

Animal Health Research Institute, Beni-Suef.

**RELATION BETWEEN BOVINE FASCIOLIASIS
AND BACTERIAL LIVER INFECTIONS AS WELL AS
OTHER INTERNAL PARASITIC INFESTATIONS
IN COWS AND BUFFALOES IN BENI-SUEF
GOVERNORATE**

(With 7 Tables and One Figure)

By

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**العلاقة بين الإصابة بالفاشيولا واصابات الكبد البكتيرية وكذلك الإصابة
بالطفيليات الداخلية الاخرى فى الأبقار والجاموس بمحافظة بنى سويف**

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وجد بالفحص الطفيلي والبكتريولوجى لعينات ابقار وجاموس ان بها بعض الاصابات الطفيلية والبكتيرية، منفردة ومختلطة (فاشيولا وبكتريا او فاشيولا وطفيل آخر) من فحص ٢١٨ بقرة وجد ان (٨,٢٦%) ، (١١,٩٣%) و (١١%) و(١,٨٣%) منها مصابة بالفاشيولا، ديدان المعدة والامعاء، البارامفستوم والكوكسيديا بالترتيب، ووجد من فحص ١٦٧ جاموسة للطفيليات ان (٥,٩٩%) ، (١٠,١٨%) ، (٩,٥٨%) و (١,٢٠%) مصاب بالفاشيولا، ديدان المعدة والامعاء، البارامفستوم والكوكسيديا بالترتيب. وقد وجد بالفحص الطفيلي لعينات ابقار وجاموس ان بها بعض معدلات اصابة اعلى فى الأبقار عن الجاموس لكل الطفيليات السابقة. اما عن الاصابات المختلطة فكانت ١٢ بقرة (من ١٨ بقرة مصابة بالفاشيولا) بها طفيليات المعدة والامعاء، بنسبة ٦٦,٦٧% وخمسة ابقار مصابة بالبارامفستوم (من ١٨ بقرة مصابة بالفاشيولا) بنسبة ٣٨,٨٩%. وعند اجراء الفحص البكتريولوجى على ١١٠ عينه اخذت من اجزاء من اكبادة الأبقار والجاموس وأيضا الحوصلة المراربه تم عزل الميكروبات التاليه بالنسب التاليه الفيوزوباكتريم نيكروفيروم (١٣,٤%) والميكروب القولونى (١٢,٣٧%) وميكروب السيزانثيا مارسيسينس (١١,٣٤%) وميكروب السيدومونس أيروجنوزا (١٠,٣١%) وميكروب الأنثيروباكتير أيروجينس (٩,٢٨%) وميكروب الأكتينوميسس بيوجينس (٨,٢٥%) والميكروب العنقودى والسبحى بالنسب الأتيه على التوالى (٧,٢١%) و (٦,١٩%) وميكروب الأنثيروكوكس فيكالييس (٦,١٩%) وميكروب البروتيس فالجاريس (٦,١٩%) وميكروب الأنثيروباكتير فاكيوم (٥,١٥%) وميكروب البروتيس ميرابيلز (٤,١٢%). وقد تم التصنيف السيرىولوجى لعترات الميكروب القولونى المعزوله كما تم دراسة حساسية كلاً من الميكروب القولونى والميكروب العنقودى والميكروب السبحى لبعض المضادات الحيويه كما تم تحديد أقل نسبه تركيز لبعض اضافات الاغذيه الدوائيه التى تقتل ميكروبى الفيوزوباكتريوم والاكثينوميسس المعزولين.

SUMMARY

Parasitological examination of faeces, gall bladders and livers as well bacteriological examination of gall bladders and livers of cows and buffaloes, revealed the presence of some parasitic and bacterial infections either single or mixed infections (*Fasciola* with bacteria or with other parasites in one animal). Faecal examination of 218 cows, indicated that 8.26%, 11.93%, 11% and 1.83% cases were infested with *Fasciola*, Gastro Intestinal Parasites (G.I.P.), *Paramphistomum* and *Coccidia* respectively. Also from 167 buffaloes faecal samples 5.99%, 10.18%, 9.58% and 1.20% were infested with *Fasciola*, Gastro Intestinal Parasites, *Paramphistomum* and *Coccidia* respectively. There were higher rates of infestations with all mentioned parasites in cows than buffaloes. As for mixed infections, 12 from 18 fasciolised cows had faecal-borne parasites (66.67%), 5 of which were infested with Gastro Intestinal Parasites (27.78%) and the other 7 cows had paramphistomiasis (38.89%). Regarding buffaloes 3 from 10 (30%) had *Fasciola* and Gastro Intestinal Parasites beside 2 from 10 (20%) had *Fasciola* and *Paramphistomum*. Bacteriological examination of 110 samples of gall bladders and inflamed livers of cows and buffaloes terminated 88 positive samples, 49 of them were mixed with liver fluke infestations and 39 were pure bacterial infection. Different species of bacteria were detected in the examined samples at different rates as follow: *Fusobacterium necrophorum* (13.40%), *E. coli* (12.37%), *Serratia marcescens* (11.34%), *Pseudomonas aeruginosa* (10.31%), *Enterobacter aerogenes* (9.28%) *Actinomyces pyogenes* (8.25%) *Staphylococcus aureus* (7.21%), *Streptococcus uberis* (6.19%), *Enterococcus faecalis* (6.19%), *Proteus vulgaris* (6.19%), *Enterococcus faecium* (5.15%) and *Proteus mirabilis* (4.12%). Serological identification of the isolated *E. coli* determined that 4 strains were O111, 4 were O29, 3 were O26 and one strain was O164. Antimicrobial susceptibility of the isolated strains of *E. coli*, *S. aureus* and *Strept. uberis* recovered from the infected livers to different chemotherapeutic agents was discussed and the minimum inhibitory concentrations (MIC) for the isolates of *F. necrophorum* and *A. pyogenes* to some antimicrobial food additives were determined.

Key words: *Fascioliasis, bacterial liver infections, internal parasites, cows, buffaloes*

INTRODUCTION

The distribution of several pathogenic helminthes infections coincides geographically with many devastating microbial diseases, including enteric bacterial infections. Many authors dissected the mechanisms by which helminthes modulate the host's response to enteric bacteria and bacteria-mediated intestinal inflammation, Khan *et al.* (2002), Weng *et al.* (2007), Silvia *et al.* (2007). Ogunrinade and Adegoke (1982) in Nigeria mentioned that *Trypanosoma* spp., *Babesia*, *Paramphistomum* and *Schistosoma* were the most commonly found parasites associated with fascioliasis. The association between pyogenic abscesses caused by *Staphylococcus aureus* and worm infection has been described by Lambertucci *et al.* (1990 and 2001), Pinilla *et al.* (2001). Lambertucci *et al.* (1998) and Rayes *et al.* (2000) also found an association between tropical pyomyositis and infection by *Toxocara canis*.

On the other hand, liver infection with bacteria in cows and buffaloes is a major economic liability to producer, packers and consumers of beef, the liver may be infected with bacteria, liver fluke or both. Bacterial infection of liver occurs at all ages and in all animals, but it has more economic importance in feedlot animals. The aggressive grain feeding programs in feedlot animals may progress acidosis and rumenitis which leading to entrance of pyogenic cocci and other microorganisms to the liver through different routes (Nagaraja and Chengappa 1998). These microorganisms coalesce together to begin liver abscess process that varies in thickness and range in size from a minute pinpoint to over 15 cm in diameter and affected from 12 to 32% in most feedlots (Brink *et al.*, 1990). Moreover liver bacterial infection may ascend the billiary passage beside liver fluke may cause liver abscess and bilateral suppurative nephritis (Braun *et al.*, 2004).

The aim of this work was to determine the infestations of cows and buffaloes with some internal parasites with or without *Fasciola* in the same animal, and also determination of some bacterial causes of liver infection in the presence and absence of liver fluke. In vitro sensitivity of the isolated bacteria to different chemotherapeutic agents was also achieved.

MATERIALS and METHODS

Parasitological examination:

- Examined animals:

A- Cows:

1-Faecal samples:

Samples were taken from 218 cows (sporadic cases aged between 8 months to 6 years), each individual animal sample in plastic bag, labeled and numbered for each animal and sent directly to Beni Suef laboratory of Animal Health Research for parasitological examination

2- Gall bladders:

Parts from livers beside the whole gall bladders of 43 feedlot beef and adult cows were collected from Beni Suef abattoir (18 of them were infested with *Fasciola*) in plastic bags, numbered for each animal and sent to Beni Suef laboratory of Animal Health Researches in an ice box for bacteriological examination

B- Buffaloes:

1-Faecal samples:

Samples were taken from 167 buffaloes aged between 1-7 years, each individual animal sample in plastic bag, labeled and numbered for each animal and sent directly to Beni Suef laboratory of Animal Health Researches for parasitological examination

2- Gall bladder

Parts from livers beside the whole gall bladder of 67 feedlot beef and adult buffaloes were collected from Beni Suef abattoir (34 of them were infested with *Fasciola*) in plastic bags, numbered for each animal and sent to Beni Suef laboratory of Animal Health Researches in an ice box for bacteriological examination.

Method of parasitological examination:

Faecal examination was carried out according to Welch *et al.* (1987) to detect *Fasciola* eggs. Concentration floatation technique was carried out according to Soulsby (1982) for detection of nematodes infected animals. Centrifugal sedimentation floatation technique for *Eimeria* was followed according to Yakhchalim and Cheraghi (2007).

Bacteriological examination:-

Samples: -

Parts from livers of 67 buffaloes and 43 cows and the whole gall bladder of them were collected from abattoir of Beni Suef Governorate

in plastic bags and transported directly to Beni Suef laboratory of Animal Health Researches in an ice box.

Media used for isolation:-

- Blood agar medium.
- MacConkey agar medium (Oxoid).
- Tryptone soya agar medium (Biolife).
- Tryptone soya broth (Oxoid).
- Brain heart infusion broth (Biolife).

Bacterial isolation and identification:-

All samples were cultured onto different media in duplicated plates and incubated under aerobic and anaerobic conditions (in anaerobic jar containing 6% carbon dioxide) at 37°C for 24- 72 h according to Cruick- Shank *et al.* (1975) and Collee *et al.* (1996). The isolated colonies were purified and identified morphologically and biochemically according to Tan *et al.* (1994) and Collee *et al.* (1996).

Serological typing of isolated *E. coli*: -(according to Ewing, 1986).

Agar slants containing generous growth of the isolated strains of *E. coli* were submitted for agglutination test using polyvalent and monovalent O, *E. coli* antisera obtained from Denka Seiken Co., LTD. Japan Seiken Set 1 Code 312001

Antimicrobial susceptibility: -

1-For the isolates of aerobic bacteria: -

The disc diffusion technique was adopted according to Koneman *et al.* (1992). Antimicrobial discs and their inhibition zones were shown in Table (1)

2- For the isolates of *Fusobacterium necrophorum* and *Actinomyces pyogenes*: -

Minimum inhibitory concentration (MIC) to some antimicrobial food additives were determined in U shape microtiter dilution plates according to Reddy *et al.*, (1982) and Rosenblatt (1986). The plates were read visually (button in the bottom of the well) for growth. The MIC value was recorded as the lowest concentration of the antimicrobial compound that inhibited growth of the organism.

Table 1: Interpretation of zones of inhibition for antimicrobial susceptibility according to manufacture company (BiomMerieux)

Antimicrobial agent	Concentration\ μg	Zone of inhibition (mm)	
		Resistant (R) \leq	Sensitive (s) \geq
Cefotaxin	30	14	18
Rifamycin	30	21	22
Gentamicin	10	12	15
Ampicillin	10	22	30
Streptomycin	10	11	15
Oxytetracycline	30	14	19
Ceftiofur	30	19	23
Lincomycin +	15	16	20
Spectinomycin	200		
Florfenicol	30	12	18
Naldexic acid	30	14	16

RESULTS

Parasitological examination:

Faecal examination of 167 buffaloes and 218 cows as in Table (2) showed that 10, 16, 17 and 2 buffaloes had infestation with *Fasciola*, *Paramphistomum*, Gastro Intestinal Parasites and *Coccidia* spp. respectively with incidences of 5.99%, 9.58%, 10.18% and 1.20%. While, 18, 24, 26 and 4 cows had infestation with *Fasciola*, *Paramphistomum*, Gastro Intestinal Parasites and *Coccidia* respectively with percentages of 8.26%, 11%, 11.93 % and 1.83%. Belonging to double infestation with *Fasciola* and other parasites as in Table (3) from 10 buffaloes 3(30%) had *Fasciola* and Gastro Intestinal Parasites while 2 (20%) had *Fasciola* and *Paramphistomum*. Regarding cows, 7 from 18 (38.89%) were infested with both *Fasciola* and *Paramphistomum* species, while 5 (27.78 %) were found infested with both *Fasciola* and Gastro Intestinal Parasites. On the other hand coccidial infection was found in 2 non-infested buffaloes and 4 non infested cows with *Fasciola*.

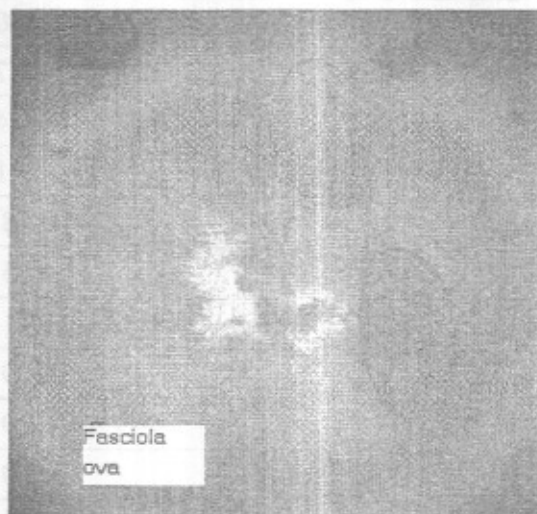
Table 2: Parasitic infestation (faecal examination).

Parasites	Buffalo		Cows	
	Infested	%	Infested	%
Fasciola	10/167	5.99%	18/218	8.26%
Paramphistomum	16/167	9.58%	24/218	11%
Gastro Intestinal Parasites (G.I.P.)	17/167	10.18%	26/218	11.93%
Coccidia	2/167	1.20%	4/218	1.83 %

Table 3: Mixed infestation with both *Fasciola* and other parasites.

Parasites	Buffaloes		Cows	
	Infested	%	Infested	%
Fasciola with Paramphistomum	2/10	20%	7/18	38.89%
with Fasciola(G.I.P)	3/10	30%	5/18	27.78%
Total	5/10	50%	12/18	66/67%

Fasciola ova



Bacteriological examination

Bacteriological examination of 110 gall bladder and parts of liver of buffaloes and cows revealed isolation of 97 isolates of bacteria from 88 positive samples as shown in Tables (4) & (5)

Table 4: Incidence of bacterial, *Fasciola* and mixed infection of liver and gall bladder of cows and buffaloes

Species	No of Examined Animals	Bacterial Infected Animals		Fasciola Infected Animals		Mixed Infected Animals		Bacterial infected animal without Fasciola		Fasciola infected animal without bacteria	
		No	%	No	%	No	%	No	%	No	%
		Cows	43	38	88.37	18	41.86	18	41.86	20	46.51
Buffaloes	67	50	74.63	34	50.75	31	46.27	19	28.36	3	4.48
Total	110	88	80	52	47.27	49	44.55	39	35.46	3	2.73

Table 5: Incidence of some bacterial isolates in infected livers of cows and buffaloes.

Species	No	%
<i>F. necrophorum</i>	13	13.40
<i>E. coli</i>	12	12.37
<i>Ser. Marcescens</i>	11	11.34
<i>Ps. Aeruginosa</i>	10	10.31
<i>Ent. Aerogenes</i>	9	9.28
<i>A. pyogenes</i>	8	8.25
<i>S. aureus</i>	7	7.21
<i>Strept. uberis</i>	6	6.19
<i>E. faecalis</i>	6	6.19
<i>Pr. Valgaris</i>	6	6.19
<i>E. faecium</i>	5	5.15
<i>Pr. mirabilis</i>	4	4.12
Total	97	100.00

Fig. 1: Frequent distribution of some bacterial isolates in infected livers of cows and buffaloes.

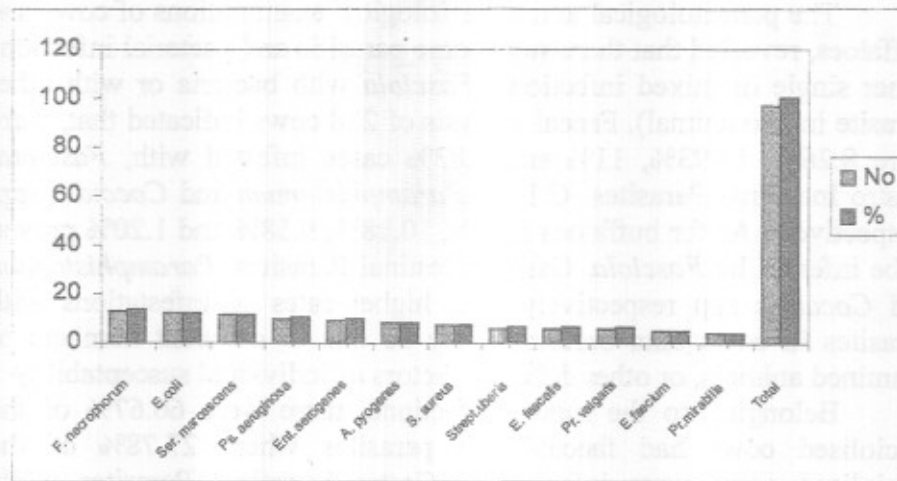


Table 6: Antimicrobial sensitivity of *E. coli*, *S. aureus* and *Strept. uberis* isolated from infected liver to different chemotherapeutic agents.

Chemotherapeutic Agents	<i>E. coli</i> (12)			Chemotherapeutic agents	<i>S. aureus</i> (7)			<i>Strept. uberis</i> (6)		
	R	S			R	S		R	S	
		No	%			No	%		No	%
Ceftiofur	0	12	100	Cefotaxin	0	7	100	0	6	100
Gentamicin	3	9	75	Rifamycin	0	7	100	0	6	100
Lincospectin+ Spectinomycin	4	8	66.67	Gentamicin	3	5	57.14	3	3	50
Naldexic acid	7	5	41.65	Ampicillin	5	2	28.57	3	3	50
Florfenicol	7	5	41.65	Streptomycin	7	0	0	4	2	33.33
Oxytetracycline	8	4	33.33	Oxytetracycline	3	4	57.14	2	4	66.67

Table 7: Minimum inhibitory concentration (MIC) for isolates of *F. necrophorum* and *A. pyogenes* to some antimicrobial food additives by (μ g/ml)

Antimicrobial food additives	<i>F. necrophorum</i>	<i>A. pyogenes</i>
Tylosin	8.9	5.5
Chlortetracycline	2.5	1.5
Oxytetracycline	3	1.2

DISCUSSION

The parasitological and bacteriological examinations of cows and buffaloes, revealed that there were some parasitic and bacterial infections either single or mixed infections (*Fasciola* with bacteria or with other parasite in one animal). Faecal analysis of 218 cows indicated that, there were 8.26%, 11.93%, 11% and 1.83% cases infested with, *Fasciola*, Gastro Intestinal Parasites (G.I.P.), *Paramphistomum* and *Coccidia* spp. Respectively. As for buffaloes 5.99%, 10.18%, 9.58% and 1.20% proved to be infested by *Fasciola*, Gastro Intestinal Parasites, *Paramphistomum* and *Coccidia* spp respectively. The higher rates of infestations with parasites in cows than buffaloes may be due to different numbers of examined animals, or other different factors as individual susceptibility.

Belonging to the mixed infections, there were 66.67% of the fasciolised cows had faecal-borne parasites where 27.78% of the fasciolised cows were infested by Gastro Intestinal Parasites while 38.89% from the fasciolised cows were infested by *Paramphistomum*. These results agreed with those of Ogunrinade and Adegoke (1982) in Nigeria who reported that 61.9% had faecal-borne parasites and 85.8% had positive bacterial isolates in the bile in cows infested with *Fasciola*. In buffaloes there were 50% had fecal-borne parasites, 30% from the fasciolised buffaloes were infested by G.I.P. and 20% were infested by *Paramphistomum*. The rate of mixed infestations with both *Fasciola* and Gastro Intestinal Parasites was higher in buffaloes than in cows, but lower in mixed infestation with both *Fasciola* and *Paramphistomum* than in cows. These mixed infections may be due to cross resistance which has also been demonstrated between *Schistosoma mansoni* and *F. hepatica*, by Christensen, *et al.* (1980) cited by Ogunrinade (1982), also Goose (1977) as cited by Ogunrinade (1982), demonstrated a depression of immune responses in *Fasciola hepatica* in rat but at the same time a "non-specific immunostimulant" effect acts against trypanosomiasis in the same animals were noticed. Mixed infestations by coccidian and *Fasciola* were n't observed in this work may be due to low rates of coccidiosis detected (1.83% in cows and 1.20% in buffaloes).

The bacteriological examination indicated that 80% of the examined samples as shown in Table (4) were positive, 44.55% of the examined samples were mixed with liver fluke infestations and 35.46% were pure bacterial infected samples. 2.73% of the examined samples contained liver fluke without bacterial infection this was due to some owners either used antimicrobial food additives in the animal rations, the

animals were administrated antimicrobial agents before slaughter or their was no factor to cause bacterial infection at all. Different species of bacteria were detected in the examined samples (Table 5) either alone or in combination with coliform bacilli which were detected at different rates as follows: *E. coli* 12.37%, *Serratia marcescens* 11.34%, *Proteus* spp. 10.31% and *Ent.aerogenes* 9.28%. These results agreed with Abdel-Fattah *et al.* (1995) who isolated coliform bacilli from 26 samples of inflamed cattle livers, Nagaraja *et al.* (1999) could isolate *E. coli*, *Proteus* spp. and other facultative bacteria from 22% of liver samples with abscess, Reinsten *et al.* (2007) who isolated *E. coli* O157: H7 from 0.54% of gall bladder of cattle and Matsumoto *et al.* (2008) who isolated coliform bacilli from 52 out of 232 bile samples of cattle. *Staphylococcus* and *Streptococcus* spp. were isolated from the infected livers at a rate of 7.21% and 6.19% respectively, similar results were recorded by Berg and Scanlan (1982) and Lechtenberg *et al.* (1988). *Enterococcus* and *Pseudomonas* species were isolated at a rate of 11.34% and 10.31% respectively, El-Dakhly *et al.* (2007) could isolate them from liver of cattle, sheep and camel at a rate of 25.92% and 3.70% respectively. *F. necrophorum* was detected in 13.40% of samples and this result agreed to some extent with Nagaraja *et al.* (1993) who isolated it from 100% of liver abscess. Abdel-Fattah *et al.* (1995) isolated *F. necrophorum* from 26 inflamed livers and 30 liver abscess of cattle. Eight isolates of *A. pyogenes* were isolated from inflamed livers and gall bladders (8.25%), this result resemble the results recorded by Nagaraja *et al.* (1999) who isolated it from 10% of liver samples. However, high rate was recorded by Abdel-Fattah *et al.* (1995) who isolated it from 17 out of 26 inflamed liver samples of cattle.

Regarding to serological identification of 12 isolates of *E.coli* obtained from inflamed liver samples and gall bladders, the serogroups O were as follows O111 (4isolates) O29 (4isolates) O26 (3isolates) and O164 one isolate. Similar results were recorded by Blanco *et al.* (1993) who isolated *E. coli* O26 from cattle with diarrhoea, Orden *et al.* (1999) and Roopnarine *et al.* (2007) who isolated *E. coli* O111 and other serogroups from cattle and calves with diarrhoea.

Regarding to antimicrobial susceptibility, Table (6) indicating that all isolated *E.coli* strains were sensitive to ceftiofur, 75% to 66.67% of the tested strains were sensitive to gentamicin and lincospectin+ spectinomycin and moderately sensitive to florfenicol, naldexic acid and oxytetracycline. These results agreed with the results recorded by Ahmed *et al.* (1994), Bywater *et al.* (2004) and Checkley *et al.* (2008). All

Staphylococcus and *Streptococcus* isolates were susceptible to cefotaxin and rifamycin, moderately resistant to gentamycin, ampicillin and oxytetracycline. Guerin *et al.* (2002) recorded that all *Strept. ubris* strains isolated from clinical mastitis were susceptible to chloramphenicol and rifampicin also similar results were recorded by Hendriksen *et al.* (2008) who reported that some *Staphylococcus* and *Streptococcus* isolated from cattle exhibited some resistant to gentamycin and tetracycline. Two isolates of *Strept. ubris* (33.33) were streptomycin sensitive and 66.66% of the isolates were resistant. This results are similar to the results recorded by Owens *et al.* (1990) who indicated that 62% out of 144 strains of *Strept. ubris* were resistant to streptomycin and Ebrahimi *et al.* (2008) who demonstrated that *Strept. ubris* isolated from mastitic milk showed high level of resistance against streptomycin.

In case of M.I.C of tylosin, chlortetracycline and oxytetracycline for *F. necrophorum* and *A. pyogenes* which were shown in Table (7), the results cleared that *F. necrophorum* isolates were susceptible to high concentrations of tylosin, chlortetracycline and oxytetracycline more than *A. pyogenes* isolates and this may be due to over use of these antimicrobial agents, these results nearly agreed with the results recorded by Lechtenberg and Nagaraja (1989), Nagaraja *et al.* (1993) and Tan *et al.* (1994).

In conclusion different species of bacteria were isolated from hyperemic and serotic livers samples and gall bladders of cattle, the occurrence of this bacteria either due to the aggressive grain feeding programs in feedlot animals which progress acidosis, rumenitis and consequently liver infection or due to the cirrhosis caused by liver fluke, which progress nephritis and liver infection or both causes coalesce together to produce liver infected with bacteria.

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