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THE INCIDENCE OF FASCIOLIASIS AND SOME TYPES OF BACTERIA INCURRED CAUSING LACK MEAT AND LIVERS QUALITY OF SLAUGHTERED ANIMALS AT AL-TAIF, KSA.

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ABSTRACT

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This study, reviews a one year period (2013), recorded of slaughtered house (sheep) at Al-Taif, KSA. It aimed for determination the prevalence of Fascioliasis and abscesses affecting the liver of slaughtered animals. A total of 18,925 sheep were slaughtered and 3501 (18.5%) livers of sheep were condemned due to fascioliasis and abscesses affections. Of the slaughtered sheep were a significantly (p0.05), higher prevalence of Fascioliasis was recorded sheep (3.1%). Fascioliasis considered the most cause of liver condemnation and was responsible for total liver condemnation for sheep as 16.9%., Out of 60cases, 20(3.3%) were apparently infected with acute suppurative hepatitis while 40 (6.6) out of 60 cases appeared to be infected with chronic hepatitis. A total of 19 bacterial isolates were recovered from acute suppurative hepatitis specimens, 7(35%) *S. aureus*, 5 (25%) *E.coli*, 6 (30%) *Clostridium perfringens* and1 (5%) *Clostridium novyi* respectively. While in case of chronic hepatitis 64 bacterial isolates were detected, 1 (2.5%) *S. aureus*, 16 (40%) *E. coli*, 5 (12.5%) *Klebsiella spp*, 25 (62.5%) *Clostridium perfringens* and1 (2.5%) *Clostridium novyi* respectively.

Key words: *Fascioliosis, Flukes, Fasciola gigantica, sheep s.aueus, E.coli*

INTRODUCTION

Fascioliasis also known as distomatosis and liver rot, is an important helminth disease caused by two trematodes Fasciola hepatica (the common liver fluke) and Fasciola gigantica. This disease belongs to the plant-borne trematode zoonoses. In Europe, the Americas and Oceania only F.hepatica is a concern, but the distributions of both species overlap in many areas of Africa and Asia (Mas-Coma *et al.,* 2005). Fascioliasis is caused by F.gigantica, a digenetic trematode which belongs to the family Fasciolidae. They are very important liver parasite of sheep (Ruminants). The adult inhabits the bile duct and gall bladder of liver in these animals. Inside their host, the liver flukes cause severe damage which may lead to the death of the animals (Anosike *et al.,* 2005). Fascioliasis is one of the helminthes trematodes that constitute both economic and public health constraints to a profitable ruminant production in tropical Africa (Adams and Mckay, 1966; Fabiyi and Adeleye, 1982). Fascioliasis has been implicated as the cause of morbidity and mortality in the production of ruminants (Onwuliri *et al.,* 1993; Okoli, 2001). Among diseases which are not often apparent to farmers but are of considerable economic and public health importance are the liver fluke infections (F.hepatica, F.gigantica) (Mas-coma *et al.,* 1997). The liver of the infected ruminant is damaged and partially or completely condemned and the sub-clinical and chronic disease usually results in decreased production of meat, milk and wool. Furthermore, secondary bacterial infections, fertility problems and great expenses with anti-helminthes therapies should be considered (Black and Froyd, 1972; Coop and Sykes, 1977; Hope *et al.,* 1977; Daryani *et al.,* 2006). Fascioliasis is an important helminth disease caused by two trematodes, F.hepatica (the common liver fluke) and F.gigantica. The disease belongs to the plant borne trematodes zoonoses. In Europe, the Amercas and Oceania only F.hepatica is a concern, but the distributions of both species overlap in many areas of Africa and Asia (Mas-Coma *et al.,* 2005). These ruminants however have also been found to harbor other helminthes mixed infections (Anosike *et al.,* 2005). Such as Dicrobothriasis, cotylophomiasis among others. This infection brings about decrease in meat production and subsequent economic loss. The nutritional and health value of the animal deteriorates leading to poor carcass quality and loses (Shar-Fisher and Say, 1989). It was shown that the estimated annual loss to live stock due to fasciolosis all over the world was more than 2000million dollars (Mungube *et al.,* 2006). Ovine fasciolosis can result in blood-loss representing a loss of metabolizable energy that has an adverse effect on weight gain (Hope, 1984; Mitchell, 2002). Demonstrated 22%, 26% and 33% weight loss with a mean of 87, 157 and 233 flukes in sheep. It is stated that even low rates of fluke infection in cattle can

cause significant reduction in performance and infection with 54 flukes/animal resulted in 8-9% reduced weight gain. Even after the animals are cleared of fluke, the initial impaired performance remains until slaughter (Coop and Sykes, 1977; Hope *et al.,* 1077). Fascioliasis is a significant live stock problem; yearly an estimated US$ 2 billion are foregone due to weight loss, reduction in milk yield and fertility in production animals. Fascioliasis is a dangerous disease leading to a huge economic losses in live stock production and causing severe illness in human livers. (Mc Manus and Dalton, 2006; Hussein and Khalifa, 2009). Liver is considered the most important organ for animal health production and reproduction. Many of the metabolic activities of the body occurred in the liver. Liver infection is an important disease that affects all kinds of meat producing animals, this lead to great losses to live-stock production and national income due to condemnation of great numbers of livers in the slaughter houses (Foster and Woods, 1970; Tomate, 1973). Fasciola plays an important role in the microbial invasion of the infected animals either by transportation or by depressing the vital resistance of the host. Besides, infected liver constitute a good media for bacterial multiplication, transportation of micro-organisms with the parasites occurs during the different stages of its life cycle either outside or inside the animal body. Anaerobic necrotic lesions of the liver produced by immature flukes occasionally provides a suitable environment for the germination of spores of Clost.novyi type B bacteria in the liver The bacteria will release toxins into the blood stream resulting in what is known as black disease in sheep and sometimes cattle. Importance of cattle fascioliasis in economic losses caused by condemnation of livers at slaughter and production losses especially due to reduce weight gain (Eguale and Abie, 2003). Bovine liver infested with mature Fasciola worms. Out 30.4% were apparently infected with acute supportive hepatitis while 69.6% appeared to be infected with chronic hepatitis. A total of 35 bacterial isolates (18 anaerobic and 17 facultative anaerobic) were recovered from acute supportive hepatitis specimens. Fuso-bacterium necrophorum was the most predominant anaerobic isolates (7 isolates) and Arcano-bacterium pyogenes was the most frequently isolated facultative anaerobic organisms (6 isolates). While, poly-microbial isolation were detected in 76.5% instances. In case of chronic hepatitis *Clost. perfringens* was the most predominant isolated anaerobe (26 isolates), E.coli was the most frequently isolated facultative anaerobe (17 isolates) and Poly-microbial isolation were detected in 87.2% instances. A total 31 Clost.perfringens isolates were tested for its toxin by using dermo-necrotic reaction in guinea pig. *Clost. perfringens* type A represented 40% and 65.4% while, type D represented 60% and 23.1% for acute and chronic hepatitis, respectively (Sohair and Eman, 2009).

The aim of the study to determined the percentage of infestation by Fascioliasis among sheep at Al-Taif governorate. This study was carried out to enumerate the pathological hepatic lesions induced by bacteria in liver infested with Fasciola and to detect the relationship between these bacterial infections and liver fascioliasis.

MATERIALS and METHODS

The study was conducted at Al-Taif slaughtered house of sheep, KSA. It was done during 2013. The study populations were sheep of different ages and body conditions for the purpose of meat production. Simple random sampling method was used to select the study units. A cross-sectional study was used to determine the prevalence of fascioliasis in sheep slaughtered. A total of samples comprising of sheep (n=355) were collected from the selected animals to determine the prevalence of fascioliasis in the abattoir. Species, age and body condition of the animal were considered as potential risk factors for the occurrence of fascioliasis. Routine post mortem inspection of liver and gall bladder of each animal was carried out to check for the presence of Fasciola. Livers and gall bladders were dissected carefully. Livers were inspected by making multiple deep incisions of the lobes and making a deep cut with a number of small sub cuts. Gall bladders were opened using a knife and thoroughly investigated for the presence of Fasciola. Concerning daily condemnation of livers for sheep suffering from Fascioliasis were recorded. The data were subjected for studying to get the percentages of liver condemnation from the period (2013) in Al-Taif slaughtered house, KSA.

Identification of Fascioliasis: Investigation and identification of Fasciola was done according to their distinct morphological characteristic following the standard guidelines (Urquhart *et al.,* 1996). The study was a retrospective slaughtered house survey which used its data for period of 2013, and involved 18.925 sheep was slaughtered at Al-Taif abattoir, KSA. Also more samples of adult worms were collected from liver which had active infection for Fasciola spp. identification. Thereafter, 60 samples randomly collected from bile duct of infected livers of sheep and were identified according to (Soulsby, 1982).

Identification of Bacteria:

Samples:

Specimens from 60 livers were collected from Al-Taif slaughter house in the period 2013. Grossly, 40 samples were found to be infected with chronic hepatitis while, 20 samples showed liver abscesses in association with liver fluke infestation. Parts of the affected livers were collected at the time of slaughter separately in plastic bags under sterile condition and transferred directly to the laboratory for bacteriological examination. The other parts were

kept in 10% neutral buffered formalin for pathological examination.

- **Bacterial isolation and characterization**:

The surface of the liver were seared with hot spatula and then incised with a sterile scalpel.

-Aerobic identification: A loopful from each affected livers was streaked onto the nutrient agar, MacConkey agar and blood agar plates, then incubated aerobically at 37 °C for 24 hrs. Isolated colonies of various types (based on morphological appearance) were picked from plates and sub cultured on blood agar plates. Bacterial isolates were identified morphologically, culturally and biochemically according (Quinn *et al.,* 2002).

- Anaerobic identification: A loopful from each affected deep tissue of livers was inoculated into two tubes of freshly prepared cooked meat broth, one of them was heated at 80 °C for 10 minutes, to eliminate non spore forming organisms while the other tube was left without heating, both tubes were incubated anaerobically at 37 °C for 48 hrs. A loopful from each heated tube were streaked onto blood agar plate for isolation of spore forming anaerobes while, loopfuls from non heated tubes were streaked onto neomycin blood agar and brain-heart infusion blood agar (BHIBA) plates for isolation of *Clost. perfringens* and non spore forming anaerobes, respectively. All plates were examined after anaerobic incubation for 2 to 3 days and each colony type was sub-cultured for identification (Konoman *et al.,* 1992). *Clost. perfringens* isolates were typed using the intra-dermal inoculation test in Albino Guinea pigs (Sterne and Batty, 1975).

Data Analysis: The data which were recorded during the study period were entered into Microsoft excel sheet. Data were summarized and analyzed using SPSS version 16 computer program. Data were analysed using Epi Info version 6 statistical software and for further compared using Chi-square test at critical probability of $p<0.05$.

RESULTS

Table 1: The prevalence of Fascioliasis among slaughtered animals

Year	No. Slaughtered	No. of infested	%
2013	18,925	3501	18.50

Table 2: The prevalence of condemned livers due to Fascioliasis and hepatic Abscessation

Disease condition	condemned %	Livers No.
		Sheep No. = 355
Fascioliasis		592 (16.9%)
Hepatic Abscessation		209 (8.3%)

Table 3: Incidence of the aerobic and anaerobic microorganisms isolated from examined livers samples.

Type of liver samples Bacterial isolates	Acute suppurative hepatitis with fasciola (No. = 20)		Chronic hepatitis with fasciola (No.=40)	
Aerobic bacteria	No.	%	No.	%
S. aureus	7	35	1	2.5
E. coli	5	25	16	40
Klebsiella spp.	-	-	5	12.5
Total	12	60	22	55
Anaerobic bacteria				
C. perfringens	6	30	25	62.5
C. novyi	1	5	1	2.5
Total	7	35	42	105

DISCUSSION

The findings of this study showed the prevalence of Fascioliasis in slaughtered animals (sheep) at Al-Taif, KSA during year 2013. The prevalence of Fascioliasis among slaughtered animals was 18.925, while condemned livers due to Fascioliasis and hepatic Abscessation was 16.90 and 8.30 respectively .Indeed, Fascioliasis was the proper cause for condemnations (Mungube *et al.,* 2006). The total infections rate of fascioliasis lower if compared with 21.9% in Riyadh governorate abattoir (Magda and Al-Megrin, 2005). While the prevalence was (0.04%-2.4%) (Mgzoub and Kasim, 1978), also it was 0.28% in KSA (Ghandour *et al.,* 1989), this different in results may be due to the bid diameter of KSA, so there are more types of climate and ecological conditions, also seasons, sources and types of animals. Fasciolosis considered the main cause of liver condemnation and was responsible for total liver condemnation in cattle as 52.06%. The economic importance of such infections in terms of lost meat and offal were also estimated as 75000 SR annually (Nabila S. Degheidy and Jamila S. Al-Malki 2012). F.gigantica was distinguished among examined samples, while F.hepatica was difficult to be differentiated from F.gigantica on morpho-metric and morpho-anatomic criteria, better to call Fasciola spp. (Mas-Coma *et al.,* 1999) and Liver is considered the most important organ for animal health production and reproduction.

It is important to evaluate the state of health of liver since this organ is involved in many disease processes either primarily or secondarily and also because any liver damage disturb metabolic processes that are vital for normal health and optimum productivity (Sayed *et al.,* 2008).

The infestion with fasciola were accompanied by liver abscesses and this may be attributed to that bacteria were acquired by the flukes in the small intestine of ruminants and during migration, that may suggest that the flukes can spread highly pathogenic bacteria (Al-Khafaji and Rhaymah, 1993).

Bacteriological examination of the samples with acute suppurative hepatitis revealed that the incidence of aerobic and anaerobic organisms were 60% and 35%; respectively Similar findings were described by Rosa *et al.* (1989), El-Sayed *et al.* (1991) and Dore *et al.* (2007). Table (2) showed The aerobic bacteria isolated from acute suppurative hepatitis, specimens have included *S. aureus and E.coli* in an incidence of 35%, 12%, respectively. While anaerobic bacteria have included *Clostridium perfringens* and *Clostridium novyi* in an incidence of 30%, 5%, respectively The presence of some members of family Enterobacteriaceae in combination with other Clostridial bacteria such as *C. perfringens* may be explained by Itman (1989) that infection with pathogenic *E. coli* may cause altering the mucous membrane of the intestine, enable the clostridial microorganisms to vegetate and release its toxins which absorbed through the damaged gut and reached to the blood circulation and then to the liver Itman (1989). On the other hand, Morshdy *et al.* (1991) considered *E. coli* as an incidental pathogen which contaminated the animal tissues during preparation of the carcasses from faecal material, skin and hides. The aerobic bacteria isolated from chronic hepatitis, specimens have included *S. aureus, E.coli and Klebsiella spp.* in an incidence of 2.5%, 40 %and 12.5% respectively. Bacteriological examination of samples with chronic fascioliasis revealed that the incidence of aerobic and anaerobic organisms were 55% and 105%; respectively.

Table (2) showed the aerobic bacteria isolated from chronic hepatitis was *S. aureus, E.coli and Klebsiella spp.*in an incidence of 2.5%, 40% and 12.5% respectively. While anaerobic bacteria have included *Clostridium perfringens and Clostridium novyi* respectively in an incidence of 62.5%, 2.5%, respectively These results agree with the previous findings of Srokina (1987); Samad and Haque (1987) who found that fasciola infestation plays an important role in stimulation of clostridium infection especially *C. perfringens* due to the damages which were attributed to toxic environment created by the organisms in liver tissue.

CONCLUSION

Fasciolasis is an endemic disease in the world and causes sever economic conditions and affecting large and small animals. Fasciolasis considered the most cause of liver condemnation and was responsible for total liver condemnation for sheep .The percentage of fasciola infection during a year 2013 was 18.925 among sheep in Al Taif slaughter house. Also fasciola worms may incriminated in aiding bacterial infections specially C. perfringenes and E. coli which lowering the hepatic viability. Generally, livers of slaughtered cattle are considered as hazardous source of different mixed bacterial species. Moreover, their low value referred to high incidence of pathological lesions.

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دراسة لنسب حدوث الاصابة بالفاشيولا وبعض أنواع البكتريا المسببة لانخفاض جودة لحوم وأكباد الحيوانات المذبوحة في الاغنام بالطائف، السعودية.

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أجريت هذه الدراسة لمدة **عام** واحدة (٢٠١٣) وسجلت حالات الأغنام المذبوحة بسلخانات الطائف، بالسعودية. كان الهدف من هذه الدراسة تحديد نسب حدوث مرض الفاشيولا وكذلك الاصابات البكترية والخراريج التى تصيب أكبد الحيوانات المذبوحة نتيجة لهجرة ديدان الفاشيولا. كان أجمالى الحيوانات التي تم فحصها ١٨٩٢٥ غنم بينما أعداد المصاب ٣٥٠١ بنسبة أصابة (١٨%) في أكباد الاغنام . بالفحص البكتريولوجي وجد أن ٢٠ من ٦٠ حالة مصابة بالتهاب الكبد القيحى الحاد بنسبة (٣و٣%) بينما ٤٠ حالة مصابة بالتهاب الكبد المزمن. تم عمل مسحات من الاكباد المصابة بالتهاب القيحى الحاد وعزل الميكروب العنقودى الذهبى ، الميكروب القولونى ، الكلوستريديوم برفيرنجيس والكلوستريديوم نوفياى بنسبة (٣٥% و ٢٥% و ٣٠% و ٥% على التوالى). كذلك تم عمل الفحص البكتريولوجى لمسحات الاكباد المصابة بالتهاب المزمن وعزل الميكروب العنقودى الذهبى ، الميكروب القولونى ، الكلوستريديوم برفيرنجيس والكلوستريديوم نوفياى بنسبة (٥و٢% و ٤٠% و ٥و١٢ % و ٥و٦٢% و ٥و٢% على التوالى.