Animal Health Research Institute,
Damanhour Branch.

STUDIES ON SOME BACTERIAL AND PARASITIC CAUSES OF LAMB DIARRHEA IN BOHAIRA PROVIENCE AND THE SUBSEQUENT BIOCHEMICAL CHANGES

(With 6 Tables)

By

A.A. BKHEET; R.S. FADLY and HANAA R. ELHOFFY

(Received at 25/8/2010)

دراسات على بعض المسببات البكتيرية والطفيلية المصاحبة للاسهالات في الحملان بمحافظة البحيرة والتغيرات البيوكيميائية المصاحبة

احمد ابو المجد بخيت ، رضا سمير فضلى ، هناء رشاد الحوفى

شملت هذه الدراسة ٧٠ من الحملان تراوحت أعمارها من ثلاثة ايام حتى شهرين منها ٦٠ حاله تعانى من الاسهال و (١٠) سليمة استخدمت كمجموعة ضابطة ومثلت هذه الحملان لانماط التربية المختلفة – مزرعة كبيرة وتجمعات صغيرة أضافة الى حالات مملوكة لأفراد بمحافظة البحيرة وكان الهدف من هذه الدراسة استبيان بعض المسببات البكتيرية والطفيلية المسببة للإسهال وكذلك دراسة التغيرات البيوكميائية المصاحبة. أظهرت الفحوص البكتيريولوجية أن الميكروب القولونى والكلبسيلا والانتيروباكتر والستروباكتر والبروتيس والسريتيا والمورجانيلا مورجانى وجدت بنسبة ٤٣,٣ و ٢٨,٣ و ١٦,٦ و ١٥ و ١٣,٣ و ١٣,٣ و ٨,٣ % على التوالى ممثلة لمجموعة الانتيروباكترياسى وتم تصنيف عناصرها جميعا بيوكيمائيا. هذا اضافة للسودموناسى اروجينوزا والمكورات العنقودية الذهبية والمكورات السبحية الشرجية والمكورات المعوية البرازية بنسبة ٨,٣ و ١٣,٣ و ٣,٣ و ٦,٧ % على التوالى. أظهرت الفحوص الطفيلية أن ٨٣,٣ % من الحملات مصابة بطفيليات معوية منها(٤٢) ٧٠ % ايجابية لاكياس الايميريا باستخدام طريقة التعويم و(١١) ١٨,٣ % ايجابية لحويصلات الكريبتوسبوريديا وأوضحت الدراسة أن (٣٩) ٦٥ % مصابة بالكوكسيديا منفرده و (٨) ١٣,٣ % مصابة بالكريبتوسبوريدا منفصلة و (٣) ٣ % مصابة بكلا الطفيليين وجميعها مصاحبة للإصابة البكتيرية وبعمل مزرعة لعينات البراز الايجابية لاكياس الايميريا فى ٥و٢% بوتاسيوم داى كرومات تم تصنيف سبعة أنواع من الحويصلات البوغية لايميريا الأغنام وكانت الايميريا كرندالز الاكثر انتشارا. تم إجراء اختبار حساسية لبعض البكتريا الهامة والأكثر عزلا لاختيار أنسب المضادات الحيوية. تمت دراسة التغيرات البيوكيميائية المصاحبة لحالات الإسهال ومقارنة التعبيرات بين السليمة ظاهريا والمصابة ببكتريا منفرده أو كربتوسبوريديا وكوكسيديا منفردتين او مشتركة وتم دراسة الأهمية الاقتصادية والصحية للاسهالات والسبل الكفيلة لمنعها.

SUMMARY

A total of 70 lambs aged from three days to 2 months were used in this study, 60 lambs showed symptoms of diarrhea while the remaining (10) lambs were healthy and used as control. The animals were belonged to a variable breeding model large farm, small holder and individually owned in Bohaira province. This work aimed to study some etiological agents, bacterial and parasitic, and studying the subsequent affects on some biochemical parameters. The bacteriological results revealed that. *Ecoli, Klebsiella, Enterobacter Citrobacter, Proteus, Serratia* spp and *Morganella morganii* as members of *Enterobacteriaceae* were detected in incidences of 43.3, 16.6, 15, 13.3 13.3, 8.3 % respectively. *Pseudomonus aerogeneosa, Staph. aureus, Strept. ini* and *Enterococcus fecalis* were detected in an incidences of 8.3, 13.3, 3.3 and 6.7 % respectively, the incidences were varied in variable breeding size. The parasitic investigations revealed 83.3 % positive samples 70% for *Eimeria* spp. by flotation technique and 18.3 % for *Cryptosporidium* spp. by modified Zeiehl Nelson technique, 65 % of cases were infested with *Eimeria* spp. Oocysts alone, 13.3 % of cases revealed *Cryptosporidia parvum* separately and 5 % were harboring mixed parasites all were coincide with bacterial infection. Seven spp. of *Eimeria* were identified by fecal culturing. *E. crandalis* was the most common one. Sensitivity tests were carried for some of important and predominant isolates of bacteria. All members of the isolated *Enterobacteriaceae* strains were biochemicaly identified. The biochemical alterations accompanied with diarrhea including changes associated with bacterial, Coccidian, Cryptosposidia infections singly or mixed in relation to apparently healthy were done. The economic importance, health hazards and reduce incidences or combating the problem were discussed.

Key words: *Lambs, diarrhea, bacterial infection, parasitic infestation.*

INTRODUCTION

Neonatal diarrhea is an important cause of death and considered to be one of the main hazards to lambs health (Mottelib *et al.,* 1992; Sharif *et al.,* 2005).

Diarrhea is chemical entity which cause serious economic loss may lead to lamb mortality, weight loss or even lead to subsequent growth retardation (Bastarous *et al.*, 2001). Diarrhea could be attributed to infection with single or multiple agents. Its severity depends partially on

non infective contributing factors and on the nature of involved organisms. (Schoenian, 2007). Several bacterial species may be involved in diarrhea and losses of neonatal lambs. The most important being is *E coli.* and other members of *Enterobacteriacae* (Sharif *et al.,* 2005; Wani *et al.,* 2008) *Klebsiella* species are incriminated in lamb diseases (Munoz *et al.*, 1996). Different disease conditions were recorded in association with *Klebsiella* species from enteritis and pneumonia. (Mottelib *et al.,* 1992; Abou EL Hassan, 1996)

Pseudomonas aeruginosa produces a wide variety of virulence which many play a role in the pathogencity of this organism. This virulence could be attributed to several extra cellular and cell associated substances (Goto, 1996; Marty *et al.,* 1998).

Parasitic gastroenteritis is primarily a disease of lambs. The major of outbreaks of coccidiosis occur in lambs up to-4 months old. Although ewes have natural immunity, they act as a source of infection by contamination of bedding with coccidian oocysts (Foreyt, 1990; Qzdal *et al.,* 2009). Fifteen *Eimerea* spp. have the capacity of infesting sheep (Platzer *et al.,* 2005).

Fourteen spp. infest sheep intestine, only one *(E. gilruthi)* in abomasums. Clinical eimeriosis only occur in lowered resistance lambs or those subjected to heavy infections.

Cryptosporidiosis is one of the main causes of morbidity and mortality in young immune suppressed animal. Human being is considered as one of the major pathogens associated with diarrhea in ruminant (Hilali *et al.,* 1998).

Hematological and biochemical alterations were recorded in newly born lambs suffering from diarrhea, moreover, significant changes in serum electrolytes, macro and microelements, some enzymes of blood serum protein were also recorded (Nassif *et al.* 2002; El-Sangery *et al.*, 2004). The concentration of plasma protein reflects the health state because they are nutritive, carrier for the transport of components for most of the plasma constituents. (Jain, 1986). In addition they have an important function in body defenses (Kaneko, 1997)

Several outbreaks and sporadic cases of diarrhea occurred in neonatal lambs till weaning at Bohaira Province in variable breeding models, large farms, small holders causing economic losses. Therefore the goal of this study was aimed to throw some light on the variable bacterial

and parasitic probable causes of diarrhea among lambs, studying the biochemical alterations and the changes in electrolytic pattern and their subsequent effects on lamb finding. The sensitivity tests for the predominant and important bacterial isolates were done.

MATERIALS and METHOD

I- Animals: A total of sixty (60) Egyptian sheep lambs of both sexes from three days up to two months old suffering from diarrhea were included in the present study and ten (10) lambs were apparently clinically healthy were served as a control. These lambs were belonged to private farm and small holders'. The diseased lambs under investigation were clinically suffered from diarrhea showing soft to fluid feces which may contain mucus and / or blood with as without foul odor.

II - Sampling:

1- **Fecal swabs:** Fecal swaps were taken aseptically from diarrheic lambs and apparently healthy for bacterial isolation and identifications according to Knoneman *et al.* (1994); Quinn *et al.* (1994).

2- **Fecal samples:** Were taken directly from the rectum in a clean plastic bags from diarrheic and control (apparently health) lambs and examined microscopically

3- **Blood samples:** Were collected from Jugular vein without anticoagulant for serum separation, from diarrhoeal lambs (n = 60), as well as clinically healthy (n=10) which were used as a control group. The collected serum samples were stored at -20°C until biochemical analysis.

Bacteriological media:

1- Liquid media, Nutrient broth, Muller Hinton broth, Seleniet -F broth, Brain heart inf. broth (Oxoid, 1982)

2- Semisolid media (soft agar): Semisolid nutrient agar (Cruickshank *et al.*, 1975)

3- Solid media: Nutrient agar, Blood agar base, MacConky agar Eosin Ethylene Blue (EMB) agar Manitol salt agar, Salmonella Shegella agar, Muller Hinton agar (Oxoid, 1982)

4- Media used for bacteriological identifications Peptone water, M.R. and V.P broth Simons citrate agar, Christensen's urea agar base, Triple sugar Iron agar, Lysine iron Agar (Oxoid, 1982)

Methods

Bacteriological examinations, isolations and identifications were carried according to Cruickshank (1975); Krig and Halts (1984); Quinn *et al.* (2002).

Parasitological investigations:

1- *Concentration floatation technique:* Using saturated salt solution to detect Eimeria Spp. Oocysts and using Sheathers solution to demonstrate the presence of *Cryptosporidium spp Oocysts* according to Soulsby, (1982)

2- Fecal culture for positive Eimeria spp. Oocysts using (2.5%) pot. dichromate solution.

3- Thin fecal smear stained with modified Ziel Nilson technique to detect C*ryptosporidium parvuma* according to Henricksen and pohlenz (1981)

4- Identification of Oocysts (morphology and morphometry according to Levine (1985)

Biochemical analysis: Was calorimetrically analyzed using test kits.

- For measuring glucose level according (Siet *et al.*, 1981)

- Total protein were carried according to (Peteres, 1968)

- Calcium was determined according to Glinder and king (1972)

- Inorganic phosphorus was estimated accorsing to Daly (1972)

- Sodium and Potassium were determined according Oser (1979)

- Anti- microbial sensitivity, was carried according to test diffusions technique as described by Koneman *et al.* (1995) on Muller Hinktion agar. A total of 12 anti-bacterial agents discs (Oxoid) were used in this investigation including Florophencol, Marbofloxacine, Enrofloxacine, Sulpha Trimethprime, Gentamycine, Streptomycine. The results were interpreted according to Quinn *et al.* (1994). Statistical analysis was carried according to Snaedecor and Cochran (1982)

RESULTS

Table 1: Incidences of isolated bacteria from diarrhoeal and apparently healthy lambs at variable breeding models

Isolates E.coli	Farm	Holder	Indevi.	Total diseased		Control	Total	
				N	%		N	%
E.coli	9	7	10	26	43.3	2	28	40
Klebsiella	6	4	7	17	28.3	1	18	25.7
Kl.oxytocea	2	3	3	8	13.3	1	9	12.8
Kl.pneumoniae	-	1	-	1	1.6	-	1	1.4
Kl.pn.ozaene	4	-	4	8	13.3	-	8	11.4
Enterobacter	4	5	1	10	16.6	3	13	18.6
Ent. agglomerance	2	1	1	4	6.6	2	6	8.6
Ent.aerogenes	2	2	-	4	6.6	-	4	5.7
Ent.cloaca	-	2	-	2	3.3	1	3	4.3
Citrobacter	4	2	3	9	15	3	12	17.1
Cit.diversus	2	1	3	6	10	2	8	11.4
Cit. freundii	-	1	-	1	1.6	1	2	2.8
Cit. amalonaitcuc	2	-	-	2	3.3	-	2	2.8
Proteus	3	1	4	8	13.3	2	10	14.3
Pr.vulgaris	2	1	3	6	10	1	7	15
Pr.mirabilis	1	-	2	3	5	1	4	5.7
Serratia	2	4	2	8	13.3	1	9	12.8
Ser.funticola	-	2	-	2	3.3	-	2	2.8
Ser.marcescens	2	2	2	6	10	1	7	10
Morganalla morganii	2	-	3	5	8.3	-	6	8.6
Pseudomonas aeruginosa	2	-	3	5	8.3	-	5	7.1
Staph. Aureus	4	2	2	8	13.3	-	8	11.4
Strept ini	-	1	1	2	3.3	-	2	2.8
Enterococcus fecalis	1	1	2	4	6.6	1	4	5.7

Table 2: Prevalence of bacterial infections among variable breeding models

	Farm		Small holder		Individual		Total	
	N	%	N	%	N	%	N	%
E.coli	9	39	7	53.8	10	41.66	26	43.3
Klebsiella	6	26	4	30.8	7	29.16	17	28.3
Enterobacter	4	17.4	5	38.5	1	4.16	10	16.66
Citrobacter	4	17.4	2	15.4	3	12.5	9	15.
Proteus	3	13.	1	7.7	4	16.66	8	13.3
Serratia	2	8.7	4	30.9	2	8.33	8	13.3
Morganella morganii	2	87	-		3	12.5	5	8.3
Total Enterobacteriacae	30	81.1	23	85.2	30	78.9	83	81.4
Pseudomonas aeruginosa	2	8.7	-		3	12.5	5	8.3
Staph .aureus	4	17.4	2	15.4	2	8.33	8	13.3
Strept ini	-		1	7.7	1	4.16	2	3.3
Enterococcus fecalis.	1	43	1	7.7	2	8.33	4	6.7
Total	37		27		38		102	

Table 3: Result of anti biogram of the important predominant isolated bacteria.

Isolate Anti- microbial	E Coli (N=10)		Klebsiella (N = 5)		Pseudomonas (N = 5)		Staph .aures (N= 5)	
	N	%	N	%	N	%	N	%
Cefteofure	7	70	3	60	2	40	4	80
Cephotaxime	5	50	2	40	1	20	3	60
Marbofloxacine	7	70	3	60	2	40	3	60
Enrofloxacine	6	60	2	40	1	20	2	40
Florophenecol	8	80	4	80	2	40	2	40
Gentamycine	3	30	3	60	-	-	3	60
Amoxicillin	2	20	1	20	-	-	1	20
Tetracycline	-	-	-	-	-	-	1	20
Lincospectine	6	60	2	40	2	40	3	60
Sulpha – trimethozel	2	20	1	20	-	-	1	20
Revampicine	2	20	1	20	-	-	1	20

Table 4: Incidence of parasitic infestation in fecal samples of diarrhoeal lambs (n=60)

	Total Examined	Total Infested		Infestation state						Total *Eimerria*		Total *Cryptosporidium*	
				Eimeria		*Cryptosporidium*		Mixed					
Diarrhoeal lambs	60	No	%	No	%	No	%	No	%	No	%	No	%
		50	83.3	39	65	8	13.3	3	50%	42	70	11	18.3
App. healthy	10												

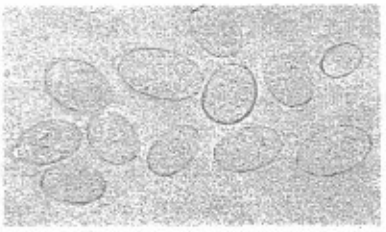
Table 5: Prevalence and characters of different *Eimeria* species isolated from diarrhoeal infested lambs (n=50)

Eimeria species	No.	%	Form	Micropile	Polar cap (um)	Oocyst (Um)	Spoulation Time (Day)
E.crandalis	30	60	Spherical elliptical	Yes lightly marked	With or without	21.9x9.4	1-3
E.ovina	23	46				31x20	2-4
E.ahsata	20	40					
E. fauri	14	28	Ellipsoidal ovoid	Yes	Yes	33.4x23	2-3
E.Parva	12	24	ovoid	yes	Yes	32x23	1-3
E.ovinodalis	8	16					
E.pallida	5	10				16.5x14	3-5
			ovoid	Yes rarefied			
					No		
			Spherial to sup spherical	No		23x18	1-3
					No		
						15.6x10.6	1-3
				indistinct			
			Ellipsoidal ovoid	No	No		
			Ellipsoidal oval				
					No		

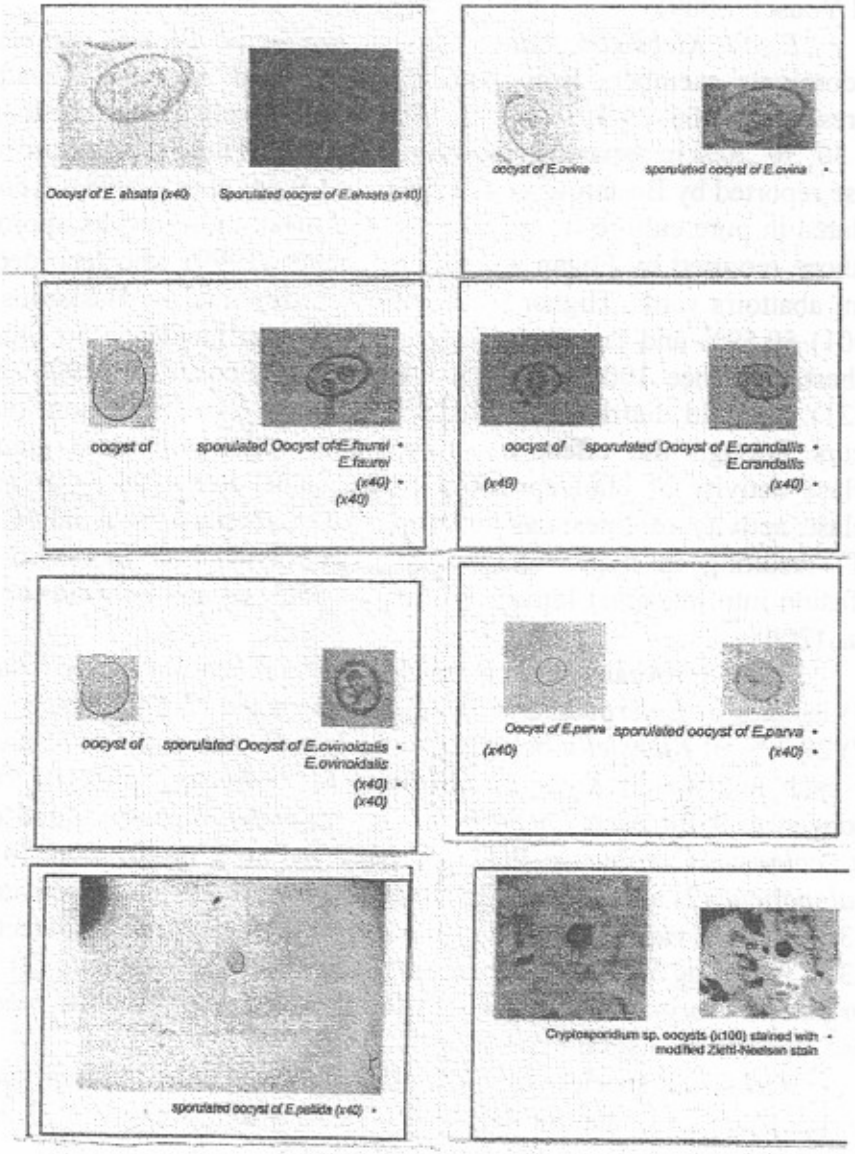
Table 6: Effect of bacterial infection or concurrent with Eimerea or Cryptosporidium on serum glucose, protein serum and mineral levels of diarrheic lams.

Variable	Bacteria	Bacteria + Eimerea	Bacteria + Cryptosporidium	Bacteria + Eimerea Cryptosporidium	(control)
Glucose, mg/dl	66.08±0.43 b	61.05±0.51 c	54.67±4.18 d	50.02 ± 0.20 c	70.70 ± 0.58 a
Total protein, g/dL	5.51±0.14 b	5.76 ± 0.06 b	5.70±0.10 b	5.78 ± 0.18 b	7.32 ± 0.10 a
Albumin, g/dL	4.22±0.05 ab	4.09 ± 0.04 ab	4.14±0.13 ab	3.98 ± 0.02 b	4.32 ± 0.09 a
Globulin, g/dL	1.28±0.09 b	1.67 ± 0.08 b	1.57±0.03 b	1.80 ± 0.20 b	3.00 ± 0.03 a
Albumin to globulin ratio	3.52±0.29 a	2.72±0.14 ab	2.64±0.14 ab	2.30 ± 0.20 bc	1.44 ± 0.03 c
Sodium mg/L	127.00±.00 b	122.10±0.44 c	125.67±3.33 bc	116.08±1.71 d	133.20±0.84 a
Potassium mg/L	5.18±0.08 bc	5.70±0.12 ab	4.97±0.13 bc	6.33±0.46 a	4.37 ± 0.09 c
Calcium, mg/dl	7.73±0.22 b	7.74±0.14 b	7.9±0.40 b	7.32±0.25 b	9.83 ± 0.05 a
Phosphorus, mg/dl	6.11±0.27 b	6.74±0.17 a	6.27±0.07 ab	6.24±0.04 ab	5.64 ± 0.10 b

Values are means and their standard errors.
Means within a row without a common letter differ significantly 41($P < 0.05$)



Mixed spp. of Eimeria oocysts



DISCUSSION

Diarrhea is of common occurrence in animals of all ages in the flock. In lambs it could resulted in significant mortalities, weight loss or even lead to subsequent growth retardation (Bastarous *et al.*, 2001; Sharif, *et al.,* 2005). Table 1 shows that the, *Enterobacteriaceae* constitute the main causes of diarrhea in lambs as 81.4 % among diarrheal lambs. The incidence varied from 78.9 % in individual breeder and reached to 85.2 % in small holder's model. These results approached to these reported by EL.Ged, *et al.* (1994); Bastarous, *et al.* (2001); Andres *et al.* (2009); Ismail and Yousef (2009).

E.coli, Klebsiella, Enterobacter, Citrobacter, Proteus spp were the predominant members from both diarrhoeal and apparently healthy as represented 4.366, 29.3, 16.66, 15, 13.3% respectively in diarrhoeal and 20, 10, 30, 30, 20% in apparently health respectively. These results agreed with those reported by Bastarous *et al.* (2001) and Wani *et al.* (2008). *E.coli* was isolated in pure culture from 26 cases (43.14 %), these results approached to those reported by Fogan and Desmatchelier (1999) who recorded 36% from abattoirs yards. Higher incidences were reported by Bastarous *et al.* (2001) 50.59% and Ismail & Yousef (2009) with incidence 56.14%. The highest incidence 100 % was recovered by Blanco *et al.* (1996) among (5–21) days old diarrhoeal lambs. Generally in *E.coli* infection, diarrhea occurs through the effect of enterotoxins which stimulate granulates cyclase activity of elial epithelium, heat stable toxin, (S.T) or adenyle cyclase activity of intestinal and capillary epithelium, heat labile toxin, (LT) resulting in hyper secretion of electrolytes and increased water diffusion into intestinal lumen resulting in acidosis and dehydration Wani *et al.* (2008).

Table 1 revealed the bacteriological examination of fecal samples (83) isolates of *Enterobacteriaceae* from diarrhoeal (26) 43.3 % of *E.coli*, (17) 28.3 % of *Klebsiella* belonged to 8 *Kleb. Oxytocea, Kleb. ozaenae* (8) for each and single *Kleb. pneumoniae, Enterobacter* spp (10) 16.6 % belonged to 4 for each *Ent. agglemerans* and *Ent. aerogenes* and only 2 *Ent. coloaca. Citrobacter* 9(15 %) belonged to *Cit. diversus* (6), *Cit. amalonaticuc* (2) and only (1) *Cit.freundii, Proteus* spp. were recovered (8) 13.3 % 6 *Prot. vulgaris* and (2) *Prot mirabilis, Serratia* were detected (8) 13.3 % including *Serratia marcescens* (6) and *Serratia funtecola* (2) finally *Morganella morganii* were detested (5) 8.3 % These results agreed with those reported by Ashraf (1996)

Klebsiella species are incriminated with lamb diseases specially enteritis among neonatal mortalities (Mottelib *et al.*, 1992; Abo EL Hassan

(1996). The severity and significance depends on the system of management and the degree of intensification, colostrums intake, birth weight, age susceptibility, housing factor and the climatic conditions (Meltzer and Shpight, 1996).

Pseudomonas aeruginosa were isolated in (5) 8.5% from diarrhoeal lambs. These results are nearly to that recorded by Amal *et al.* (2002). *Pseudomonas aeruginosa* is an opportunistic pathogen that rarely cause disease in healthy animals. That pathogen cause abroad spectrum of infection as urinary, respiratory and intestinal tract infection and other sites although it contributes to high morbidity and mortality rates (Hotack and Majt, 1997).

Tables 1 and 2 show that *Staphylococcus auereus* were detected in infection rate of 13.3 % among diarrheal lambs while it was negative in apparently healthy lambs. *Staphylococcus auereus* is an important pathogen responsible for mortalities in newly born lambs as septicemic pathogen (Nevin *et al.*, 2001)

Tables 1 and 2 revealed *Streptococcus ini and Enterococcus fecales* in incidences of 3.3, 6.6 % in diarrheal lambs, these organisms presented as commensally pathogens become invadors when disturbed immunity.

The pattern of antibiotic sensitivity of the most prevalent and harmful intestinal pathogens were done in vitro and the obtained data as in Table 3 revealed that Florofenecol, Cephteofure, Marbofloxacine were the most sensitive drug with incidence of 80, 70, 70% among 10 isolates of gained *E.coli* isolates followed by Enrofloxaciu and Lincospectin with 60%, these results approached to those recorded by Khaled (2004).

Concerning sensitivity of *Klebsiella spp.* as in Table 3, Florofenecol, Cephtecfure, Marbofloxacine and Gentamycine were the more sensitive with 80, 60, 60 and 60 % followed by Lincospectin and cephotaxim with 40% lowest sensitivity were detected for Sulpha-methexazol, Amoxicillin and Ampicilline as 20% of isolates revealed sensitive. Moreover, Oxytetracycline was resistant for all *Klebsiella* spp isolate as *E.coli*. These results agreed with and Khaled (2004).

In the present investigation the antibiogram of *Pseudomonas aeruginosa* isolates were studied. The selection for treatment of it is often problematic due to its antibiotic resistance Kovac, *et al.* (1998).

Table 3 denotes that Revampicine, Sulpha methexazol, Tetracycline, Amoxicillin and Gentamycine were resistant for the isolated *Pseudomonas*. These results agreed with Ghoniem *et al.* (2002) *Pseudomonas aeruginosa* were sensitive for Cephteofur Marbocyl, florophenecal and Lincospectin in 40 %, these results agreed with Bert

et al. (1998). However lower incidence could be attributed to low number of examined isolates.

The sensitivity of *Staphylococcus aureus* as in Table 3 revealed that Cephteofur Marbofloxacine, Cephotaxim, Lincospectin and Gentamycine are sensitive with rate 80, 60, 60, 60 % respectively, these results agreed with Guta *et al.* (2002) while disagreed with Hatem (2003).

Amoxicillin, Tetracycline, Sulpha methanol and Revampicine were lower as only (1) 20 % were sensitive. These results agreed with the finding of Hatem *et al.* (2003) and Ashraf (1996).

Those protozoan parasites *Eimeria* and *Cryptosporidia* are important enteric diseases of lambs resulting in diarrhea, inefficient weight gain and occasionally death (Foreyt 1990).

The parasitological examination of fecal samples revealed that 50 (83.3 %) out of 60 examined lambs were infested with *Eimeria* and *Cryptosporidium* (Table 4). These results are in accordance with Foreyt (1990); Causapé *et al.* (2002); Ozdal *et al.* (2009). *Eimeria oocysts* were identified in 42(70%) by floatation technique. This result agreed with Yakhchali and Golomi, (2008) (69.6%) in Iran and relatively similar to Ozdal *et al.* (2009) 60.6% in Turkey, but our results was lower than those reported by Khalifa *et al.* (1986) 84% in Egypt. Boshra, (1994) 91.33% in Egypt and Bastarous, *et al.* (2001) 100 % in Egypt, However a Lower percentage than our results was recorded by Aly (1990) 24.56 % in upper Egypt, Mottelib, *et al.* (1992) 20% at AL-Quassim, Abou EL Hadid and Lotfy (2007) 29% in Egypt and Yakhchali and Zarei (2008) 16.7 % in Iran., these different results, may be attribute to location, season, age, sex and health condition of examined lambs Seven species of *Eimerea* were detected in the present study the predominant was *E.crandalis* 59.5 % (Table 5). This result agreed with that reported by Reginsson and Richter (1997); Bastouerous *et al.* (2001) in Egypt. Lambs could harbored mixed infection with at least three species of Eimera Dittmar *et al.* (2010). Parasitological examination of fecal samples for the presence of *Cryptosporidium* parvum by Modified Zeihl Niealsen Technique (acid fast stain) revealed spherical red-pink stained oocysts with granular appearance and single layer wall 4.5-5 micron in diameter against green background this results agreed with description of Abu-Eisha (1994), Selim, (1995) and, Abu-Elwafa, *et al.* (2007) *Cryptosporedium* spp. were detected in 11(18.3 %) of 60 lambs. Table (4), the same result recorded by Abu-Elwafa *et al.* (2007) in Egypt.

The pervious studies that have been conducted on prevalence of *Cryptosporidium* spp. in lambs, based on microscopy, have reported prevalence ranging from 10.10% to 68.3% for *Cryptosporidium* spp.

(Abu Eisha (1994); Selim (1995); Abd. ELwahed (1999); Nassif, *et al.* (2002) in Egypt, Majewska, *et al.* (2000) in Poland, Ulutas and Voyvada (2004) Sari, *et al.* (2008); Ozdal *et al.* (2009); Turkey, Misic *et al.* (2006); Serbia, Soltan *et al.* (2007) Tunisia) Our result Fall within the range reported in pervious studies that mentioned above. These differences between results might attribute to geographical Location, overcrowded or bad hygienic condition, health conditions of examined Lambs and environmental conditions, breading size.

There is no totally effective therapy for Cryptosporidiosis Other than a healthy immune system good management, sanitary conditions and quarantine of sick lambs (Foryet, 1990; Misic, 2006) Veterinarians and breeders should be aware of the disease in order to avoid greet losses among lambs and to prevent its transmassion to human (Selim 1995; Abd. ELwahed 1999).

Concerning the biochemical alterations in diarrhoeal lambs which accompanied with Bacterial and / or parasitic affections. Serum glucose levels showed significant decrease in variable affections compared with those apparently healthy (Table 6), these findings agreed with those reported by El-Sengary, *et al.* (2004); El-Dessouky and Nabila, (2005); Osman and Sadick (2008); Ismail *et al.* (2009); Shahira and Sahar (2009). who postulated factors causing hypoglycemia as anorexia, decrease intestinal glucose absorption, a low level of glucose reserves is young age and alterations in tissue metabolism caused by decreased blood flow and oxygenation associated with the hypovolemic shock which present in the hypoglycemic diarrheic animals.

Concerning the results of total serum protein the results as illustrated in Table 6 revealed significant hypoproteinemia, hypoalbuminemia and hypo globulinemia. These results agreed with the findings of Jain (1986); Ahmed (2002); Nassif, *et al.* (2002); Shahira and Sahar (2009).

The decrease in blood serum protein in the present study may be attributed to inability of the gut of infected animal to absorb and assimilate the heamobiotic principals regarding blood serum total protein, albumin and globulin. Jain (1986) declared that the loss of protein during inflammation or ulcerations of gastro intestinal tract lead to impaired absorption as well as increase protein loss due to injured mucosal cell.

Serum electrolytes showed significant decrease in serum sodium among the variable causative agent of diarrhea in lambs where, the highest reduction in those affected by bacteria, *Eimerea* and *Cryptosporidium*. The reduction of values was less in single than double causes of diarrhea in comparison with apparently healthy one. That significant decrease in

sodium level ($P<0.05$) hyponatremia could be attributed to losses of body fluids and the impairment of absorption through the damaged intestinal mucosa (Ismail *et al.*, 2009; Shahira and Sahar, 2009). Nearly similar results were also obtained by Assad and Nizar (2004).

Concerning blood serum potassium level in the present study as illustrated in Table 6 revealed a significant increase (hyper Kalemia) compared with apparently healthy lambs. These results were supported by the finding of El Dessouky and Nahila (2005); Osman and Sediek (2008); Shahira and Sahar (2009). The results could be attributed to the subsequent acidosis associating with long standing enteritis in which false positive increase in K. values in attempts to compensate the occurring metabolic acidosis which known as K/H exchange (Radostits *et al.*, 2000).

Hyper Kalmia is a common problem associate with the acid base and electrolyte disturbances that occurs in diarrheic lambs (Alan *et al.*, 2008)

In the present results, Table 6 showed significant decrease in serum calcium level when compared with those control apparently healthy lambs, these results agreed with Shigehero *et al.* (2003); Shahira and Sahar (2009); Ismael and Fatma (2009). The decrease in blood serum calcium level could be attributed to losses of body fluid in hyper secretary diarrhea and impaired absorption through the damaged intestinal mucosa as reported by the previous authors.

Duncan *et al.* (1994) attributed the decrease in calcium level to the hypo albuminemia where decrease albumin concentration lower the total calcium level while both ionized and complex calcium levels remain normal.

In conclusion it is obvious that diarrhea is clinical sign of major importance. *Enterobacteriacae* specially *E.coli*, infection, must be considered in diarrheic lambs, other bacterial causes as *Pseudomonas* and *Staph.aureus* infection singly or combined with parasite specially *Crytosporidia* and coccidiosis cause biochemical alterations firstly attention to the mangemental factors and nutrition of the dams to insure adequate colostrums intake will help in the minimization of the infection. Moreover the proper planning and adequate knowledge of the back ground of the diseases and application of appropriate preventive programs at right time leading to elimination of diarrhea, the hygiene and good management will lead to combating, prevention of parasitic infestations. The adequate knowledge of biochemical alterations and proper interference will compate the lethal effects of diarrhoeal acidosis and dehydration.

REFERENCES

Abd–El Wahed, M.M. (1999): Cryptosporidium infection among sheep in Qalubia Governorate, Egypt. J. Egypt soc. Parastiol. 29 (1): 113-118.

Abo El Hadid, S.M. and Lofty, H.S. (2007): Some studies on enteric parasites of sheep in Beni–Suef Governorate Bs. Vet. Med. J. 17, (1): 11–18.

Abo-ElHasaan, D.G. (1996): Neonatal diarrhea in lamb and goat Vet. Med. J. Giza 44 (2): 371-380.

Abu–Elwafa, S.A.; Elsheikha, H.M. and El–Shazly, A.M. (2007): cryptosporidiosis inflock of sheep and ther nomad shepherds. 5 th int. Sci, conf. Mansonra 1389–1400.

Abu–Eisha, A.M. (1994): Cryptosporidial infecticon in man and farm animals in Ismailia Governorate. Vet. Med. J. Giza Hz (2): 107–111.

Ahmed, N.S. (2002): Serum protein profile in cattle calves infested with cryptosporedia species Egy. j. comp. and dinic path. 15 (1): 149–155.

Alan, D.W.; Moise, N.S. and Rebhun, W.C. (2008): Hyperkalemic Atrial Standstill in neonatal calf diarrhea Journal of veterinary internal Medicine. 6, 5: 294-297.

Aly , U.A.H. (1990): Studies on parasitic and bacterial diarrhea in sheep. M.V. Thesis. Fac. Vet. Med. Assiut Univ.

Amal, A.M.G. and EL-Taher, E.G. (2002): Occurrence of C. jejuni and P. aerogenosa in Ewes j. Egypt. Vet. Med. Ass. 62 (2): 175–185.

Andres, S.J.; Menez, A.; Sauchez, J.; Alonso, J.; Gomez, L.; Lopez, F. and Rey, J. (2009): Evaluation of some etiological factors presedposing to diarrhea in lamb in laserna (south west Spain) small ruminant Research, 70 (2): 272–275.

Ashraf, M.A. (1996): Studies on bacterial associated neonatal calf diarrhea. M.V.Sci thesis Fac. Vet. Med Tanta Univ.

Assad, A. and Nizar, A. (2004): Studies on the clinical hematological and Biochemical changes in diarrheic calves, Alex. Vet. Med. J. 21 (2): 712–719.

Bastarous, A.F.; Thabet, A.E.R.; Abd Elhafeez, M.M.; Sayed, A.M. and Arafa, M.I. (2001): Studies on diarrhea in lambs in Assiut Governorate Isolation and identification of causative bacteria and parasitic agents Assiut Vet. Med. J. 46(91): 98-108.

Bardisy, M.M. (1994): Studies on the role of clostridia organisms and other bacteria in calf diarrhea with special reference to their susceptibility to some antibacterial agents. Assiut Vet. Med. J., 30: 194–215.

Bert, F.; Maubec, E.; Bruneau, B.; Berry, P. and Lanbert, Z.V. (1998): Multi-resistant *Ps.aerogenosa* outbreaks associated with contaminated tap water in a neurosurgery intensive care unit. J. Hosp. Inf, 39 (1): 153–62.

Blanco, J.D.; Blanco, J.E.; Blanco, M.; Ruis Santo Quiteiria and Dela Fuente, R. (1996): Serogroups *E. coli* strains isolated from diarrheic lambs in Spain Vet. Microbiol. 47 (3–4): 209–217.

Boshra, M.A. (1994): Some studies on coccidiosis in sheep M.V.Sc. Thesis Fac. Vet. Med. Bani-Suif.

Causape, A.C.; Quilez, J.; Sanchez-Acedo, C.; Del CaCho, E. and Lopez-Bernad, F. (2002): Prevalence and analysis of potential risk factors for Cryptosporidium Parvum infection in lambs in zaragoza (northeastern Spain). Vet. Parasitol., 104: 287-298.

Clinder, E.M. and King, J.D. (1972): Rapid colorimetric determination of calcium in biological fluids with methylenblue. Am. J. Clin, Path. 58: 376–382.

Cruickshank, R.; Duguid, J.P.; Marmion, B.P. and Swain, R.H.A. (1975): Medical Microbiology 12th vil II Churcgill Livingston Edinburgh London and Newyourk.

Daly, J.A. (1972): Colorimetric method for determination of serum inorganic phosphorous Clin, Chemist 18: 263.

Dittmar, K.; Mundt, H.C.; Gtzonka, E.; Daug Schies, A. and Bangoura, B. (2010): Ovine coccidiosis in housed lambs in Saxony–Anhalt (central Germany) Berl Munch–Tierarztl wochen. 123 (1–2): 49–57.

Duncan, J.R.; Qrsses, K.W. and Mohaffy, A. (1994): Veterinary Laboratory Medicine, Clinical Pathology 3rd Ed. Ames Lowa State Univ. Press.

EL-Dessouky, S.A. and Nabila, M.E. (2005): Effect of cryptosporidium parvum infection on the hematological and blood biochemical changes of buffalo calves with special reference to the prevalence of infection among buffaloes. Assiut. Vet. Med, J. 51 (105): 108–120.

EL-Ged, A.; EL Seyed, Z.M.; Khalid, A.; EL-Caber, G.A.; EL-Rahman, M.A. and EL-Abo EL-Hassan, D.G. (1994): Postnatal diarrhea in lamb and goat kids. Vet. Med. J. 44 (2): 371.

El-Sangary, F.H.; ElKabany, M.F. and Nagwa, A. Helmy (2004): Someimmuonlogical, hematological and bacteriological studies on kids suffering from diarrhea Alex. Vet. J. 21(1): 276-292.

Fogan, Ni and Desmarchelier, P. (1999): Shiga toxin producing *E Coli* in sheep and pre-slaughter lambs in eastern Australia. Lett Appl Microbiol. 28(5): 335–339.

Foreyt, W.J. (1990): Coccidiosis and cryptosporidiosis in sheep and goats. Vet. Clin North Am Food Anim. Pract, 6 (3) 655–670.

Ghoniem, A.M.; EL Taher, E.G. and Ezeldeen, A.N. (2002): Occurrence of C.jejuni and *Ps. aerogenosa* on eur J. Egypt. Vet. Med. Assoc. 62, 2: 175–185.

Glinder, E.M. and King, J.D. (1972): Rapid calorimetric determination of calcium in biological fluids with methylene blue. Am. J. Clinic., Path. 58: 376-382.

Goto, S. (1996): Recent progress in identification of pathogenic factors of *Ps.aeregenosa*. J. Infect. Chemothe., 2: 111–116.

Guta, C.; Sebunya, T.K. and Gashe, B.A. (2002): Antimicrobial susceptibility of Staphylococcus species from cow milk originating from davey farms around Gabon. Botswana, East. Afr. Med. J.79 (1): 45–48.

Hatem, M.E.; Mona, S.Z.; Asmaa, A.H. and Mouse, A. ELshaboury (2003): Bacteriological, histopatholgical, Clinic pathological and studies on respiratory affections in sheep and goat in Egypt J. Egypt. Vet. Med. Assoc. 63 (1): 97–109.

Henricksen, S.A and Pohlenz, J.F.L. (1981): Staining of Cryptosporidia by modified Ziehl neelsen technique Acta Vet. Scand., 22: 594-596.

Hilali, M.; Fatma, A. and ElKharss, A. (1998): Diagnosis of cryptosporedium parvum infesting sheep and goats suffering from diarrhoea at El-Kharj,Saudia Arabia Alex J. Vet. Sci. 14 (1): 91-96.

Hotack, A. and Mail, N.V. (1997): Sero-typing and virulence factors of *Ps.aerogenosa* clinical isolates. Acta Microbial Immuinol Hung 44 (2) 141–146.

Ismail, S.M. and Yousef, F.M.C. (2009): Clinco diagnostic studies in Bacterial causes of diarrhea in lambs at Ismailia Govern at 6th Int. Sci. Con Mansoura 341–353.

Jain, N.C. (1986): Schlam's veterinary hematology 4th Ed. Lea Febiger, pheladelfia. USA.

Khaled, M.K. (2004): Studies in the role of Escherichia ship to neonatal calf diarrhea ph.D. Thesis. Fac. Vet. Med, Tanta Univ.

Kaneko, J.J. (1997): Serum protein and dysprotinemias in: Clinical biochemistry of domestic animals 5 th Ed, Academic Press London.

Khalifa, R.M.A.; Omaran, L.A.A. and E. Saahy Moneib, M. (1986): Coccidiosis of sheep in Assiut. Vet. Med: J., 16 (31): 135–147.

Koneman, E.W.; Allan, S.D.; Janda, W.M.; Schreekenb erger, P.C. and Winn, W.C. (1994): Introduction to Diagnostic Microbiology, J.B. Lippincott company Philadelphia, U.S.A.

Kovac, K.; Paterson, D.L. and Yav, L.C. (1998): Antimicrobial thereby of *Ps. aerogenosa* therapeutic issues, resistance pneumonia, endocarditic and infections of GE tract bone and jeint and urinary tract infect. Med. 15(6) 385–394.

Krieg, N.R. and Hoet, J.G. (1984): Bergeer's manual of systemic Bacteriology. Vol. William and Wilkins Company Baltimore London.

Levine, N.N. (1985): Veterinary protozoa logy. 1st ed. Iowa State University Press Ames.

Majewska, A.; Werner, A. and Sulima, P. and Luty, I. (2000): Prevalence of cryptosporidium in sheep and goat bred on Eiv farms in west – central region of Poland. Vet. Parasite, 89 (4): 269–275.

Marty, N.; PasQuier, C.; Dournes, J.L.; Chemin, K. and Montrozier, H. (1998): Effect of characterized P.aerogenosa Lipopolysachariedes on adherence to human tracheal cells. J. Med, Microbiol., 47: 129–134.

Meltzer, R. and Shpigal, N.Y. (1996): Etiological and epidemiological aspect of calf diarrhea in Israel, dairy farm B.C.V. A Edinburgh P.1-5.

Mottelib, A.A.; Haroun, E.M.; Magzoub, B.M. and Bashear, E. (1992): The effect of gastrointestinal parasites on blood picture in sheep and goat at Al-Qusaim Assiut Vet. Med. J. 28(55): 215-223.

Misic, Z.; Katic, R.S. and Kulisic, 2. (2006): Cryptosporidium infection in lambs and goat kids in Serbia Acta. Veterinaria 56 (1): 49–54.

Munoz, M.; Alvarez, M.; Ian, Za and Carmenes, P. (1996): Role of enteric pathogens of the etiology of neonatal diarrhea in Lambs and goat kids in Spain Epidemiol. infect. 117 (1): 203–211.

Nassif, M.N.; Amer, S.A. and Osman, S.A. (2002): Some studies on ovine and caprine Cryptosporidiosis concerning prevalence and electrophoretic pattern of blood serum protein Assiut Vet. Med. J. 47, 94: 249–262.

Nevin, M. Sibhy; Manal, M. Abu EL makarem; Gehan, G. Shehab and Gehan, A. Hosny (2001): Bacteria and Mycoplasma as cause of pneumonia in goat with special reference to the associated histopatholgical changes Egypt. Vet. Med. Associ, 61(3): 25–44.

Oser, B.L. (1979): Hawks physiological chemistry 14 th Ed MC Graw Hill Company LTD London.

Osman, F.A. and Sadiek, A. (2008): Role of cryptosporedial infection as a pathogen of neonatal calf diarrhea in Assiut Governorate 13th Conf. of Fac. Vet. Med. Assiut Univ.

Oxoied (1982) Manual of culture media ingredient and other laboratory service. Wade Road Basing tok Hampshire. U.K

Ozdal, P.T.; Yasar Gas, S.D. and Suleyman, K. (2009): Parasitic Protozoan (Eimerea, Giardia and cryptosporidium) in lambs with Diarrhea in the van province (Turkey). Bull–Vet. Inst. Pulawy, 53 K 47–51.

Ozdal, P.T.; Yasar Gas, S.D. and Suleyman, K. (2009): Parasitic Protozoan (Eimerea, Giardia and cryptosporidium) in lambs with Diarrhea in the van province (Turkey). Bull–Vet. Inst. Pulawy, 53 K 47–51.

Peters, T. (1968): Colorimetric determination of total protein in serum based on the principle of burette reaction Clin. Chem., 14: 1147.

Platzer, B.H.; Presol, M.; Cienlick, A. and Joachim (2005): Epidemiology of Eimerea infections in an Austrian milking sheep flock and control with diclazunil Vet. Parasitol. 129: 1 – 9.

Quinn, P.J.; Carter, M.E.; Markery, B.K. and Carte, G.R. (1994): Microbiology year book Wolfe publishing Europe limited.

Quinn, P.J.; Qrter, M.E.; Markeys, B.K.; Donnelly, W.J.C. and Leonard, F.C. (2002): Veterinary Microbiology and Microbial diseases Greet Britain by MPG Book Ltd Bedmin Cornwelt U.K.

Radostits, OM.; Blood, D.C.; Hincheloff, K.W. and Gay, C.C. (2000): Veterinary Medicine Text book of diseases of cattle, sheep, pigs. goat and horses 9th Ed. W.B. Saunders, London.

Reginsson, K. and Richter, S.H. (1997): Coccidian of the genus Eimerea in sheep in Iceland Buvisindi. 11: 99–106.

Sari, B.; Arslam, M.O.; Gicik, Y.; Kara, M. and Tasci, G.T. (2008): The prevalence of cryptosporidium species in diarrheic lambs in kars province and potential risk factors. Trop. Anim–Health Prod.

Sharif, Obeidat, J. and AL-Ani, F. (2005): Risk factors for lamb and Kid mortality in sheep and goat farms in Jordan. Bulgadian J. of Vet. Med., 8, (2), 99–108.

Schoenian, S. (2007): Diarrhea (Scour) in small ruminants. Mary land Research and Education Center Univ. of Maryland cooperative Extension www.sciencedirect.com.

Selim, M.A. (1995): Prevalence of Cryptosporidiosis in animals and humans being in contact in Sharkea province Alex. J. Vet. Sci., 11 (4): 527-533.

Siet, G.; Henny, J. and Schiele, F. (1981): Interpretation des examines de laboratior. Karga Ed. PP206.

Shahira, H.M. and Sahar, S.A. (2009): Field trail for treatment of diarrhea in kids at sharkia governorate 6th intern. Sci conf 447-461.

Shigehiro, I.; Kazuyuki, S.; Yohko, S.; Kouji, I.; Eiichiro, K. and Ryuj, (2003): Effect of intravenous Infusion of hypotonic Lactated ringer's solution on calves with diarrhea. J. Vet Med. Sci. 65 (9): 1033–1036.

Snaedecor, G.W. and Cochran, W.G. (1982): Statistical methods 8th ed Lowa State, University press, USA.

Soltan, R.; Guyo, TK.; Del, Cas E. and Ayadi, A. (2007): Prevalence of cryptosporidium spp. (Eucoccidiorida Cryptosporedia) inseven species of farm animals in Tunisia Parasite, 14: 335–338.

Soulsby, E.J.L. (1982): Helminthes, Arthropods and Protozoa of domesticated animals, 6th ed. published by Balliere Tindall and Cassel LTD. London.

Ulutas, B. and Voyvada, H. (2004): Cryptosporidiosis in Diarrheic lambs on sheep farm. T. Parazitol Derg 28 (1): 15–17.

Wani, S.A.; Hussain, L.; Fayaz, I.; Mir, M.A. and Niyhikawa, (2008): Subtype analysis of stx 1, stx2 and eaegebes in shiga tokin prosucing Esherichiaenic Ecoli (EPEC) from lambs in india WWW.Sciencedirect.com

Yakhchali, M. and Golomi, E. (2008): (Eimerea infection, coccidian: Eimeridare) in sheep of different age groups in sanandi city Iran. Vet. Archive 78 (1): 57–64.

Yakhchali, M. and Zarei, M.R. (2008): P revalence of eimeria in fection in sheep of Tabriz Suburb, Iran. Iranian J. of Vet. Res. 9(3): 277-280.