

Animal Health Research Institute.  
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**STUDIES ON DIARRHOEA IN LAMBS  
IN ASSIUT GOVERNORATE  
I- ISOLATION AND IDENTIFICATION  
OF CAUSATIVE BACTERIAL  
AND PARASITIC AGENTS  
(With 5 Tables)**

By

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دراسات عن الإسهال في الحملان بمحافظة أسيوط  
١- عزل وتعريف البكتريا والطفيليات المسببة للإسهال

الفونس فخرى بطساوروس ، عبدالراضى ثابت ، محمد محمد عبدالحفيظ ،  
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شملت الدراسة عدد ٧٩ حالة من صغار الحملان تراوحت أعمارها من أقل من أسبوع إلى ستة أشهر منها ٤٩ حالة تعاني من الإسهال الحاد، ٣٠ حالة سليمة ظاهريا من المزارع الحكومية في محافظة أسيوط. أظهر الفحص الإكلينيكي وجود درجات متفاوتة من الإسهال بينما أظهر الفحص البكتريولوجي ميكروب القولون الممرض وغير الممرض في الحالات المرضية والسليمة ظاهريا أما منفردا (٥٣،٤٥%)، (٤٤،٤٤%) على التوالي أو مشتركا مع ميكروب الانتروباكتريز (٥،١٧%) والستروباكتريز فرينداي (٥،١٧%) والكليبيلا أوكسي توكا (١،٧٢%) من الحالات المرضية فقط. وقد تم تصنيف باقي الميكروبات المعزولة لعائلة الانتروباكتريسي حيث تم التعرف منفردا على ميكروب الكليبيلا نيموني، الكليبيلا أوكسي توكا، البروتيس ميرابلينز والانتروباكتريز أروجينسي بنسبة (١١،٧٦%)، (٣،٥٣%)، (١٢،٩٤%)، (١٢،٩٤%) على التوالي. كما أظهرت نتائج الفحص الطفيلي تواجد الإصابات الطفيلية بنسبة ٦٦،٢% وكانت نسبة الإصابة بالكوكسيديا ١٠٠% في حالات الإسهال الحاد، ٨٦،٧% في حالات الحملان السليمة ظاهريا وكانت نسبة الإصابة بالديدان ١٢،٢٥% في حالات الإسهال الحاد. وتم دراسة الأهمية الاقتصادية والصحية لهذه المشكلة وكذلك اقتراح السبل الكفيلة لحل هذه المشاكل.

## SUMMARY

Faecal samples from 79 newly borne lambs including 49 severely diarrhoeic ones and 30 apparently healthy cases at age less than one week "5 days" to six months old collected from governmental farm in Assiut Governorate, were examined for enteropathogens (bacteriological examination) and parasitic infections. Enteropathogenic *E.coli* and untypable ones were isolated from faeces severely diarrhoeic and apparently healthy lambs either singly (53.45%), (44.44%) respectively or mixed with *Enterobacter aerogenes* (5.17%), *Citrobacter freundii* (5.17%) and *klebsiella oxytoca* (1.72%) from severely diarrhoeic cases only. Single isolated member of Enterobacteriaceae were *E.coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* *Proteus mirabilis* and *Enterobacter aerogenes* at incidence of (50.59%), (11.76%), (3.53%), 12.94% and 12.94%, respectively. On the other hand, parasitological examination of the same samples revealed that the parasitic infection was 96.2%. Coccidial infection was 100% and 86.7% in faecal samples collected from severely diarrhoeic and apparently healthy lambs, while helminths infection was 12.25% in case of faecal samples collected from severely diarrhoeic lambs. The economic and public health importance of this problem has been discussed and suggested measures for solving this problem are given.

**Key words:** Lambs, Diarrhoea, Isolation, Causative agents.

## INTRODUCTION

Diarrhoea is a clinical entity which causes serious economic losses as it may lead to lambs mortality, weight loss or even late growth. It is caused by a combination of many risk factors.

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 (Tzipori, 1981). 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*. These pathogens are responsible for great mortality and various morbidity changes and at the same time constitute a hazard to public health (Orden *et al.*, 2000).

Parasitic gastroenteritis is primarily a disease of lambs and occasionally older sheep. It includes coccidiosis and gastrointestinal helminths. The major of outbreaks of coccidiosis occur in lambs 1-4 months old. Although ewes have a natural immunity, they act as a source of infection by contamination of bedding with coccidial oocysts (Eddie, 1992). Gastrointestinal helminths are major contributors to reduced productivity and lower the production of mutton, milk and wool. Also they can cause outbreaks of diarrhoea when considerable number of larvae on pasture coincide with the presence of susceptible lambs (Martin and Aitke, 2000). In Egypt parasitic gastro-enteritis of sheep was studied by several authors (Ahmed, 1983; El-Akbawy, 1987 and Mostafa, 1990 and others).

Several outbreaks and sporadic cases of diarrhoea occurred in neonatal lambs in Assiut Governorate. Therefore, the present work was aimed to study the role of *Enterobacteriaceae* and the parasitic causes of gastroenteritis as a causative agents of diarrhoea in lambs.

### MATERIAL and METHODS

#### Samples:

Faecal samples were collected from diarrhoeic (49) and apparently healthy (30) lambs. The age of the examined lambs ranged from less than one week (5 days) up to six months. Each sample was divided into two portions, the first portion was examined bacteriologically for *Enterobacteriaceae* 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 over night at 37°C. Identification of different isolates mainly on the basis of morphology and biochemical reactions. Serological identification of the isolates, that produced biochemical reaction simulating *E. coli*, was carried out after their purification by determination of the group antigens using slide agglutination test, against the *E. coli* antisera obtained commercially from AG, Marburg, Germany and following the instruction of the manufactures. On the other hand, other members of *Enterobacteriaceae* were identified only by biochemical tests as their respective immune sera were not available.

The second portion of each faecal sample was used for detection of the parasitic infestation through:

- a) Macroscopic examination: Each sample was examined by naked eye for: consistency, presence of blood, mucous, whole, segments or parts of worms.
- b) Microscopic examination:
  - 1) Direct faecal smear (Soulsby, 1982).
  - 2) Concentration flotation technique with concentrated salt solution (Levine, 1985).
  - 3) The identification of helminths eggs was based on the description given by Soulsby (1982), and culture of these eggs previously done by Monib and Arafa (2000), *Eimeria* oocysts were identified according to Levine (1985).

## RESULTS

Results are illustrated in Tables 1, 2, 3, 4 & 5.

## DISCUSSION

Diarrhoea is of common occurrence in animals of all ages in the flock. In lambs it can result in significant mortality while in older animals growth rates are reduced and weight loss can occur. 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 sometime tinged with blood "it does become chocolate - coloured due to haemorrhage". Sometimes faeces were profuse clay to grayish mucoid and contained blood streaks.

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 (68.24%) and apparently healthy lambs (31.76%) 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).

Single isolated members of *Enterobacteriaceae* that recovered from both diarrhoeic and apparently healthy lambs were *E.coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Enterobacter aerogenes* while. *Klebsiella oxytoca* was isolated only from diarrhoeic lambs (table 1). The association of these bacteria with lambs diarrhoea was

previously reported by Hassanin (1979), Richard et al. (1980) and Ahmed (1983). In addition to that, the mixed isolates were recorded only from diarrhoeic ones. As clear in table (1) the mixed infection were absent in all faecal samples of examined apparently healthy lambs. Meanwhile *Enterobacter aerogenes*, *Citrobacter freundii* and *Klebsiella pneumoniae* mixed with *E.coli* were recovered from 3 (5.17%), 3 (5.17%) and 1 (1.72%) respectively.

It is clearly seen that *E.coli* was isolated in pure culture from 50 faecal samples, either singly 43(50.59%) or mixed with *Enterobacter aerogenes*, *Citrobacter freundii* and *Klebsiella oxytoca* at an incidence of (3.53%), (3.53%) and (1.18%) respectively (Table 1).

Recorded results here somewhat were 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). On the other hand, recorded data, nearly simulates those reported by Hassanin (1979) who isolated O<sub>125</sub>: K<sub>70</sub> of *E.coli* which associated with 52.6% of bacteria cause enteritis in local breads lambs.

Isolated *E.coli* strains were identified serologically as 18.42% *E.coli* O<sub>88</sub>: B<sub>7</sub>, 13.16% *E.coli* O<sub>11</sub>: B<sub>4</sub>, 2.63% *E.coli* O<sub>44</sub>: K<sub>74</sub> "L" and 65.79% untypable strains (Table, 2). The association of these serotypes with lambs diarrhoea were reported by Akmedova and Agdami (1963), Karmy and Ragab (1983) and Blanco et al. (1996).

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). Generally in *E.coli* infections, diarrhoea occurs through the effect of enterotoxins which simulate guanylate cyclase activity of the ileal epithelium [heat stable toxin (ST)] or adenylate cyclase activity of intestinal and capillary epithelium [heat labial toxins (LT)] resulting in hypersecretion of electrolytes particularly Na<sup>+</sup> and HCO<sub>3</sub> and an increased diffusion of water into lumen of the intestine which resulted in acidosis and dehydration (Kaske, 1993).

Diarrhoea of neonatal lambs is often treated with antimicrobial drugs. However, antibiotic therapy is frequently ineffective, partly due to the presence of drug – resistant strains and the failure to identify drug sensitivity. This subject is discussed in details in part two.

This study cleared that gastro-intestinal parasites play a significant role as a causative agents of diarrhoea in lambs, where they detected in 96.2% of examined animals (Table, 3).

*Eimeria* species were detected in all animals (100%) suffering from severe diarrhoea and in 86.7% of apparently healthy animals. Nine species of *Eimeria* were detected in this present work and the highest of them were *E. crandalis* and *E. Parva*, where they represented (79.75%) , 78.5% respectively (Table 4). This high incidence of *Eimeria* species indicates it is widely spreading among sheep. This result coincided with Khalifa, et al. (1986) but it more higher than that recorded by Ahmed (1983) and Mostafa (1990) where they recorded it in (4.49 %) and 24.56% respectively. This difference might be attributed to the stress of the environmental conditions as well as overcrowded or bad hygienic conditions of the tested farm.

Gastro-intestinal helminthes (G.I.II.), were detected here in 37.97% of diarrhoeic lambs and apparently healthy ones. In severe diarrhoeic lambs G.I.H. were detected in 12.25% while in apparently healthy ones it was 80.0% (Table 3).

*Trichostrongylus* infection represented the most common species of the helminthes infection where it was 29.1% (Table 5). This is strongly supported by the previous work of Reid (1976), who recorded that the main cause of diarrhoeic syndrome especially at 4-10 months old was *Trichostrongylus* and sometimes given it the name "black scour". This result was also coincided with both El-Akbawy (1987), Mostafa (1990) and Mottelib et al. (1992).

In conclusion it is obvious that diarrhoea, although readily identifiable as a clinical sign of major importance, however, is unavoidable by many sheep farmers. This should not be so because with proper planning, an adequate knowledge of the background of disease and the application of appropriate preventative programmes at the right time, the occurrence of diarrhoea in the flock can be virtually eliminated.

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Table (1): Single and mixed isolates of *Enterobacteriaceae* isolated from diarrhoic and apparently healthy lambs.

<i>Enterobacteriaceae</i> Isolates	Diarrhoic Lambs		Apparently Healthy		Total	
	No.	%	No.	%	No.	%
<i>E. coli</i>	31	53.45	12	44.44	43	50.59
<i>Klebsiella pneumoniae</i>	5	8.62	5	18.52	10	11.76
<i>Klebsiella oxytoca</i>	3	5.17	-	00.0	3	3.53
<i>Proteus mirabilis</i>	7	12.07	4	14.81	11	12.94
<i>Enterobacter aerogenes</i>	5	8.62	6	22.22	11	12.94
<i>E. coli</i> + <i>E. aerogenes</i>	3	5.17	-	00.0	3	3.53
<i>E. coli</i> + <i>Citrobacter freundii</i>	3	5.17	-	00.0	3	3.53
<i>E. coli</i> + <i>K. oxytoca</i>	1	1.72	-	00/0	1	1.18
Total	58	68.24	27	31.76	85	

Table (2): Serological identification of isolated Enteropathogenic "*E.coli*" strains

Serotype	Condition of Lambs					
	Severe Diarrhoeic		Apparently Healthy			
	No.	%	No.	%	No.	%
<i>E.coli</i> O <sub>88</sub> : B <sub>7</sub>	7	18.42	-	-	-	0.00
<i>E.coli</i> O <sub>11</sub> : B <sub>4</sub>	5	13.16	-	-	-	0.00
<i>E.coli</i> O <sub>44</sub> : K <sub>74</sub> (L)	1	2.63	-	-	-	0.00
Untypable <i>E.coli</i>	25	65.79	12	100	12	100
Total	38		12		12	

Table (3): Incidence of parasitic infection in diarrhoeic lambs

	Examined Animal	Inf. Animals with parasites		Single Helminthe		Single Coccidia		Mixed infection		Total	
		No.	%	No.	%	No.	%	No.	%	No.	%
Severe diarrhoeic cases	49	49	100.0	-	-	43	87.75	6	12.2	49	100%
Apparently healthy cases	30	27	90.0	1	3.3	3	10.0	23	7.7	26	86.7
Total	79	76	96.2	1	1.3	46	58.2	29	36.7	75	94.9
										30	37.97

Table (4): Incidence of different Eimeria species in lambs.

Species of Eimeria	Severe diarrhoea		Mild diarrhoea		Total	
	No. of infected Animal	%	No. of infected Animal	%	No. of infected Animal	%
E. cranidialis	39	79.6	24	80.0	63	79.75
E. parva	36	73.47	26	86.7	62	78.5
E. fauri	28	57.14	8	26.7	36	45.6
E. pallida	28	57.14	6	20.0	34	43.04
E. ovina	27	55.10	13	43.3	40	50.6
E. abasta	24	48.97	5	16.7	29	36.1
E. intercala	9	18.37	3	10.0	12	15.2
E. ovinooidalis	8	16.33	4	13.3	12	15.2
E. granitosa	3	6.12	-	-	3	3.8

Table (5): Incidence of different Helminthes in lambs.

Species of Eimeria	Diarrhoea Lambs		Apparently Healthy		Total	
	No. of infected Animal	%	No. of infected Animal	%	No. of infected Animal	%
<b>Nematod:</b>						
Trichostrongylus	2	4.08	21	70.0	23	29.1
Oesophagostromum	2	4.08	14	46.7	16	20.25
Bonostomum	2	4.08	18	60.0	20	25.5
Trichuris	1	2.04	-	-	1	1.3
Ostertagia	2	4.08	9	30.0	11	13.9
Chabertia	1	2.04	13	43.3	14	17.7
Decyocatus	1	2.04	1	3.3	2	2.5
Haemonchus	-	-	1	3.3	1	1.3
Skrajabinema	-	-	1	3.3	1	1.3
<b>Cestode:</b>						
Moniezia	-	-	1	3.3	1	1.3