

قسم : طب الحيوان وأمراض الدواجن .
كلية : الطب البيطري - جامعة أسيوط .
رئيس القسم : أ. د. / إبراهيم سكر .

دراسات عن تفشي مرض الاسهال في عجول الفريزيان حديثة الولادة

١ - التغيرات الاكلينيكية والدوائية والباثولوجية

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شمل هذا البحث مجموعتين من العجول الفريزيان :

الأولى : أظهرت حالة شديدة من الاسهال وكانت سبعة حيوانات نفق منها ثلاثة قبل البدء في العلاج ،

الثانية : تكونت من تسعة عجول في حالة اسهال ولكنها أخف حدة وشدة من الحالة الأولى ، في حين كانت هناك سبعة عجول لم يبدو عليها أية أعراض واستخدمت كضوابط .

كانت تلك الحيوانات تابعة لقطيع من عجول وأمهات الفريزيان بمدينة الخارجة بمحافظة الوادي الجديد . سجلت الأمراض الاكلينيكية للحيوانات المصابة بالاسهال والتي كانت مطابقة تماما لأمراض التهاب بكتريا القولون . كما فحصت العجول الثلاثة النافقة وكانت مطابقة تماما لحالات التهاب بكتريا القولون لوحظ أيضا نقص عدد كرات الدم الحمراء وكذلك نسبة الهيموجلوبين التي صاحبت هذا النقص في بعض حالات المجموعة الثانية مع زيادة واضحة في عدد كريات الدم البيضاء خاصة الخلايا البلعية (منقسمة النواه) ، الا أنه قد لوحظ نقص واضح في عدد الخلايا الليمفاوية في كلتا المجموعتين خاصة منها المجموعة الأولى . لم يلاحظ أى تغيير في نسبة الهيماتوكريت في حيوانات المجموعة الثانية في حين سجلت زيادة واضحة في تلك النسب في حالات المجموعة الأولى .

أظهرت الفحوص البكتريولوجية والتي استخدمت فيها مسحات شرجية كذلك مسحات من الاعضاء والاحشاء الداخلية ودم القلب وجود ميكروب القولون وبالفحص السيرولوجي للعترات المعزولة وجدت الانواع ٠٢ ، ٠٤ .

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**STUDIES ON AN OUTBREAK OF COLIBACILLOSIS AMONG HOLSTEIN
FRIESIAN CALVES IN THE NEW VALLEY, EGYPT
I. CLINICAL, HAEMATOLOGICAL, AETIOLOGICAL
AND PATHOLOGICAL ALTERATIONS**
(With 3 Tables & 6 Figs.)

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(Received at 9/4/1983)

SUMMARY

This investigation comprised two classes of calves showing neonatal diarrhoea, severely dehydrated calves (7 animals), and moderately dehydrated calves (9 animals). Seven apparently healthy calves served as control throughout the period of investigation. This investigation was carried out at El-Kharga governmental farm station, New Valley. The clinical signs recorded were of the typical profuse watery diarrhoea, characteristic for coli infection. Necropsy and microscopic findings for the dead calves were typical colisepticaemia infection.

Oligocythaemia was evident in some affected calves with a comparable decrease in haemoglobin concentration. Obvious leucocytosis with marked neutrophilia (segmented cells were remarkably increased) and marked lymphopaenia in both groups specially the severely dehydrated ones was also observed. No demonstrable changes for the heamato-crit values in the moderately dehydrated calves, while distinct rise was detected among the severely dehydrated conditions.

Bacteriological examination of rectal swabs and swabs from the internal organs and heart blood of the necropsied calves showed the presence of *E.coli* micro-organisms. Polyvalent 03 and polyvalent 04 were found upon serotyping.

INTRODUCTION

Diarrhoea in young calves has been recognised as a serious condition leading to severe losses for more than 2000 years (WATT, 1965). Of noteworthy to mention that the incidence of diarrhoea appears to be increasing (OTTSEN, 1959), possibly the increase due to the increasing concentration of livestock.

With the discovery of bacteria and in particularly the activity of coliformorganisms in calves affected with diarrhosa, successive generations of veterinarians and associated workers have gathered and impressive indictment against bacteria in general and coliform in particular (WATT, 1965). RADOSTITS (1965) stated that bacteria belonging to family Enterobacteriaceae of which *E.coli* is the most important factor in the pathogenesis of diarrhoea in calves under 10 days of age.

Clinical symptoms that were prominent among calves with acute colibacillosis were studied by a lot of workers (SMITH, 1962; PENHALE, 1965; WATT, 1965 and MOTTELIB 1972).

Of special interest to report here that variable levels in the haematological picture were detected in buffalo calves affected with diarrhoea due to many different causative agents in the studies of MOTTELIB (1972) and COLES (1975).

Haematocrit values in healthy calves, diarrhoeic calves surviving, and diarrhoeic dying calves were studied by FISHER and MARTINEZ (1975) proved to be a reliable guide in the prognosis of calf diarrhoea.

The purpose of the present study is twofolds; firstly to observe and record the clinical, haematological findings in a group of calves suffering an acute spontaneous diarrhoea with rise in their mortality; secondly to find out the real causative agent (s) responsible for such outbreak and register the most prominent pathological alterations characteristic for such causative agent (s).

MATERIAL and METHODS

The investigation was conducted on a herd of Holstein friesian homebred new-born calves kept under a wide range of agricultural conditions belonging to a governmental farm at the New Valley Governorate. Their age ranged from birth up to twelve weeks. A previous history of suffering a pneumoenteritis syndrome in the farm had occurred in 1960. In 1981 again the farm suffered an outbreak of neonatal diarrhoea in calves from 4 to 10 weeks with a mortality rate of 10 to 15%. In 1982. The veterinarian supervising this farm complain that 19 new born calves, 8-12 days old suffered a severe watery, yellowish-green profuse diarrhoea which in some cases lead to death. Morbidity among the new born calves all over the farm became much greater than 50% of the total 135 animals. Twenty-three calves for the present study after notification were selected according to the state of dehydration they suffered. The first group included seven calves with serious clinical signs of the severe dehydration, while the second group included nine moderately dehydrated animals. The rest, being seven apparently healthy animals and served as control.

Blood Samples:

An anticoagulated blood samples were withdrawn from the jugular vein for the haematologic study. Total erythrocytes (Terra/L.), and total leucocytes (Giga/L.) were estimated using improved Neu Bauer Haemocytometer, while for haemoglobin concentration (Hb) determination, test kit (Biomerieux, France) was used. Haematocrit value was determined using microhaematocrit centrifuge. Blood films were prepared by the Two-slides method soon after collecting the blood, stained with Giemsa stain and examined microscopically for differential leucocytic count Bacteriological Examination.

A bacteriological examination including an antibiotic sensitivity test was performed on the rectal swabs and on postmortem material (liver, heart, lung, kidneys, intestinal tracts, and mesenteric lymph nodes).

Postmortem Examinations:

Was carried out and samples of the liver, lung, kidneys, heart, spleen, stomach, intestine, and mesenteric lymph nodes were fixed in 10% neutral buffered formalin solution. The material were embedded in paraffin. Sections 5-7 μ , were stained by Harris haematoxylin and eosin and subjected to microscopical study.

RESULTS

Clinical Symptoms:

The physical condition of the two classes of calves, showing diarrhoea (severe and moderate) were registered and recorded. The first group (severely dehydrated condition) showed the typical signs of the severe dehydration viz., lassitude, sunken eyes appearance in the orbits, turgor of the skin, calves were so weak and lethargic, their rectal temperatures were almost normal or subnormal. Heart rate varied from 90 to 120/minute, pale, dirty mucous membranes. Affected animals could not rise to their feet, and were in fact in a recumbent position, despite obvious discomfort. If offered milk it may drink few mouth falls, then apparently lose interest in its surroundings. If it is stimulated it responds in a drugged disinterested manner. Finally the animal became completely comatose with shallow rapid respirations and three of them died before the commencement of treatment. While for the class II (moderately dehydrated), the most prominent clinical signs they exhibited were some degrees of inanition, diminished food intake and high faecal water content of badly offensive odour, that wet the perineum and hind legs. The physical condition was worse and the animal was weak and inco-ordinate.

Haematological Findings:

Fluctuating levels for the total erythrocytes in both groups (Tables 1,2 & 3) were detected that lies within the physiological limits, while a characteristic and marked increase in the number of leucocytes for the first group (14.00 ± 2.44 G/L.) with accompanied neutrophilia (5952 ± 7.07 G/L.). Marked decrease than normal in the number of lymphocytes (65.00 ± 3.26 37.5 ± 5.44 G/L.) respectively while fluctuating levels were detected for basophils and eosinophils (Tables 1,2 & 3). Of great interest of record here that an obvious rise in the haematocrit value of the group of calves suffering severe diarrhoeal infections ($40.00 \pm 1.63\%$) while for control ones ($27.14 \pm 1.86\%$) tables (1,2, & 3), while for the second class of calves, variable levels of haematocrit value were observed and lies within the normal physiological limits.

Bacteriological Findings:

Bacteriological examination conducted on rectal swabs from the affected calves and swabs taken from the different internal organs including heart blood of the necropsied calves showed the presence of large numbers of recognized pathogenic strains of *E.coli*. Upon serotyping, polyvalent O3 and polyvalent O4 were found. Sensitivity tests indicated that the isolated pathogenic strains of *E.coli* were sensitive to chloramphenicol.

Macroscopic Examinations:

The lungs were voluminous and firm. Large, red patches of congestion were distributed all over the lung tissue. Areas of consolidation were observed in the apical and cardiac lobes. On section, the bronchial tree showed thick mucous exudate in some areas. The liver were swollen, soft, dark red in colour, and the cut surface showed pale irregular areas of different sizes. On cut section various amounts of blood oozed out. Examination of the heart revealed that, the pericardial sac was mostly distended with thin transudate. The myocardium was flabby and in one case opaque, gray coloured area could be observed. The kidneys were enlarged and soft in consistency. Their capsules striped off easily. On sectioning, the corticomedullary junctions were congested. The abomasum showed congested mucosa. The intestinal content was yellowish-green in colour and watery in consistency. The mucosa was covered with moderate amount of mucous and appeared congested. The mesenteric lymph nodes were congested and oedematous. On the surface of the spleen petichaeal haemorrhages were observed in one case.

Microscopic Findings:

In the lung sections, the interstitial tissue as well as the interalveolar septa were thickened by congested vessels, oedema and some mononuclear cell infiltrations. The bronchioles showed degenerative and desquamated changes in their epithelial lining. The pulmonary vessels showed prominent intimal and medial degenerative changes. Areas of compensatory emphysema and others of atelectasis could be observed (Fig.).

In the liver (Fig.) severe necrobiotic changes were seen in the hepatocytes. Areas of coagulative necrosis in, which massive number of bacilli could be observed. Disse spaces showed cedema and reticuloend-othelial cells showed slight activation. Bile canaliculi in some areas were obstructed with bile cylinders. Oedema of the portal triads was a constant feature.

On microscopic examination the heart revealed diffuse myocardiosis, focal myocardial necrosis and intermyocardial haemorrhages. Coronary vessels showed degenerative changes. In areas showing necrobiotic changes the bacilli could be observed. In one case aged infact was observed (Fig.

The epithelium of the urinephrous tubules (Fig.) specially the cells lining the convoluted tubules and ascending limb of Henle's loop showed necrobiotic changes. Some glomeruli were swollen and the renal vessels showed intimal degenerative changes.

The abomasum showed catterhal gastritis associated with degenerative changes in the glandular epithelium and mononuclear leucocytic cellular infiltrations.

The micromorphological alterations noticed in the intestinal tract were restricted to the small intestine and varied from cattarhal to mucopurulent enteritis.

The mesenteric lymph nodes showed congestion, neutrophilic cellular infiltration.

On microscopic examination of the spleen, congestion, slight degree of lymphoid exhaustion as well as vascular degenerative changes were observed.

DISCUSSION

In the present study bacteriological examination of all the infected calves of both groups showed the presence of *E.coli* strains upon rectal swabs and the swabs taken from the different internal organs as well as heart blood of the necropsied calves (three). These results are in consistent with those published by EDGSON (1964), LOOSEMORE (1964), GAY (1965), RADOSTITS (1965), MOTTELIB (1972) and BOYD, BAKAR, and LEYLAND (1975) that *E.coli* considered to be the most pathogenic microorganisms responsible for diarrhoeal outbreak in calves under 10 days. SMITH (1962) and PENHALE (1965) were of the opinion that deficiency of gamma globulins play a key role in the predisposition of young calves to enteric infection and septicaemia by coliform organisms. Furthermore, TENNANT (1971) stated that the pathogenesis of this group of diseases is complex and may involve environmental and nutritional factors acting together with infectious agents.

In the view of the blood changes in neonatal diarrhoea (Tables 2 & 3), it was found that marked leucocytosis was detected in both classes of calves showing both severe and moderate dehydration. This was accompanied by a marked increase in the number of segmented neutrophils and an obvious decrease in the number of lymphocytes. These results are in close agreement with those reported by MOTTELIB (1972) and BOYED *et al.* (1974). The latter author gave the values of 12.56 ± 5.60 and 20.18 ± 8.94 G/L. for total leucocytes in both moderate and severe affected

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calves respectively. MOTTELIB (1972) stated that the degree of leucocytosis met with in different individuals was influenced by the severity of infection or inflammation, the virulence of the infectious agent and the susceptibility of the calf to the infection. The prominent lymphopaenia observed in our study were attributed in the view of COLES (1980) to the increased activity of the adrenal glands when the tissues are invaded by these pathogenic microorganisms. Varying degrees of polycythaemia was evident (Table 2 & 3) that correlated closely with the slight increase in their haemoglobin concentration. These findings are closely agreed with those reported by MOTTELIB (1972) who explained such changes to the marked haemoconcentration due to the severe loss of fluids in faeces. On the contrary, MOTTELIB (1972) recorded also some cases of oligocythaemia with a correlated values in their haemoglobin and attributed such phenomena to aplasia of the bone marrow, increased destruction of blood cells and failure of their formation.

Tables 2 & 3 showed no demonstrable changes in the haematocrit values for the moderately dehydrated calves, while a marked rise in these values was observed for the severely dehydrated calves. The former findings are in consistent with those of DALTON *et al.* (1965), while the latter observations were in agreement with those of TENNANT *et al.* (1972), DOYD *et al.* (1974) and FISHER and MARTINEZ (1975). These reports showed that the consistent observed features of severe illness due to neonatal diarrhoea were increased packed cell volumes indicating dehydration and hypovolaemia attributing the moribund clinical signs in particularly the obvious tachycardia and slow jugular vein filling.

Because the bacteria could be observed in liver and heart tissue sections, that may indicate bacteremia, our cases could be considered as colisepticaemic and not isocolibacillosis.

Vascular changes, manifested in intimal and medial degenerative changes as well as adventitial oedema could be attributed to the thrombo embolic effect of *E.coli* infection, (OSBORNE, 1967). In spite of aged myocardial infarct was observed in one case, vascular degenerative changes in coronary ramifications were observed in the three cases. Coronary thrombosis could not be observed in any of the cases.

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Table 1 - Clinical and Hematological Findings of Apparently Healthy New Born Calves.

Animal No.	Rectal Temperature (°C)	Heart Rate (Per min.)	Respiratory Rate (Per min.)	Hemoglobin Concentration (g/L)
1	37.3	59.0	28	118.40
2	37.5	68.0	26	109.10
3	37.0	66.0	26	110.70
4	37.4	58.0	26	98.55
5	37.6	68.0	12	115.76
6	37.8	72	30	118.80
7	37.0	64	24	110.10

Mean
± SD

37.37 ± 0.20 63 ± 5.0 26.05 ± 3.2

110.37 ± 0.82

8.640 ± 0.49

30.57 ± 5.53

1.14 ± 0.69

65.00 ± 3.26

27.18 ± 1.86

Table 2 - Clinical and Hematological Findings in neonatal calves with Acute Infective Infections (Severely Dehydrated Conditions).

Animal No.	Rectal Temperature (°C)	Heart Rate (Per min.)	Respiratory Rate (Per min.)	Hemoglobin Concentration (g/L)
1	36.8	120.	38	124.62
2	39.4	120	16	136.40
3	40.3	93	36	128.32
4	36.8	68	40	158.46*
Mean ± SD	38.17 ± 1.92	107 ± 15.3	37.5 ± 1.91	146.95 ± 12.22

110.37 ± 0.82

8.640 ± 0.49

30.57 ± 5.53

1.14 ± 0.69

65.00 ± 3.26

27.18 ± 1.86

Table 3 - Clinical and Hematological Findings in neonatal calves with Acute Infective Infections (Moderately Dehydrated Conditions).

Animal No.	Rectal Temperature (°C)	Heart Rate (Per min.)	Respiratory Rate (Per min.)	Hemoglobin Concentration (g/L)
1	39.5	76	30	128.46
2	39.5	64	28	98.08
3	40.4	84	30	76.92
4	40.2	64	32	136.40
5	38.5	80	35	108.20
6	39.6	88	20	125.44
7	38.5	100	34	124.40
8	38.5	96	30	140.10
9	39.8	80	28	98.48
Mean ± SD	39.8 ± 0.73	81.33 ± 12.48	30.33 ± 2.91	113.46 ± 20.77

110.37 ± 0.82

8.640 ± 0.49

30.57 ± 5.53

1.14 ± 0.69

65.00 ± 3.26

27.18 ± 1.86

Hemoglobin %

(%)

26

28

28

25

30

28

25

27

34.55

7.34

Hemoglobin %

(%)

40

38

42

31

37.5

5.44

59.24

14.00

16.200

16.40

16.200

14.00

11.00

8.500

3.750

11.00

6.750

8.500

11.00

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