FASCIOLA AS A ZOONOTIC PARASITE IN SLAUGHTERED ANIMALS AT KALYOBIA ABATTOIRS

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A B S T R A C T

A total of 360 cattle and 360 buffaloes carcasses of different ages and sexes were examined at Kalyobia abattoirs from January to December, 2011. The obtained results indicated that the prevalence of Fasciola species in the slaughtered cattle (3.67%) was lower than that in slaughtered buffalo (5.56%). Generally, females (6.67% & 11.67%) were more susceptible to infection with fasciolosis than males (2.08% & 2.5%) among slaughtered cattle and buffalo, respectively. However, The Histopathological examination of the liver infested with fasciola showed newly formed bile ductules with inflammatory cells infiltration and fibrosis associated with hyperplasia in the lining epithelium with polyps formation as well as the portal area showed severe fibrosis with inflammatory cells infiltration. Regarding PCR technique, the using of Eael restriction endonuclease enzyme as a genetic marker for *F. hepatica* is greatly effective when the enzyme uniquely fragmented the SrRNA gene into two bands without digesting the gene of *F. gigantica*. Out of 200 stool samples collected from human of different ages and sexes at Kalyobia province, 4%were positive for Fasciola eggs. Thus, children between one and fifteen years old represent the highest infection (5.88%) than individuals between sixteen and thirteen (4%). On the other hand, the lowest infection was observed in individuals between thirty one and forty five (1.75%).

KEY WORDS: Abattoirs, Fasciolosis, PCR.

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1. I N T R O D U C T I O N

Fascioliasis is a serious infectious parasitic disease infecting domestic ruminants and humans. It has a worldwide distribution in a large variety of grass-grazing animals as sheep, goats, cattle, buffaloes, horses and rabbits. In Egypt, donkeys and camels are hosts for Fasciola species [17]. Furthermore, the disease constitutes a major public health problem in several areas of the world, including the highlands of Bolivia, Ecuador and Peru, the Nile Delta in Egypt, and central Vietnam [18].

Human fascioliasis causes serious hepatic consequences due to the severe damage which occurs in the liver cells mainly during the early migrating stages of the flukes. The disease affects the general immune status of man, and there is no accurate method adopted for early diagnosis of such disease before the time of egg deposition [11].

Fasciola hepatica and Fasciola gigantica can generally be distinguished on the basis of their morphology, but the use of molecular methods and markers are necessary for species confirmation and to distinguish the intermediate forms [2, 30]. The two species and their intermediate forms can be discriminated by sequences of the first (ITS-1) Internal Transcribed Spacers, the 5.8S, and second (ITS-2) Internal Transcribed Spacers (ITS) of the nuclear ribosomal DNA (rDNA), 28S

ribosomal ribonucleicacid (rRNA) [23, 24].

2. MATERIAL AND METHODS

2.1. *Sampling*:
Collection of samples from slaughtered animal was done thorough routine post mortem examination of slaughtered animals. Thus, gross inspection was done on each slaughtered animal including, the whole carcass and the internal organs.

2.2. *Detection of tissue parasites*:
Examination of Fasciola species by traditional method was carried out according the technique recommended by Carleton [6].

2.3. *Histopatholgical examination*:
Histopatholgical examination of naturally infested tissues was applied according to Banchroft et al. [3].

2.4. *Characterization of Fasciola species by PCR*
2.4.1. *Extraction of genomic DNA*
The total genomic DNA of the two species of Fasciola hepatica and Fasciola gigantica was extracted by using the UNSET lysis solution according to the technique recommended by Hugo et al. [22]. 1µl of the suspended pellet was checked by 0.8% gel electrophoresis for the presence of DNA.

2.4.2. *Nuclear subunit ribosomal RNA (Sr. RNA) gene detection*
The nuclear small Sr RNA genes of the two species of Fasciola were detected by using the following primers and the program of PCR for amplification of nuclear SrRNA was 30 cycles for 1 minute at 94°C, 2 minutes at 45°C and 3 minutes at 72°C according to Stohard and Rollinson [36].
SSU1:
(5, CGACTGGTTGATCCTGCCAGTAG- 3)
SSU2:
(3, TCCTGATCCTTCTCAGGTTCAC – 5,)

2.4.3. *Restriction fragment length polymorphisms profiles*
Restriction endonuclease represented by Eael (Roche Applied Science) was used to identify and differentiate the nuclear small subunit ribosomal RNA (SrRNA) gene of the two species of Fasciola. For each digestion reaction, 1µl was used together with 1.2µl of the particular enzyme buffer for a final volume of 12.2µl. The digestion was carried out for 3.5 hr at 37°C and the digestion products were evaluated on 2% TE agarose gels and stained with ethidium bromide. Accordingly, the restriction patterns were detected upon ultraviolet transillumination and photographed.

2.4.4. *Analysis of PCR amplified products*
Accurately, PCR amplified products were analyzed by agarose gel electrophoresis on 1.4% gel containing ethidium bromide dye (0.5µl/ml).

2.5. *Collection and Examination of Human stool specimens*
Accurately, 200 stool samples were collected according to the technique adopted by Garcia and Bruckner [15] and examined by: Direct smear method [4] and Kato thick smear method [25].

3. RESULTS AND DISCUSSION

3.1. *Prevalence of Fasciola species*:
The present results achieved in Table (1) declared that the overall prevalence of fasciola species in slaughtered cattle (3.61%) this result is found within the range recorded by previous authors [16, 27] in Egypt (3.54%) and in Coast province (3.5%). On the contrary, higher prevalence was reported formerly [5, 33] in Zambia (53.9%, 28.24%). On the other hand, the present results were higher than those recorded in earlier studies (2.34%) [40].
The seasonal dynamics of *Fasciola* species in cattle and buffalo regarding Table (1&2) it is evident that the highest percentage was detected in autumn

(5.56%) and (7.78%), followed by Spring& Summer (3.33%) and (5.56%) then Winter (2.22%) and (3.33%), respectively. The highest prevalence in autumn may be explained as *Fasciola* cercaria and Lymnaea snails have been found to survive better at 25-20 c which explains the higher prevalence at Autumn, however, the most important factors influencing the prevalence of Fasciola are temperature and moisture which affect the hatching of fluke ova, the viability of encysted metacercaria and population of snails [32]. Our results agree with former studies [7, 37]. On contrast, earlier authors [26] reported that higher incidence of fasciolosis was showed at winter (39.08%) followed by spring (29.50%), autumn (20.33%) and summer (12.92%). These differences may be attributed to the variation in agro-ecological conditions favorable to the parasite and the intermediate host such as altitude, rainfall, temperature, livestock management system, and suitability of the environment for survival and distribution of the parasite as well as the intermediate host. One of the most important factors that influence the occurrence of fasciolosis in a certain area is availability of suitable snail habitat [38].

Concerning *Fasciola* species in buffalo, Table (2) declared that their prevalence in slaughtered buffalo (5.56%) was higher than that in slaughtered cattle (3.61%). Nearly similar findings were recorded by earlier studies [29] 5.86%. On contrary, higher prevalence was noted previously 78.73% [35] and 62.7% [12]. The lower prevalence of fasciolosis (1.58%) was mentioned by former authors [17]. In general, females (6.67%&11.67%) were more susceptible to infection with fasciolosis than males (2.08%& 2.5%) among slaughtered cattle and buffalo, respectively as shown in Tables (1&2). These results agree, quite well, with Phiri et al. [33].

3.2. *Histopathological changes due to fasciola species*

The histopathological examination of the liver due to *fasciola* species revealed the portal area showed newly formed bile ductules with inflammatory cells infiltration and fibrosis (Fig. 1). Part of the parasite was embedded in the lumen of the bile ducts associated with hyperplasia in the lining epithelium with polyps formation and periductal inflammatory cells infiltration (Fig. 2). The fibroblasts were originated from the portal areas and dividing the hepatic parenchyma into lobules (Fig. 3). These results come in accordance with those reported previously [1, 9].

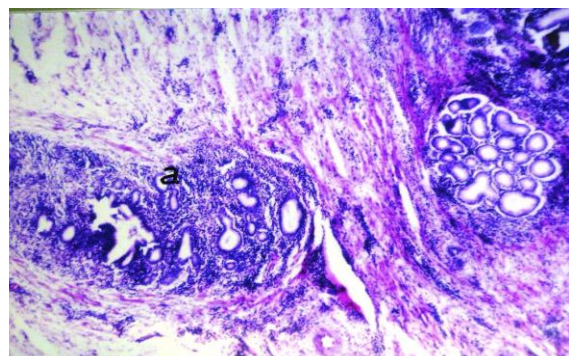

Fig. 1 Liver of cattle showing fibrosis in the wall of bile duct with inflammatory cells infiltration and newly formed bile ductules (a)

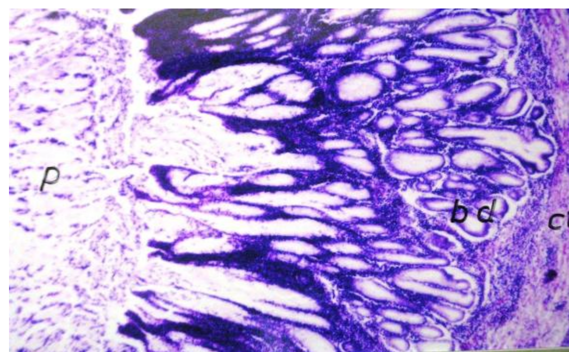

Fig. 2 Liver of cattle showing part of the parasite embedded in lumen of bile ducts (p) associated with hyperplasia in the lining epithelium of bile ducts (bd) with polyps formation and periductal inflammatory cells infiltration (ct).

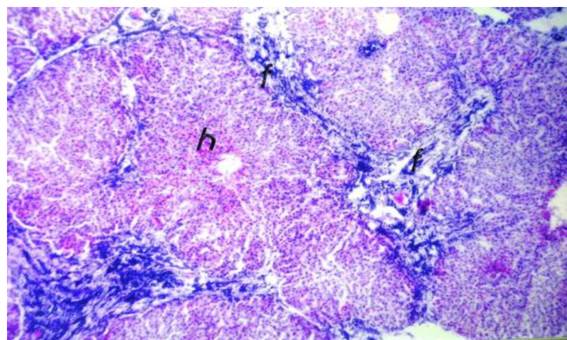

Fig. 3 Liver of cattle showing fibrosis (f) arising from the portal area dividing the hepatic parenchyma (h) into lobules.

3.3. *Characterization of Fasciola Species by PCR*

Concerning the application of PCR for differentiation of two species of *Fasciola* by using specific primers of *F. hepatica*, results achieved in fig (4) indicated that 8 samples had positive bands related to *F. hepatica* and 2 negative bands representing *F. gigantica*. Consequently, the using of Eael restriction endonuclease enzyme as a genetic marker for *F. hepatica* was greatly effective when the enzyme uniquely fragmented the SrRNA gene into two bands without digesting the gene of *F. gigantica*. The current results were nearly similar with those obtained by previously [21, 29). On the other hand, simple and rapid PCR- RFLP to distinguish *F. hepatica* from *F. gigantica,* based on a 618 bp long sequence of 28 SrRNA gene recently obtained from liver fluke populations, and found few nucleotide differences between both *Fasciola* species and no intraspecific variation between each species [30]. However, a genetic variation between *F. gigantica* and *F. hepatica* with amplification fragment based on a 400-500 bp is described formerly [34]. Accordingly, one can confirm that PCR is simple, rapid and accurate tool for differentiation of the two species of *Fasciola* as compared with those of morphological, pathological or immunological techniques.

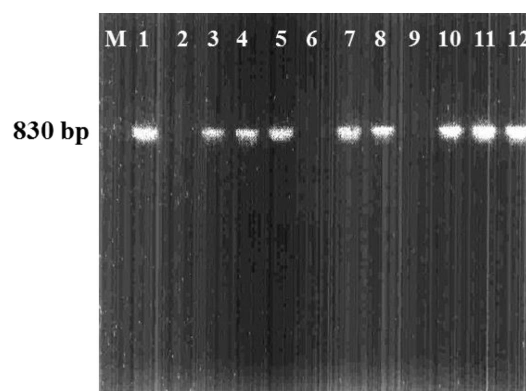

Fig. 4 Gel electrophoresis of PCR amplified products using specific agarose primer for characterization of Fasciola hepatica. *Lane M*: 860 bp ladder as molecular DNA marker. *Lane 1*: control positive for *Fasciola hepatica*. *Lane 2*: control negative for *Fasciola hepatica*. *Lane 3, 4, 5, 7, 8, 10, 11, 12:* positive (*Fasciola hepatice*). *Lane 6, 9:* negative (*Fasciola gigantica*).

Table 1 Prevalence of *Fasciola species* in liver of slaughtered cattle

Gender	Winter			Spring			Summer			Autumn			Total		
	No.	+ve	%	No.	+ve	%	No.	+ve	%	No.	+ve	%	No.	+ve	%
Males	60	1	1.67	60	1	1.67	60	1	1.67	60	2	3.33	240	5	2.08
Females	30	1	3.33	30	2	6.67	30	2	6.67	30	3	10.00	120	8	6.67
Total	90	2	2.22	90	3	3.33	90	3	3.33	90	5	5.56	360	13	3.61

Table (2): Prevalence of *Fasciola species* in livers of slaughtered buffaloes

Gender	Winter			Spring			Summer			Autumn			Total		
	No.	+ve	%	No.	+ve	%	No.	+ve	%	No.	+ve	%	No.	+ve	%
Males	60	1	1.67	60	2	3.33	60	1	1.67	60	2	3.33	240	6	2.5
Females	30	2	6.67	30	3	10.00	30	4	13.33	30	5	16.67	120	14	11.67
Total	90	3	3.33	90	5	5.56	90	5	5.56	90	7	7.78	360	20	5.56

3.4. *Fasciola eggs in human stools*

Table (3) indicated that 4% of the examined cases were positive for *Fasciola* eggs. The obtained result was in agreement with the result given by previous study [20], whereas, lower results was reported by (39) (3%). Other authors reported higher positive rates (10.4%) [10].

Results achieved in table (4) declared that children between one and fifteen years old represent the highest infection (5.88%) than individuals between sixteen and thirteen (4%). On the other hand, the lowest infection was in individuals between thirty one and forty five (1.75%). The obtained result was in agreement with result given by earlier authors [12].

Table (5) indicated that females (4.67%) were more exposed to infection more than males (3.23%). The obtained result was in agreement with the result given by former authors [8, 31]. This may be due to immune suppression of females by other physiological activities such as menstruation and pregnancy. Also females are associated more with the washing of clothes and kitchen utensils and meal preparation in houses and management of freshwater plants that potentially carry attached metacercariae [13].

Table 3 Demonstration of *Fasciola* eggs among examined cases by Kato thick smear and direct stool examination

Locality	No. of examined cases	Positive cases	%
Toukh	150	7	4.67
Benha	25	-	-
Shebeen El-Kanater	25	1	4.00
Total	200	8	4.00

Table 4 Age distribution among positive cases as detected by Kato thick smear and direct stool examination

Age (Year)	Total	Positive cases	Negative cases	%
1 - 15	68	4	64	5.88 %
16 -30	75	3	72	4.00 %
31 -45	57	1	56	1.75 %

It is evident from the results recorded in table (6) that infection was higher in rural areas (4.38%) than urban areas (2.5%). These results come in accordance with those reported formerly [8, 12].

Table 5 Sex distribution among positive cases as detected by Kato thick smear and direct stool examination

SEX	Total	Positive cases	Percent
Females	107	5	4.67 %
Males	93	3	3.23 %

Table 6 Prevalence of *Fascioliasis* in correlation to residence as detected by Kato thick smear and direct stool examination

Locality	Rural			Urban		
	No.	+ve	%	No.	+ve	%
Benha	20	-	-	5	-	-
Toukh	125	6	4.8	25	1	4.00
Shebeen El - Kanater	15	1	6.67	10	-	-
Total	160	7	4.38	40	1	2.5

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