

CLINICO – DIAGNOSTIC STUDIES ON BACTERIAL CAUSES OF DIARRHEA IN LAMBS AT ISMAILIA GOVERNORATE

By

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ABSTRACT

A total number of (57) lambs from one week to 2 months old was used in this study. (45) lambs showed symptoms of diarrhoea, inappetence, loss of body weight, weakness and dehydration while the remaining (12) lambs were apparently healthy. The animals were belonged to private farms in west kantara – Ismailia Governorate.

This work aimed to study some etiological agents of diarrhoea, haematological and biochemical changes associating it in newly born lambs. The estimated blood parameters include Hb, R.B.Cs, W.B.Cs, P.C.V% , MCV, M.C.H. and M.C.H.C. Moreover, serum electrolytes, calcium, inorganic phosphorus, magnesium, glucose and total protein, in addition to the electrophoretic pattern of blood serum protein was studied.

The results of presented study revealed that, E.coli, Klebsiella spp. and Proteus spp. were the most important bacterial isolates from diarrhoeic lambs and represented by (46.67%), (15.56%) and (13.33%), respectively.

Haematological investigations of blood samples of diarrhoeic lambs revealed a significant decrease in M.C.H.C. while total leukocytic count , RBCs count and M.C.V. were significantly increased. The biochemical studies revealed a significant decrease in the blood serum levels of sodium, chloride, calcium, inorganic phosphorus, magnesium, glucose and total protein while total globulins, Alpha and Gamma-globulins revealed a significant increase in diarrhoeic lambs.

INTRODUCTION

Neonatal diarrhoea is an important cause of death and considered to be one of the main hazards to lambs health (**Mottelib et al., 1992 and Sharif et al., 2005**). The immune system of animals at young age is not well developed and the maternal immunity would not withstand variable infection (**Holland, 1990, Mahmoud, 1993 and Schoenian, 2007**). Diarrhoea is still most common and costly disease affecting neonatal ruminant (**Pugh, 2002 and Andres et al., 2009**) Diarrhoea can be attributed to infection with a single agent (in very young or stressed animals) or more commonly to multiple agents. Its severity depends partially on non – infective contributing factors and on the nature of involved organisms (**Elfaki, 2000 and Schoenian, 2007**). Several bacterial species may be involved in diarrhoea and losses of neonatal lambs. The most important being is certain strains of *E.coli* that possessing virulent factors and also other members of enterobacteriaceae (**Sharif et al., 2005 and Wani et al., 2008**).

These pathogens are responsible for great mortality and various morbidity changes and at the same time contribute a hazard to public health (**Orden et al., 2000 and Sharif et al., 2005**). Diarrhoea is a symptom caused by different agents of different natures with high incidence of mixed infection. The proposed causes of diarrhoea in lambs are many and including bacteria, viruses, protozoa, environmental factors such as overfeeding, artificial feeding, over population, cold, temperature, bad hygiene and colostrum deprivation (**El-Sangary et al., 2004 and Schoenian, 2007**). Diarrhoea in lambs is caused by a variety of aetiological agents including *Escherichia coli*, *Salmonella* spp. and *Campylobacter* spp. (**Wani et al., 2008 and Andres et al., 2009**).

Haematological alterations were recorded in newly born lambs suffering from diarrhoea. Moreover, a significant changes in serum electrolytes, macro-and micro elements, some enzymes and electrophoretic pattern of blood serum protein were also recorded (**Nassif et al., 2002 and El Sangery et al., 2004**).

Several outbreaks and sporadic cases of diarrhoea occurred in neonatal lambs at Ismailia Governorate, therefore, the goal of this study was aimed to throw some light on the probable causes of diarrhoea among lambs, studying the haematological and biochemical alterations and the changes in electrophoretic pattern of blood serum protein accompanying diarrhoea.

MATERIALS AND METHODS

1- Animals (Lambs) :

Fourty five (45) Egyptian sheep lambs of both sexes from one week and up to 2 months old suffering from diarrhoea were used in this study. Twelve (12) lambs were apparently clinically healthy and served as a control. These lambs were belonged to private farms at West Kantara city and its surroundings, Ismailia Governorate. The diseased lambs under investigation were clinically suffered from diarrhoea showing soft and fluidy faeces which may contain mucous and blood and with or without foul smelling.

II. Samples and adapted methods :

Samples were taken from both healthy and diseased cases as follow :

1- Faecal Swabs :

Faecal swabs were taken aseptically from diarrhoeic lambs for bacteriological examination where isolation and identification of Enterobacteriaceae was carried out on the basis of **Koneman et al., (1994)** and **Quinn et al., (1994)**. It was streaked directly on three specific selected solid media (MacConkey agar, Brilliant green and S&S agar plates) and incubated overnight at 37 °C. At the same time, Salmonellae spp. were detected by culturing faecal sample on Selenite "F" broth and incubated at 37°C for 18 hours. Then subculture was done on MacConkey's agar, Brilliant green and S&S agar plates and incubated overnight at 37°C. Identification of different isolates mainly on the basis of morphology and biochemical reactions.

2- Blood Samples :

Two blood samples were collected from each animal through jugular vein puncture. Anticoagulant one for haematological examination and the other separated serum for biochemical assay according to **Jain (2000)**.

III- Diagnostic Procedures :

(a) **Clinical examination** : All animals under study were subjected to clinical examination.

(b) **Laboratory diagnosis** :

1- Haematological Procedures :

Determination of total erythrocytic count, haemoglobin concentration, packed cell volume and total leukocytic count were carried out according to **Jain (2000)**.

2- Biochemical Studies :

Serum samples were colorimetrically analysed using test kits (Bicon – Germany and SGM – Italian) for measuring glucose levels (**Siet et al., 1981**) total proteins (**Peters, 1968**). Protein electrophoresis was done using SDS-Polyacrylamide gel electrophoresis according to **Laemmli (1970)**, calcium (**Glinder and King, 1972**), magnesium (**Fragay, 1974**) and Inorganic phosphorus (**Daly, 1972**). While sodium, potassium and chloride were determined using flame photometer according to (**Oser, 1979**).

IV- Statistical analysis : The data were statistically analysed according to **Snedecor and Cochran (1982)**.

RESULTS

The results of bacteriological examination was illustrated in table (1). Haematological and biochemical alterations were demonstrated in tables (2 & 3). While the results of serum protein electrophoretic pattern were shown in table (4).

Table(1) Incidence and bacterial causes of diarrhoea in lambs

Etiological microorganism	Diarrhoeic lambs		Apparently healthy		Total	
	Number	Percent %	Number	Percent %	Number	Percent %
- <i>Escherichia coli</i>	21	46.67	4	33.33	25	43.86
- <i>Klebsiella</i> spp.	7	15.56	3	25.0	10	17.54
- <i>Proteus</i> spp.	6	13.33	1	8.33	7	12.28
- <i>Enterobacter aerogens</i>	4	8.89	4	33.33	8	14.04
- <i>E.coli</i> + <i>E. aerogens</i>	2	4.44	0.0	0.0	2	3.51
- <i>E.coli</i> + <i>Klebsiella</i> spp.	2	4.44	0.0	0.0	2	3.51
<i>E. coli</i> + <i>Citrobacter</i> spp.	3	6.67	0.0	0.0	3	5.26
	45	78.95	12	21.05	57	

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Table (2) Mean values of Haemogram picture in clinically healthy and diarrhoeic lambs

Conditions Parameters		Healthy lambs (Control)	Diarrhoeic lambs
Hb	gm /dl	10.6 ± 1 .31	9.14 ± 0.37
R.B.Cs	10⁶ /µl	8.2 ± 0.4	9.6 ± 0.3 *
W.B.Cs	10³ /µl	9.7 ± 0.4	11.2 ± 0.1 *
P.C.V	%	31.6 ± 2.4	35.2 ± 1.7
M.C.V	F1	30.78 ± 2.3	38.15 ± 1.4 **
M.C.H	Pg	10.32 ± 2.4	9.93 ± 2.9
M.C.H.C.	gm /dl	33.53 ± 3.1	26.03 ± 1.3 *

* Significant at (P < 0.05).

** Highly significant at (P < 0.01).

Table (3) Mean values of blood serum parameters in clinically healthy and diarrhoeic lambs

Conditions Parameters		Apparently healthy lambs (Control)	Diarrhoeic lambs
Sodium	mmol/L	147.26 ± 1.38	129.23 ± 3.1 ***
Potassium	mmol /L	4.29 ± 0.18	4.58 ± 0.16
Chloride	mmol/L	110.0 ± 1.12	96.42 ± 1.05 ***
Calcium	mg /dl	10.21 ± 0.23	9.57 ± 0.30
Inorg. Phosphorus	mg /dl	6.59 ± 0.20	7.54 ± 0.62
Magnesium	mg /dl	2.34 ± 0.04	1.94 ± 0.02 **
Glucose	mg /dl	43.78 ± 3.30	36.78 ± 1.14 *
Total protein	gm /dl	6.64 ± 0.14	6.14 ± 0.12 *
Albumin	%	35.52 ± 0.77	33.11 ± 1.24
Globulin	%	64.48 ± 0.78	66.89 ± 1.24 *

* Significant at (P < 0.05)

** Highly significant at (P < 0.01)

*** Very highly significant at (P < 0.001)

Table (4) Mean Values of serum protein electrophoretic pattern in clinically healthy and diarrhoeic lambs

Parameters \ Conditions	Healthy lambs (Control)	Dierrhoeic lambs
Alpha – globulin %	21.61 ± 0.58	23.87 ± 0.47 **
Beta – globulin %	13.47 ± 1.04	11.98 ± 0.84
Gamma– globulin %	29.40 ± 0.95	31.04 ± 0.75 *

* Significant at (P < 0.05)

** Highly significant at (P < 0.01)

DISCUSSION

Diarrhoea is of common occurrence in animals of all ages in the flock. In lambes, it could be resulted in significant mortality while in older animals, growth rates are reduced and weight loss can occur (Naveed et al., 1999, Bastauerous et al., 2001 and Sharif et al., 2005).

The clinical signs observed on diarrheic lambs in this study were depression, weakness, decrease milk suckling, weight loss and intermittent diarrhoea. Moreover, various degrees of diarrhoea were noticed during clinical investigation of diarrhoeic lambs were profuse in some cases, watery and yellow in colour. In others, faeces were offensive, semifluid watery, yellowish, containing mucous and sometimes tinged with flood. Some cases of diarrhoeic lambs were died and represented by 6 lambs (13.33%) which were suffering from severe weakness, severe watery diarrhoea and were positive for both *Escherichia Coli* and *Kellsella* spp. infection.

Similar results were recorded by Elfaki, (2000) and Schoenian, (2007). This clinical findings may be attributed to fluid loss, electrolytes imbalance, malabsorption and protein losing enteropathy (Sharif et al., 2005).

Table (1) shows that the Enterobacteriaceae constituted as one of the main cause of diarrhoea in lambs. Difference in the recovery of these bacteria between diarrhoeic (78.95 %) and apparently healthy lambs (21.05%) was due to the enhanced growth of facultative pathogens in diarrhoeic lambs and their intermittent excretion in the faeces of apparently

healthy ones (El Ged et al., 1994, Elfaki, 2000, Bastauerous et al., 2001 and Andres et al., 2009).

Single isolated members of Enterobacteriaceae that recovered from both diarrhoeic and apparently healthy lambs were *E.coli*, *Klebsiella* spp. *Proteus* spp. and *Enterobacters aerogens* (Table,1). The association of these bacteria with lamb diarrhoea was previously reported by Ahmed (1983), Bastauerous et al., (2001) and Wani et al., (2008). In addition, the mixed isolates were recorded only from diarrhoeic lambs while the mixed infections were absent in all faecal samples of examined apparently healthy lambs.

It is clearly seen that *E.coli* was isolated in pure culture from 32 (56.14%) faecal samples, either singly 25 (43.86%) or mixed with *Enterobacter aerogenes*, *Klebsiella* spp. and *Citobacter* spp. at an incidence of (3.51%) , (3.51%) and (5.26%), respectively (Table,1).

The recorded results in this study were somewhat high as compared with that reported by Fegan and Desmarchelier, (1999) who recorded incidence of 36% of 72 lamb faecal samples from abattoir yards while Blanco et al., (1996) reported higher incidence (100%) of *E.coli* isolated from 144 diarrhoeic lambs (5 to 21 days old). However Wani et al., (2008) isolate Enteropathogenic *E.coli* from 6.1% of 230 lambs with diarrhoea. The disease syndromes associated with *E.coli* are usually referred to as colibacillosis and include enteric colibacillosis which includes diarrhoea and toxemia and systemic colibacillosis which is caused by invasive strains (Wray et al., 1993, Elfaki, 2000, and Schoenian, 2007). Generally, in *E.coli* infections, diarrhoea occurs through the effect of enterotoxins which stimulate granulate cyclase activity of the ileal epithelium [(heat stable toxin, (ST)] or adenylate cyclase activity of intestinal and capillary epithelium [(heat labile toxin, (LT)] resulting in hypersecretion of electrolytes particularly Na^+ and HCO_3^- and an increased diffusion of water into lumen of the intestine which resulted in acidosis and dehydration (Kaske, 1993, Elfaki, 2000 and Wani et al., 2008).

The haematological findings in all diarrhoeic lambs showed a significant reduction in M.C.H.C. and significant increase in R.B.Cs counts, total leukocytic count and M.C.V. (Table,2) This result may be attributed to the occurrence of electrolytes imbalance and hemoconcentration arising from diarrhoea while the increase in W.B.Cs may reflect the condition of bacterial enteritis which may be primary or secondary to parasitic infestation (El-Sangary et al., 2004 and Schoenian, 2007).

Serum electrolytes showed significant decrease in serum sodium and chloride levels of all diarrhoeic lambs. While there is no significant variance in the serum potassium level (Table,3)

Similar results were recorded by **Massip, (1979)** and **Radostits et al., (2000)** who reported that sodium and chloride are particularly exposed to loss in diarrhoeic stools as they are components of the gastrointestinal secretions. Concerning serum calcium, inorganic phosphorus and magnesium levels, the obtained results showed a significant decrease in serum magnesium level of all diarrhoeic lambs.

This can attributed to the presence of single or double bacterial agents and their effect on general health condition of lambs. This results were in aggrement with the findings obtained by **Ramadan et al., (1985)** and **El-Sangary et al., (2004)** who recorded a significant hyponatremia, hypochloremia, hypomagnesemia and hyperkalemia in diarrhoeic calves and kids respectively. **Coles (1986)** and **Duncan et al., (1994)** attributed the decrease in serum calcium level to hypoalbuminemia where decreased albumin concentration lowers the total calcium level, while both ionized and complex calcium levels remain normal.

There is a significant decrease in serum glucose level of all diarrhoeic lambs. This finding agrees with the results previously reported by **(Lewis et al., 1975, Coles, 1986 and El-Sangary et al., 2004)** who postulated other factors causing hypoglycemia as anorexia, decreased intestinal glucose absorption, a low level of glucose reserves in young age and alterations in tissue metabolism caused by decreased blood flow and oxygenation associated with the hypovolemic shock which present in the hypoglycemic diarrhoeic animals.

The electrophoretic pattern of serum protein of diarrhoeic lambs (Tables,3,4) showed a significant decrease in total albumin, and a significant increase in total alpha and gamma globulins of all diarrhoeic lambs. However, total Beta-globulin showed a significant decrease in bacterial infection,

In conclusion, it is obvious that diarrhoea as a clinical sign of major importance, Enterobacteriaceae especially E.coli infection must be considered in diarrhoeic lambs where they cause haematological and biochemical alterations, so, attention to the management factors and the nutrition of the pregnant ewes to ensure adequate colostral production and to the care of young lambs to ensure adequate colostral intake will help in the minimization of these infections. Moreover, proper planning, an adequate knowledge of the background of disease and the application of appropriate preventive programmes at right time, occurrence of diarrhoea in the flock can be virtually eliminated.

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الملخص العربي

دراسات تشخيصية إكلينيكية على المسببات
البكتيرية

للإسهال في الحملان بمحافظة الإسماعيلية

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شملت الدراسة على عدد (٥٧) حالة من صغار الحملان تراوحت أعمارها من أسبوع إلى شهرين منها عدد (٤٥) حالة كانت تعاني من الإسهال وفقدان للشهية ونقصان في وزن الجسم وضعف عام وجفاف بينما عدد (١٢) حالة كانت سليمة ظاهريا هذه الحملان كانت تتبع مزارع خاصة بمدينة القنطرة غرب وضواحيها – محافظة الإسماعيلية – وكان الهدف من هذه الدراسة هو معرفة الأسباب البكتيرية لحالات الإسهال لهذه الحملان والتغيرات الدموية والبيوكيميائية المصاحبة لها – حيث كانت نسبة الإصابة الأعلى بالميكروب القولون الممرض هي (٤٦,٦٧%) يليها الإصابة بميكروب الكليبسيلا بنسبة (١٥,٥٦%) ثم ميكروب البروتيس الذي يمثل (١٣,٣٣%) في الحملان المصابة بالإسهال وقد تمت دراسة التغيرات الدموية والبيوكيميائية المصاحبة لحالات الإسهال ومقارنتها بالحالات السليمة ظاهريا – وقد أشرنا إلى ضرورة إرضاع الصغار لبن السرسوب لرفع المناعة وذلك لمقاومة هذه الأمراض البكتيرية وإتباع طرق التغذية السليمة للحملان.