DIARRHOEA IN NEONATAL KID-GOATS WITH SPECIAL REFERENCE TO BACTERIAL CAUSES, CLINICAL, HAEMATOLOGICAL AND BIOCHEMICAL ALTERATIONS

By

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ABSTRACT

A Survey was carried out on 130 kid-goats aged from 2 days to 3 months, from different private farms in El-Mounofia and Kalubia Governorates. Out of these animals, 100 were suffering from diarrhoea. Bacteriological examination of the faecal samples revealed the presence of E. coli (58%), Salmonella, (27%), and Shigella (15%), as the main causative agents of diarrhoea. They were sensitive to common antibiotics and less sensitive to 10% garlic extract and 40% Hibiscous subdarifa. Haematological studies revealed significant decrease in haemoglobin content (Hb), erythrocytic (RBCs) count. On contrary, haematocrit value (PCV%) showed significant increase in affected animals. A significant decrease was detected in the values of serum total proteins, albumin, iron, copper, and growth hormone. On the other hand, there was a significant increase in cortisol hormone, lactate dehydrogenase (LDH), and alkaline phosphatase enzymes. The authors emphasize that the demonstrated diarrhoea caused many harmful clinicopathological effects, reduced growth hormone, and caused severe anaemia in kid-goats.

Additional keywords: Kid-goats - diarrhoea – haemogram – Salmonella – *E.coli* - serum biochemistry – LDH – alkaline phosphatase – hormones – trace elements - garlic extract - Hibiscous subdarifa.

INTRODUCTION

With the increasing application of intensive husbandry methods and the various causes of ill-thrift in sheep and goats have attracted increasing attention. The results of many investigations showed that the

greatest losses among these species occurs in the neonatal period (**Snodgrass and Angus, 1983**). Neonatal diarrhoea in kid-goats is a common problem with not a very well understood cause (**Snodgrass *et al.*, 1977**). This syndrome has been described to a variety of causes such as nutritional imbalance, faulty management and infectious agents (**Durham *et al.*, 1979**). Infectious diarrhoea affecting kid-goats occurs mainly where intensive systems of breeding which use paddocks, pens and indoor sheds are employed. Such systems unless very carefully managed, encourage the progressive build-up of infection (**Angus *et al.*, 1982 and Aly *et al.*, 1996**).

The aim of the present work was to study the causes of diarrhoea, the clinicopathological changes in blood of infected kid-goats and the suitable antibiotics for treatment.

MATERIAL AND METHODS

Animals used:

One hundred and thirty kid-goats (100 diarrhoeic + 30 apparently healthy as a control group), aged from 2days to 3 months were used in this study. These kid-goats belonged to different localities in El-Mounofia and Kalubeia Governorates and under semi-intensive management system.

Sampling:

All animals were sampled once before administration of any treatment.

Bacteriological and parasitological Studies:

Two faecal samples were taken directly from the rectum of all animals in this investigation. One sample was taken in a clean dry plastic containers for parasitological examination to detect gastrointestinal parasites (**Coles, 1986**) and the second using sterile swabs for further bacteriological analysis. These swabs were immediately inoculated on Carry and Blair's transport medium and were cultured on selective and differential culture media at 37°C for 24 hours and the isolated colonies were then identified according to **Carter, (1984)** and **Baily and Scott, (1990)** as follows: isolated colonies from MacConky's agar plate were examined to be either lactose fermenting or non-lactose fermenting. Lactose fermenting colonies appeared to be rose pink in color and non-lactose fermenting as pale yellow colonies. Isolated colonies were stained by Gram's stain. Colonies, which appeared as Gram negative bacilli were then described for further identification of Gram negative isolates. These were then subjected to biochemical reactions such as indol production, methyl red and Voges Proskauer tests (MR/VP), citrate utilization, hydrogen sulphide production, reaction on triple sugar iron agar (TSI), urease production and oxidase test.

Detection of K99 antigen was performed by slide agglutination test (SAT) according to **Baily and Scott, (1990)**, with specific antisera. Cryptosporidia were examined in faecal smears on glass slides which were air dried, fixed in methanol and stained with Geimsa stain according to **Abou-Zaid and Nasr, (1995)**.

Haematological Studies:

Whole blood samples with EDTA were obtained from the jugular vein for determination of haemoglobin content, haematocrit (PCV%) value and erythrocytic (RBCs) count according to **Coles, (1986)**.

Biochemical and Hormonal Studies:

The blood samples were centrifuged for serum separation and used for determination of copper and iron by atomic absorption according to **Issac and Kerber (1971)**, total proteins (**Henary *et al.*, 1974**), albumin (**Doumas *et al.*, 1971**), alkaline phosphatase (**Belified & Goldberg, 1971**), lactate dehydrogenase (**Buhl and Jackson, 1978**). Cortisol hormone was measured according to **Kuchn and Burvenich, (1986)**. Growth hormone was measured by special kits according to the method described by **Ronge and Blum, (1988)**.

Sensitivity Test:

1. Sensitivity test using common antibiotics: The following chemotherapeutic agents were used in testing the sensitivity of the isolated micro-organisms:

Gentamycin (10mcg/disc), chloramphenicol (30mcg/disc), rifamycine (30mcg/disc), tetracycline (30mcg/disc), ampicillin (10mcg/disc), streptomycin (10 mg/disc), nalidixic acid 30 mg/disc), and colistin (10 mcg/disc).

2. Sensitivity test using Garlic aqueous solutions: The isolates were incubated in about 10% garlic aqueous solution at 28°C till the colonial broth become evident. The degree of inhibition was compared to control (**Zaki *et al.*, 2001**).

3. Sensitivity test using dry *Hibiscus subdarifia* flowers: The flowers were extracted with 75% ethyl alcohol using Soxhlet apparatus till complete exhaustion occurs. Alcohol was then evaporated to obtain a semisolid extract. Dilutions to 40% were obtained by dissolving the extract in distilled water. The resultant dilutions that used to test the microorganisms which streaked with 0.4 mm loop on the extract into the gutter avoiding it over flow on the surface (**Zaki *et al.*, 2001 and Radostits, 1992**).

Statistical analysis:

All data were subjected to statistical analysis using T- test according to **Gad and Well, (1967)**.

RESULTS

Kid-goats were divided after careful clinical and bacteriological examination into three groups as shown in Table (1).

Table (1): Bacterial examination of faecal samples of diarrhoeic kid-goats

The organism	Number of isolates	% of isolates
E. Coli	58	68.84
Salmonella spp.	27	44.68
Shigella spp.	15	1.05
Control (-ve)	30	0.00

-ve = negative from bacteria

Clinical Signs:

Diseased kid-goats showed severe depression, unable to stand or move and some of them showed sternal or lateral body recumbent. Soft to watery of faeces tinged with mucus or occult blood or both and having putrefied odour. Varying degrees of dehydration and severe losses of skin elasticity. Contaminated skin of anal region, rough hairs, dry muzzle, increase of body temperature, pulse and respiratory rates.

Bacteriological Studies:

Bacteriological examination of the faecal samples of diarrhoeic kids revealed that 100 samples were positive for pathogenic bacteria. The distribution of these indicated that enteropathogic; *E. Coli* and Salmonella spp. constituted the high incidence while Shigella spp. recorded with lower incidence. The increase in packed cell volume (PCV%) reflected the severity of dehydration occurred in diarrhoeic kids with bacterial enteritis in group 2 (infected with *E. coli*) and group 3 (infected with Salmonella spp.) than in group 4 (infected with Shigella spp.) and apparently healthy kids (group 1). This reflect the severity of diarrhoea caused by enterotoxins produced by enterotoxigenic bacteria proliferation in the intestine which lead to toxaemia and that in turn aggravates the dehydrations. The most characteristic features in diarhoeic kids faeces was watery and contained mucus or occult blood or both and was having putrefied odour which explain the high incidence of isolated enteropathogenic; *E. coli* and Salmonella spp. However the presence of other pathogenic bacteria was also suggested but their incidence was very low as Shigella spp.

Concerning sensitivity test; the result indicated that *E. coli* and Salmonnella spp. were highly sensitive to gentamycine, chloramphenicol, rifamycine, and tetracycline, less sensitive to ampicillin and nalidixic acid and resistant to streptomycin and colistine. Moreover, *E. Coli* was moderately sensitive to *Hibiscous subdarifa* and garlic solution (Table 2).

Table (2): Results of sensitivity test against different chemotherapeutic agents

Chemotherapeutic agents	Disc conc.	*E. coli*	Salmonella spp.
Gentamycin	10 mcg	+++	+++
Chlorampheni-col	30 mcg	+++	+++
Rifamycine	30 mcg	+++	+++
Tetracycline	30 mcg	+++	+++
Ampicillin	10 mcg	++	+
Streptomycin	10 mcg	-	-
Naildixic acid	30 mcg	++	+
Garlic aqucous solution 10%	10%	++	++
Hibiscous extract 40%	40%	++	+
Colistine	10%	-	-

+++ = 0.58 mm. ++= 0.38 mm. + = 0. 23 mm. Conc. = Concentration.

Results of haematology and biochemistry:

A significant decrease in haemoglobin content (Hb), erythrocytic (RBCs) count while, haematocrit values (PCV%) count showed significant increase in affected animals with *E. coli* (group2) and Salmonella spp. (group3) than the control healthy animals (group 1) as shown in Table (3).

Table (3): Means ± SE of haemoglobin (Hb), haematocrit (PCV%) and erythrocytic (RBCs) count in both healthy and diarrhoeic kid-goats.

Animals groups	Number of animals	PCV%	RBCs ($X10^6/\mu l$)	Hb (g/dl)
Group 1 (control)	30	24.25 ± 0.12	10.20± 0.23	9.80± 0.20
Group 2 (*E. coli*)	58	40.00 ± 0.02 **	8.24± 0.24**	8.00± 0.14**
Group3 (Salmonella spp.)	27	34.00 ± 0.10**	8.10± 0.13**	8.23± 0.74**
Group 4 (Shigella spp.)	15	23.74 ± 0.72**	9.42± 0.40	9.03± 0.72

** = Highly significant at p ≤ 0.01 SE = Standard error.

As shown in Tables (4, 5), there were a significant decrease in total proteins, albumin, growth hormone, iron, and copper. On the other hand, there was a high level of cortisol hormone, lactate dehydrogenase, and alkaline phosphatase in diarrhoeic kid-goats in comparison with the control one.

Table (4): Means ± SE of iron, copper, cortisol and growth hormones in serum of both healthy and diarrhoeic kid-goats.

Animals groups	No. of animals	Iron (mg/dl)	Copper (mg/dl)	Cortisol (ng/dl)	Growth Hormone (ng/l)
Group 1 (control)	30	250 ± 2.30	185 ± 3.4	0.098 ± 0.73	11.0 ± 0.08
Group 2 (*E. coli*)	58	178 ± 1.54**	130 ± 4.7**	0.130 ± 0.28**	8.0 ± 0.11**
Group3 (Salmonella spp.)	27	180 ± 3.53**	134 ± 4.0**	0.140 ± 0.30**	7.8 ± 0.20**
Group 4 (Shigella spp.)	15	168 ± 4.01**	148 ± 2.0 **	0.150 ± 0.40**	7.1 ± 0.30**

** = Highly significant at p≤ 0.01 SE = Standard error.

Table (5): Means ± SE of total proteins, albumin, lactate Dehydrogenase (LDH), alkaline phosphates (ALP) in serum of both healthy and diarrhoeic kid-goats.

Animal groups	No. of animals	Total proteins (g/dl)	Albumin (g/dl)	LDH (U/l)	ALP (U/l)
Group 1 (control)	30	9.3 ± 0.40	4.90 ± 0.27	252 ± 23	15.3 ± 0.80
Group 2 (*E. coli*)	58	8.2 ± 0.27**	3.80 ± 0.14**	263 ± 31**	18.7 ± 0.50**
Group3 (Salmonella spp.)	27	7.0 ± 0.10**	3.40 ± 0.72**	270 ± 14**	19.1 ± 0.60**
Group 4 (Shigella spp.)	15	6.8 ± 0.78**	2.86 ± 0.73**	260 ± 26**	19.0 ± 0.54**

** = Highly significant at $p \leq 0.01$ SE = Standard error.

DISCUSSION

Infectious diarrhoea is a common condition affecting kid-goats specially those which are bred under intensive system of breeding in this study. Faecal samples screened the presence of the common enteropathogenic organisms; *E.Coli*, Salmonella species and Shigella species which causing diarrhoea. *E. Coli* seems to be the dominant enteropathogen which plays the major role among diarrhoeic kid goats (**Tzipori *et al.*, 1981; Angus *et al.*, 1982; Carter, 1984; Farid *et al.*, 1987 and Rodostits, 1992**). Isolation of Salmonella species from diarrhoeic kid-goats confirmed the opinion that Salmonellosis is a sporadic cause of enteritis and cause loss in young kid-goats and buffaloe-calves (**Bhullar and Tiawana, 1985**). *E. Coli* and Salmonella species were sensitive to garlic 10%. While with *Hibiscous sabdorifa* flowers 40%, *E. Coli* is sensitive but Salmonella species is less sensitive.

The significant decrease in serum total proteins, albumin, iron and copper, in diarrhoeic kid-goats may be referred to the cause of diarrhoea. Where, there was significant increase in bacterial enteritis This could be explained by impaired absorption of these trace elements through the damaged intestinal epithelium resulting from enterotoxins produced by these bacteria in the small intestine (**Kasari, 1990 and Aly *et al.*, 1996**). Concerning serum protein and albumin, they showed significant decrease in diarrehoeic kid-goats than the control group. Such drastic reduction may be attributed to diarrhoea, which lower the synthetic power of albumin in the liver due to microorganism. This opinion is supported by the finding of **Aly *et al.*, (1996)** and **Zaki *et al.*, (2001)**. The significant increase in alkaline phosphatase, lactate dehydrogenase was observed in diarrhoeic kid-goats. Similar results were observed by **Sadiek, (1987) and Zaki *et al.*, (2001)**.

A highly significant decrease in serum iron was noticed in diarrhoeic kid-goats, this result agreed with those obtained by **Aly *et al.*, (1996) and Zaki *et al.*, (2001)**. The decrease of iron was accompanied by decrease of copper and this lead to anaemia. (**Radostitis, 1992**)

Concerning cortisol hormone, an increase of this hormone can be considered as an expression of stress and helps the organism to counteract this

stress, haematological, metallic and endocrine changes enhanced protein catabolism and gluconeogenesis during endotoxaemia (**Dvorak *et al.*, 1974**). Growth hormone concentrations tended to be decreased in diarrhoeic kid-goats. An effect which was probably in part mediated by tumour necrotic factor (**Walton and Cronin, 1989**). Similar result in diarrhoeic lambs was recorded by **Zaki *et al.*, (2001)**.

It could be concluded that a substantial, bacteriological haematological, biochemical, and hormonal changes occur in diarrhoeic kid-goats when the cause of diarrhoea is enterotoxigenic bacteria. This means, the quick interference with therapeutics to decrease the damage of intestinal epithelium and supporting the body immune status during infection along side with the traditional electrolyte therapy.

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REFERENCES

Abou-Zaid, A. A and Nasr, M. Y. (1995): "Some studies on enterotoxaemia in calves". Alex. J. Sci.. 11: 10 5-111.

Aly, A.O.; Zamzam, H.A.; Kohilo, K.H and El-Sheikh, A. R. (1996): "Some studies on clinical, haematological and biochemical changes in diarrheic neonatal buffaloe-calves with reference to hygienic condition". Assiut Vet.Med. J. 35(69): 91-101.

Angus, K.; Appleyard, W.T.; Menzies, J.D; Campbell, I. and Sherwood, D. (1982): "An outbreak of diarrhoea associated with Cryptosporidiosis in naturally reared lambs". Vet. Record; 110: 129 – 130.

Baily, W. R. and Scott, E.G. (1990): "Diagnostic Microbiology. A text Book for The Isolation and Identification of Pathogenic Microorganisms". C.V. Mosby Company, Saint Lowis.

Belified, A. and Goldberg, D. M. (1971): "Colorimetric determination of alkaline phosphatase activity". Enzyme, 12:561.

Bhullar, M. S. and Tiawana, M. S. (1985): "Factors affecting mortality among buffalo-calve". Indian J. Anim. Sci., 55: 599-601.

Buhl, S. N. and Jackson, K. Y. (1978): "Determination of serum lactate dehydrogenase". Clin. Chem., 24: 824

Carter, G.R.(1984): "Diagnostic Procedures in Veterinary Bacteriology and Mycology'. 1st Ed., Charles, C., Thomas Publisher, USA.

Coles, E.H. (1986): "Veterinary Clinical Pathology". 4th Ed., W.B Saunders Co. Philadelphia, London, Toronto, p 215.

Doumas, B. T.; Watson, W. A.; and Biggs, H. G. (1971): "Albumin standards and the measurement of serum albumin with bromocresol green". Clinica Chimica Acta, 31: 87 – 96.

Durhm, P.J.K; Stevenson, B.J. and Farquharson, B.C. (1979): "Rotavirus and coronavirus associated diarrhoea in domestic animals". N.Z. Vet. J., 27: 30-32.

Dvorak, M.; Lebduska, J. and Oplish, M. (1974): "Adrenocortical response to E. Coli endotoxin in mediated and non-mediated calves'. Acta. Vet. Brno 43: 23- 32.

Farid, A. F.; Nashed, S.A. and Marcell, K.S. (1987): "Salmonellosis in bufaloe- calves in Upper Egypt". J. Egypt. Vet. Med. Ass. 47(182): 153-160.

Gad, W. and Well, G. (1967): "Statistical Methods". 6th. Ed. the Iowa stat Univ. Press Iowa, USA.

Henary, R.J.; Cannon, D.C. and Winkleman, J.W. (1974): "Clinical Chemistry Principles and Techniques." 2nd Ed. Harper and Roe, New York.

Issac, R.A. and Kerper, I. (1971): American Madison; 17.

Kasari, T.R. (1990): 'Metabolic acidosis in diarrhoeic calves: "The importance of alkalinizing agents in therapy". Vet. Clin. of North America: Food animal practice, 6: 29-43.

Kuchn,C. and Burvenich, C. (1986): "Cortisol and thyroid hormones after endotoxin administration in lactating goats". Arch. Int. Physiol. Biochem., 94: 37-38..

Radostits, O.M. (1992): Proc. Aust. Assoc . conference. Addlaides, P. 153, Cited in Vermunt, J.J. (1994): "Rearing and management of diarrhoea in calves to wearing". Aust. Vet. J., 7(2): 33-41.

Ronge, H. and Blum, J.W. (1988): "Somatomedin C and other hormones in dairy cow around parturition , in newborn calves and milk". J. Anim. Nutr., 60: 168-176.

Sadiek, A.H. (1987): "Clinical and some biochemical blood changes accompanying alimentary and respiratory manifestations among fattening buffalo-calves". M.V.Sci. Thesis Fac. Vet. Med. Assut Univ.

Snodgrass, D.R and Angus, K.W. (1983): "Diseases of Sheep". 1st Ed, Blackwell Scientific Publications, London, 43-48 pp.

Snodgrass, D.R; Herring J.A.; Linklater K.A. and Dyson , D.A. (1977): "A survey of rotaviruses in sheep in Scotland". Vet. Rec., 100: 344.

Tzipori, S.; Angus, K.W.; Cambell, and Clerihew, L.W. (1981): "Diarrhea due to Cryptosporidium infection in artificially reared lambs". J. Clin. Micro., 14: 100-105.

Walton P. and Cronin, M.J. (1989): "Tumour necrosis factor – alpha inhibits growth hormone secretion from cultured anterior pitutitary cells". Endocrinol., 125: 925-929.

Zaki, Mona, S.; Bayoumi, F.S. and El-Batrawy, N. (2001): "Some microbial and clinicopathological studies on lambs suffering from diarrhoeia". Egypt. J. Comp. Path. & Clinic. Path., 14(1): 10-17.

الملخص العربي

الإسهال في صغار الماعز حديثي الولادة مع الإتجاة الخاص إلى الأسباب البكتيرية والأعراض الإكلينيكية والتغيرات الدموية والبيوكيميائية

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تم عمل مسح لعدد ١٣٠ من صغار الماعز تتراوح أعمارها من يومين إلى ثلاث أشهر في مزارع خاصة بمحافظتي المنوفية والقليوبية، وكان من بين هذه الصغار ١٠٠ تعاني من الإسهال بدرجاته المختلفة.

وقد تم الفحص البكتريولوجي على عينات من الروث حيث أظهرت النتائج وجود الميكروب القولوني الإشرشيا كولاي (*E.coli*) بنسبة ٥٨ % والسلمونيلا بنسبة ٢٧ % والشيجلا بنسبة ١٥ % ، وتعتبر هذه الميكروبات من أهم مسببات الإسهال. وقد وجد بعد إجراء إختبار الحساسية أن هذه الميكروبات أكثر حساسية للمضادات الحيوية الشائعة، وأقل حساسية بالنسبة لمستخلصي الثوم ١٠% و زهور الكركادية ٤٠%.

بالنسبة للتغيرات في صورة الدم كانت هناك نقص معنوي في تركيز الهيموجلوبين وعدد كرات الدم الحمراء، مع زيادة معنوية في نسبة حجم خلايا الدم المضغوطة.

وأما بالنسبة للتغيرات البيوكيميائية في مصل الدم فقد كان هناك نقصاً معنوياً في مستوى كل من البروتين الكلي والألبيومين والحديد والنحاس وهرمون النمو، وعلى العكس من ذلك كانت هناك زيادة معنوية في مستوى هرمون الكورتيزون ونشاط إنزيمي كل من الفسفاتيز القلوي (Alkaline phosphatase) واللاكتيت ديهيدروجينيز (Lactate dehydrogenase) .

ومن هذا يتضح أن الإسهال يسبب العديد من التأثيرات الباثولوجية الإكلينيكية الضار، حيث إنه يسبب الأنيميا الشديدة ونقص هرمون النمو في صغار الماعز.