretion which represented 24 % of the cases (Blood et al . 1983). Immunofluorescence was used as confirmatory test on ELISA postive cases, but it was clear from the results obtained that ELISA was more sensitive than IFA. The presence of IBR antibodies in the sera of the examined animals which were detercted by ELISA, could be attributed to maternal immunity or previous active infection.

The microscopical examination of the small intestines revealed atrophic changesin the villi, which varied from moderately short to completely disappeared villi. This is attributed to the direct effect of the virus which resulted in significant loss and desquamation of the surface epithelium over a relatively short period of time, so the villi contracted as the size of the functional compartment was diminshed.The lamina propria appeared moderately hypercellular which was due to condenstation of cells as a result of atrophy on one hand and to mononuclear and reticular cells proliferation on the other (Mebes *et al.* 1973). Oedema and haemorrhage seen in most cases were due to hyaline degeneration and fibrinoid necrosis of the submucosal and mesentric vessels.

The second virus which had been detected in faecal material of diarrhoeic calves was BVD virus which was known to be intermittent in such disease, but it was proved to be one of the causative agents of neonatal calf diarrhoea (Blood *et al.* 1983). Serological examination of the serum samples for BVD antibodies also showed a high sensitivity of ELISA for the detection of BVD antibodies than any other technique (Westenbrink *et al.* 1986).

The microscopical examination of the small intestines showed severe changes mainly of necrotic nature which involved the whole villus or rarely only the tips accompanied with denudation and sloughing . The involvement of blood vessels in the process of degeneration and necrosis can partially explain the necrotic changes (Bielefeldy 1983). But, the main explanation is due to the action of BVD virus on the intestinal epithelum and the underlying tissue (Bolin et al . 1988). The exhaustion of

lymphoid elements of the intstines particularly the germinal center could be attributed to the affinity of BVD virus to localize in lymphoid tissues, from which the virus was released and spread to all parts of the alimentary tracy (Bolin et al . 1988). The increased macrophages and lymphocytic cellular infiltration in the lamina propria were mainly due to migration of lymphoytes from destroyed lymphatic tissue and the aggregated macrophage in order to enhance engulving of the virus and the necrosed tissue debris (Bloin et al. 1988).

Because of the similarity of liver changes in both IBR and BVD infected animals it appeared that, there was no correlation between the hepatic changes and the reported types of enteritis.

So, hepatic changes due to viral infection , in general , started with mild degenerative changes to focal areas of necrosis (Jobb et al 1985); mononuclear cells were seen in and around the necrosed areas, around blood vessels and in the portal areas. Mixed infection was reported in few cases which were either by two viral agents (BVD & IBR), or by one of these viruses and *Samonella* spp. or *E.coli* . Mixed infection was well known in severl records , besides that the animal may be permanent viraemic for BVD and superinfected by IBR or latenty infected with IBR and superinfected by BVD . The percentage of bacterial infection due to *Samonella* spp. or *E.coli* were negligible with regard to the viral isolation which could be attributed to the continuous treatment with antibiotic. Abortive attempt done to isolate reotavirus in the faecal material is most probably due to prevalence of other viral agents or need more modification of the technique.

The ration containing aflatoxin could be considered as non-infectious cause of diarrhoea (Bielefeldy 1983) . The results of serum protein fractionation revealed a normal total protein with decrease in gamma globulin . This could be attributed to inadequate quantity in ingested colostrum , or due to the action of pathogen on the immune system leading to suppression of response to any pathogenic agents.

The aforementioned results and discussion draw the attention for the importance of the viral enteritis which has a great share in diarrhoea syndrome. Field and laboratorty investgation have indicated that there may not be a single etiology of calf -diarrhoea. The cause is complex and usually an interplay between virus and bacteria which aggravated the condition . Also, the inter-related epidomiological factors have been associated with high incidence of calf diarrhoea.

So, the laboratory approach described in this study may be helpful to establish

an etiological diagnosis of neonatal calf diarrhoea and can contribute to a better understanding of this complex disaese. It is suggested that, vaccines are only part of a total management program necessary for the prevention of the enteritis calf disease.

REFERENCES

1. Adamesteanu, T., O. Adaestenu, I. Baba , N. Danielscu, N.A. Maldovanu and O. Rotaru. 1974. Liver cirrhosis in newborn calves as a result of palcental transmission of aflatoxin . Deutsche Tierartztliche Wochenschrift, 81, (6).
2. Bielefeldy, Ohmann, H. 1983 . Pathogenesis of bovine viral diarrhoea, mucosal disease : Distribution and significance of BVDV antigen in diseased calves. Res. Vet. Sci., 34: 5 -10.
3. Blood, D.C, O. M. Radostits and J. A. Henderson. 1983. Veterinary Medicine. 6th. Ed., Bailliere Tindall , a division of Cassell Ltd, Ist Annes Road East Bourhe BN 21, 3 UN pp. 55 -774.
4. Bolin, S.R, V. Moenning , N.E.K. Gourly and J. Ridpatr. 1988. Monoclonal antibodies with neutralization activity segreate isolates of viral diarrhoea virus into groups . Archives of Virology, 99 (1/2) : 117-123.
5. Cruickshank. R, J.R.I. Duguid, B.P. Marmion and R.H.A. Swain. 1975. Text book of Medical Microbiology, 12 th Ed. Churchill, Livingstone, Edinburgh and New York.
6. Culling, C.P.A. 1963. Hand book of Histopathological techniques 2 nd Ed. , London, Butter Worth & Co. Ltd.
7. Davis , B.J. 1964. Disc electrophoresis method and application to human serum proteins . J. Ann . N.Y. Acad. Sci., 121 : 404.
8. Farid, A.F. , S.M. Nashed and M.K. Saad. 1987. Salmonellosis in buffalo-calves in Upper Egypt. J. Egypt . Vet. Med. Assoc., 47 : 153-160.
9. Henry, R.J, C.E. Sobel and N. Palmer. 1975 . Anim. Chem ., 92 : 1491.
10. Jobb, K.V.F. ,P.C. Kennedy and N. Palmer. 1985. Pathology of domestic animals. 3 rd Ed. , Vol. 2 , Academic Press, Inc. Harcoust brace Jovanovich Palisher, P.51-77,95.
11. Kruiningen, H. J. , L. H. Van Kharallah, V.G. Sasseville , M. S. Wyand and J.E.

Post. 1987. Calfhood coronavirus enterocolitis : A clue to the aetiology of winter dysentry. Vet. Path. , 24 (6): 564-567.

12.Majewska, H.,J. Zmudibski and Z. Nbaczynski. 1984. The use of different immunofluorescence methods for identification of IBR/IPV and BVD/ MD viruses isolated from pathological material. Bull. Vet. Inst. in Pulawy, 27 (1-4) : 60-64.

13.Marsolais. G, R.I. Assef, C. Montpetit and P. Marois . 1978. Diagnosis of viral agaent associated with neonatal calf diarrhoea. Can. J. Comp. Med., 42 (2) : 168- 171.

14.Mebas , C.A., E.L. Stair, M.B. Rhodes and M.J. Twehaus. 1973. Pathology of neonatal calf diarrhoea induced by coronavirus like agent. Vet. Path. 10:45-64.

15.Nagy, B., L. Csontose , V. Palfi, G. Nagy and M. Bozso. 1986. Results of diagnostic tests on calf diarrhoea of multiple aetiology occurring in the first month of life. Wiener Tierarztliche Monatschrift, 73 (6) : 181-184.

16.Pospischil. A. R. G. Hess, P.A. Bachmann and B. Baljer.1986. Experimental infection of caesarean derived colostrum deprived calves with bovine rotavirus and enter-toxigenic Escherichia coli (ETEC) : A histological electron microscopic , and morphometric investigation . J. Vet. Med.B., 33(3) : 196-205.

17.Qviedo M.T., L. N. Araya and F. Hernandez. 1987. Bacterial , parasitic and viral agents involved in the aetiology of calf diarrhoea in Costa Rica . Ciencias, Veterinatias, Costa Rica, 9 (1) : 29 -34.

18.Vallet, A. and H. Navetal. 1988. Diseases of calves from two weeks to three months of age. Revue de Medicine Veterinatire, 139 (8-9) : 801-814.

19.Voller, A., D.E. Bidwell and A. Bartlett .1979. The enzyme linked immunosorbent assay (ELISA) . A guide with abstracts of microplate applications. Flowine Press, Guernsey, P. 1, - 125.

20.Westenbrink, Middel, W.G. J., P. J. Straver and P.W. de Leeuw. 1986. A blocking enzyme-linked immunosorbent assay (ELISA) for bovine virus diarrhoea virus serology. J. Vet . Med. , B, 33 (5) : 254-301 .

دراسات عن الإلتهاب المعوى فى العجول حديثة الولادة

على عبد السلام حجازى ١ ، نبيهة رمضان على ١ ، ابراهيم محمد اسماعيل ١
عفاف سعد الدين فهمى ٣ ، ممدوح عبد الغنى ، لمياء عبد الحميد الفراش ٢
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تم فحص عدد ٢٥٠ عجلاً مصابة بالإسهال المعوى للاختبارات الفيرولوجيه والسيرولوجيه والبكتريولوجيه . كذلك تم فحص الآفات المرضيه على العجول النافقة . أظهر الفحص الفيرولوجي اصابتها بفيرس الحنجرة والقصيه الهوائيه وفيروس إسهال الأبقار من ٢٤ ٪ ، ٤ ، ١٠ ٪ على التوالى بينما اظهر الفحص البكترولوجى اصابتها بميكروب السالمونيلا والميكروب القولونى بنسبة ٤ ٪ كما وجدت سموم الافلاتوكسين في مزرعتين . أظهر الفحص الهستوباثولوجى وجود التهاب معوى بدرجات مختلفة ويدل ذلك على الميكروب المسبب .