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PCR-based Differentiation Of Zoonotic Fascioliasis

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ABSTRACT

Out of 100 samples from slaughtered cattle of different ages were examined at Kalyobia province slaughterhouses Takh center from October 2010 to October, 2011 in order to identify associated risk factors, 8% gave positive results (6 *Fasciola hepatica* and 2 *Fasciola gigantica*). Out of 100 stool samples examined from men occupationally contacts with cattle, 2% were positives to *fasciola hepatica* only.

The histopathological examination of the cattle liver infested with fasciola species 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 in both fasciola species lesions.

Regarding PCR technique, the using of EaeI restriction endonuclease enzyme as a genetic marker for *F.hepatica* is greatly effective when the enzyme uniquely fragmented the SrRNA gene into two bands. That means PCR could help to evaluate the relative zoonotic potential of *Fasciola hepatica* and *Fasciola gigantica*, especially in those regions, which have huge increases in the incidence of human fascioliasis in recent years.

INTRODUCTION

Fascioliasis is an important disease caused by *Fasciola hepatica* and *Fasciola gigantica*. The distributions of both species overlap in many areas of Asia and Africa including Egypt. Fifty adult *Fasciola* worms were collected from livers of cattle slaughtered in slaughterhouses, Cairo, Egypt. They were subjected to morphological and metric assessment of external features of fresh adults, morphological and metric assessment of internal anatomy of stained mounted worms (1). Fascioliasis, an economically important disease of livestock, is largely caused by *Fasciola hepatica* in temperate climates and by *Fasciola gigantica* in tropical regions. The distributions of these two species overlap, however, in many countries of Asia and Africa (2), the disease is now recognised as an emerging food-borne zoonosis, with an estimated 17 million people infected with *Fasciola* world-wide, and 180 million at risk of such infection (3). The incidence of human infection appears to be increasing in

rural regions. Diagnosis of the disease is achieved by detecting the ova either in feces or duodenal drainage or by imaging techniques proved to be the most useful method for confirming the diagnosis and follow-up of fascioliasis. Small, often peripheral, nonenhancing, hypodense nodules with tortuous, linear, branching tracts in CT scans, which decrease in size after successful therapy, are highly suggestive of the disease. Immature flukes can produce ectopic masses or abscesses in various locations and during the acute phase of the disease, other structures such as subcutaneous tissue, heart, lungs, pleura, abdominal wall, brain, cecum, epididymis, and stomach can be involved (4). In a particularly unusual report, direct peritoneal involvement with granuloma formation has been reported. Eosinophilic reactions can be exhibited in the body as pleuritis and pericarditis (5). Here, we present an extremely unusual radiologic incident of fascioliasis involving multiple organs.

MATERIAL AND METHODS

100 liver samples were collected from slaughtered cattle in the Takh center of Kalyobia province slaughterhouses (6). Also 100 stool samples were collected from individuals occupationally in contact with cattle including apparently healthy and clinically diseased in the same location.

Examination of human stool samples

Human fecal samples were directly obtained from the rectum or immediately after defecation and placed in polyethylene bags, closed well labeled with a serial number, locality and date of collection. Closed fecal polyethylene bags were placed in plastic bags, and then transported to the laboratory with a minimum of delay (7).

Direct smear method (8).

Kato thick smear

Approximately 1 g of feces is placed on filter paper (5 cm square) and pressed through a 100 mesh (150 μ m pore size) sieve. The sieved stool sample, containing helminthes eggs, but no particles larger than the sieve mesh size, is scraped off the wire surface of the sieve with the edge of a microscope slide and packed into the head of a disposable syringe, previously calibrated to deliver 30 mg. This amount is put on a slide, which is then inverted and pressed firmly down on a thick glass cover slip. The diameter of the fecal smear is usually about 25 mm. The slide should be examined before drying out occurs within 2 hours. The efficacy of the modified technique was estimated by attempting to retrieve known numbers of eggs that had been added to a stool from an uninfected subject. These eggs were obtained from heavily infested human stools by sieving, sedimentation, washing, and centrifugation of the emulsified stool. The number of eggs in a drop of suspension placed on a slide was counted under the microscope. One gram of human stool negative for helminthes then added to the slide, and the eggs and stool were thoroughly mixed. Six 30-mg amounts were taken according to the method outlined above,

and the eggs in each sample were counted under the microscope ($\times 40$) (9).

Examination of samples from slaughtered cattle

The liver samples were obtained thorough routine post mortem examination of slaughtered animals. Data recorded from each animal included: species, owner's name and the time of sampling. Thus, gross inspection was done on each slaughtered animal including also the whole carcass and the internal organs (10).

Histopathological examination of naturally infested tissues

Samples from cattle liver were collected and fixed in neutral buffered formalin 10%, washed in tap water overnight and exposed to ascending concentrations of ethanol (100%), cleared in xylene and embedded in paraffin. Sections of the tissues (4-5 μ thick) were prepared and stained with Hematoxylin and Eosin for subsequent histopathological examination (11).

Characterization of *Fasciola* species by PCR

Determination of genomic DNA

The total DNA of the two species (*Fasciola hepatica* and *Fasciola gigantica*) was extracted using the UNSET lysis solution (12). One μ l of the suspended pellet was checked by 0.8% gel electrophoresis for the presence of DNA.

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:

SSU1 (5, - CGACTGGTTGATCCTGCCAGTAG - 3,)

SSU2 (3, - TCCTGATCCTTCTCAGGTTTCAC - 5,)

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 (13).

Restriction fragment length polymorphisms profiles

Restriction endonuclease represented by EaeI (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, one μ l was used together with 1.2 μ l of the particular enzyme buffer for a final volume of 12.2 μ l. The digestion was carried out for 3.5 hrs 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 ultra violet transillumination and photographed.

Analysis of PCR amplified products
Accurately, PCR amplified products were analysed by agarose gel electrophoresis on 1.4% gel containing ethidium bromide dye (0.5 μ l /ml).

RESULTS

Table 1. Infectious rate of *Fasciola hepatica* and *Fasciola gigantica* in Cattle in Kaloubia Province: Numbers of examined animals (100)

Fasciola species	<i>Fasciola hepatica</i>	<i>Fasciola gigantica</i>	Total Numbers
Infectious rate	6%	2%	8%

Table 2. Infectious rate of fascioliasis in human in Kaloubia Province Numbers of examined human (100)

Species	Numbers of examined Human	Infectious rate
Human	100	2%

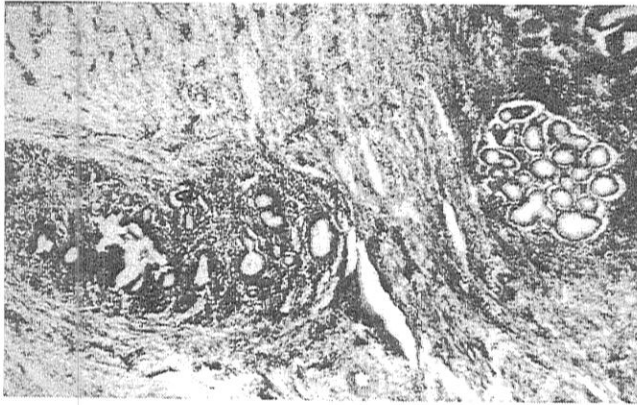


Fig. 1. Electromicrograph of liver of cattle showing fibrosis in the wall of bile duct with inflammatory cells infiltration and newly formed bile ductules (a)

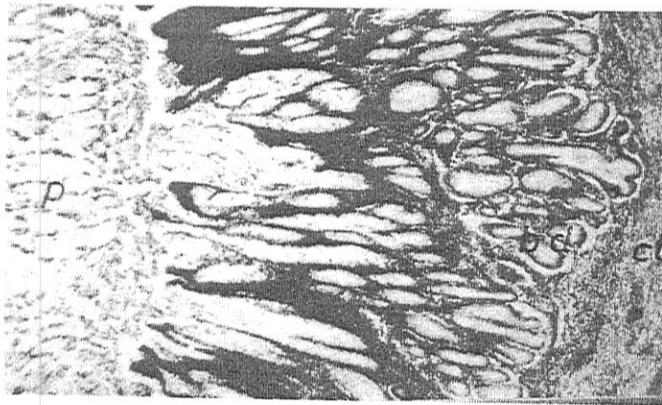


Fig. 2. Electromicrograph of liver of cattle showing part of the parasite embedded in lumen of bile ducts (b) associated with hyperplasia in the lining epithelium of bile ducts (bd) with polyps formation and periductal inflammatory cells infiltration (ct)

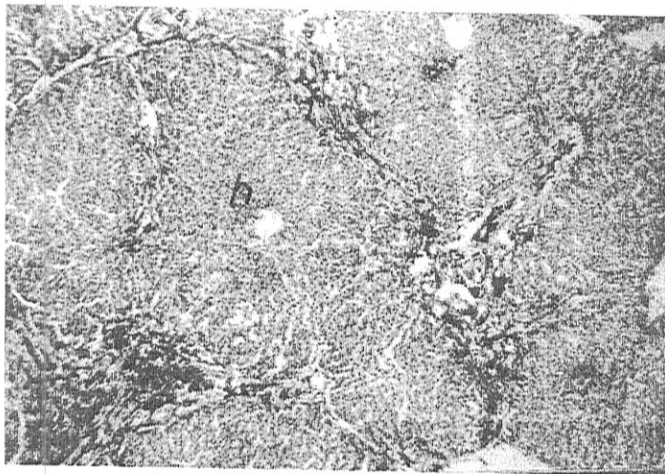


Fig. 3. Electromicrograph of liver of cattle showing fibrosis (f) arising from the portal area dividing the hepatic parenchyma (h) into lobules

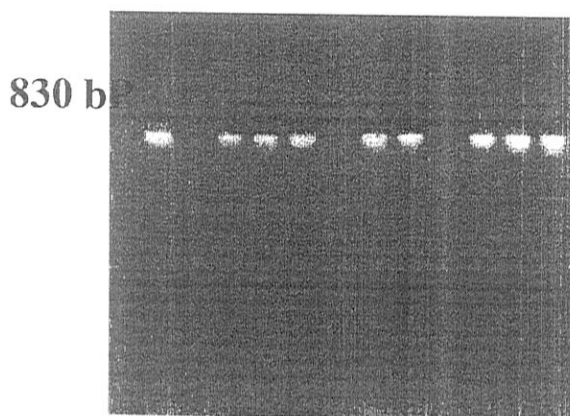


Fig. 4. Gel electrophoresis of PCR amplified products using specific agarose primer for characterization of *Fasciola hepatica*

DISCUSSION

The results of postmortem examinations of *Fasciola hepatica* and *Fasciola gigantica* are represented in Table 1. Infections among slaughtered cattle in Kaloubia Province Tuh center slaughterhouses revealed that out of 100 examined cattle, 6 were infected with *F. hepatica* and 2 with *F. gigantica*, with an infectious rate of 6% and 2% respectively (Table 1). This may be due to immune suppression and / or associated with eating of freshwater plants that potentially carry attached metacercariae (14,15).

Table 2 shows the coprologic examinations of fascioliasis, infections among human in Kaloubia Province, revealed that out of 100 examined human stool in both sex, 2 were positives with infection rate 2 %, the low infectious rates may be due to developed sewerage system, socio-economic level and education. Eating raw vegetable salads is a significant risk factor through which families may acquire the infection in endemic areas. A finding that similar to previous study (3).

From the histopathological examination of the liver infected with *Fasciola* species we observed that the portal area showed newly

formed bile ductules with inflammatory cells infiltration and fibrosis (Figure 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 (Figure 2). The fibroblasts were originated from the portal areas and dividing the hepatic parenchyma into lobules (Figure 3). The Histopathological examination of the postmortem examination to the liver of infected animals did not differentiate between the two *Fasciola* species. These results come in accordance with those previously reported (16,17).

Figure 4 illustrate the results of the application of PCR for differentiation of the two *Fasciola* species in man and cattle by using specific primers of *F. hepatica*. These results indicated that 8 samples had positive bands related to *F. hepatica* and 2 negative bands representing *F. gigantica*. Consequently, the using of EaeI 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 in several studies in China (18,19).

On the other hand, simple and rapid PCR – RFLP are used 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 (20). However, a genetic variation between *F. gigantica* and *F. hepatica* with amplification fragment based on a 400-500 bp was described (21).

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.

Infection in humans is often but not always associated with endemic disease in livestock, and there is a need to investigate the role of domestic and wild animals as 'reservoir hosts' for human infection. The PCR-based assays described here clearly have a range of potential epidemiological applications, particularly where *Fasciola hepatica* and *Fasciola gigantica* co-exist (3).

CONCLUSION

We can conclude that human fascioliasis is found in a high proportion to the relatives of index cases, and this should be taken into account when fascioliasis is detected; it is usually present in most members of a family. Eating raw vegetable salads is a significant risk factor through which families may acquire the infection in endemic areas. It is recommended that patients presenting abdominal pain and low to high eosinophilia, who have recently visited an area endemic for *F. hepatica*, should be carefully studied so as to rule out this parasitosis and the study could be expanded to include other travel companions or family members as well. In

endemic areas, adult are the most affected age-group, and the adult groups usually present the highest infection rates, which is consistent with our results. Apparently, good hygienic habits decrease the risk of acquiring fascioliasis. People with good hygienic habits may be less likely to contract the infection, may having less contact with the contaminated environment than those with poor hygienic habits.

REFERENCES

- 1.Hoda H , Abeer M A , Naglaa said M , Waheed M A and Abeer S A (2012): Molecular, biochemical, and morphometric characterization of *Fasciola* species potentially causing zoonotic disease in Egypt Parasitology Research Volume 111, Number 3 (2012), 1103-1111, DOI: 10.1007/s00436-012-2938-2.
- 2.Mas-Coma S (2005): Epidemiology of fascioliasis in human endemic areas. J. Helminthol., 79: 207–216.
- 3.Mas-Coma M S , Esteban J G and Bargues M D (1999): Epidemiology of human fascioliasis: a review and proposed new classification. Bull. Wld. Hlth. Org., 77: 340-346.
- 4.Pulpeiro J R , Armesto V , Varela J , Corredoira J (1991):Fascioliasis: findings in 15 patients. Br J Radiol, 64:798-801.
- 5.Haçarız O , Sayers G and Baykal A T (2012): A proteomic approach to investigate the distribution and abundance of surface and internal *Fasciola hepatica* proteins during the chronic stage of natural liver fluke infection in cattle. J Proteome Res. 6;11(7):3592-604.
- 6.Faust E C, Russell P E and Jung R C (1970): Clinical Parasitology, 8th ed. Lea & Febiger, Philadelphia, 64: 67.
- 7.Garcia L S and Bruckner D A (1993): Macroscopic and Microscopic examination

- of Fecal Specimens. Diagnostic Medical Parasitology. 2nd edition. Edited by: Garcia LS, Bruckner DA. Washington. American Society for Microbiology: 501-535.
8. **El-Rahimy H H, Mahgoub A M, El-Gebaly N S, Mousa W M and Antably A S (2012):** Molecular, biochemical, and morphometric characterization of *Fasciola* species potentially causing zoonotic disease in Egypt. *Parasitol Res. Sep*;111(3):1103-11. Epub 26.
 9. **Ekong P S, Juryit Dika R N M, Nguku P and Musenero M (2012):** Prevalence and risk factors for zoonotic helminth infection among humans and animals - Jos, Nigeria, 2005-2009. *Pan Afr Med J.* ;12:6. Epub 12.
 10. **Rapsch C, Schweizer G, Grimm F, Kohler L, Bauer C, Deplazes P and Braun U and Torgerson P R (2006):** Estimating the true prevalence of *Fasciola hepatica* in cattle slaughtered in Switzerland in the absence of an absolute diagnostic test. *Int J Parasitol.* 2006 Sep;36(10-11):1153-8.
 11. **Banchroft J D, Stevens A and Turner D R (1996):** Theory and practice of histological techniques. Fourth Ed. Churchill Living stone, New york.
 12. **Hugo A, Stewart V, Gast R and Byars T (1992) :** Purification of mt – DNA using PCR procedure. In "Protocol in protozoology" Lee, J. and Soldo, A. Eds, Eoc. Protozoologist, Lawrence pp. 71-74.
 13. **Stohard J and Rollinson D (1997):** Molecular characterization of *Bulinus globosus* and *Fasciola* in Zanzibar and an investigation of their roles in the epidemiology. *Sco. Trop. Med. Hyg.*, 91: 353-357.
 14. **Maco V, Marcos L A and Terashima A (2002):** Fas2-ELISA y la técnica de sedimentación rápida modificada por Lumbreras en el diagnóstico de la infección por *Fasciola hepatica*. *Rev. méd. Herediana*, 13: 49-57.
 15. **Esteban J G, Gonzalez C and Bargues M D (2002):** High fascioliasis infection in children linked to a man-made irrigation zone in Perú. *Trop. Med. Int. Hlth*, 7: 339-348.
 16. **Duff J P, Maxwell A J and Claxton J R (1999) :** Chronic and fatal fascioliasis in llamas in the U.K. *Vet. Rec.* 145 (11): 315-316.
 17. **Ansari-Lari M and Moazzeni M (2006)** A retrospective survey of liver fluke disease in livestock based on abattoir data in Shiraz, south of Iran. *Preventive. Vet. Med.*, 73 (1): 93-96.
 18. **Huang W Y, Wang H B and Zhu C R (2004):** Characterization of *Fasciola* species from Mainland China by ITS-2 ribosomal DNA sequence. *Vet. Parasitol.* .. 120(1-2): 75-83.
 19. **Lin R Q, Dong S J, Nie K, Wang C R, Song H Q, Li A X, Huang W Y and Zhu X Q (2007):** Sequence analysis of the first internal transcribed spacer of rDNA supports the existence of the intermediate *Fasciola* between *F. hepatica* and *F. gigantica* in mainland China. *Parasitol Res.*, 101(3): 813-817.
 20. **Marcilla A, Bargues M D and Mas – Coma S (2002):** A PCR – RFLP assay for the distinction between *F. hepatica* and *F. gigantica*. *Mol Cell Probes.*, 16 (5): 327-333.
 21. **Ramadan N I and Saber L M (2004):** Detection of genetic variability in non human isolates of *F. hepatica* and *F. gigantica* by the RAPD-PCR technique. *J. Egypt. Soc. Parasitol.*, 34 (2): 679-689.

الملخص العربي

التمايز للمتورقة الكبدية المشتركة باستخدام اختبار PCR

عادل محمد عبد العزيز نويشى

قسم الأمراض المشتركة - كلية الطب البيطرى - جامعة بنها

من ١٠٠ عينة من الماشية من مختلف الأعمار المذبوحة في مسالخ مركز طوخ بمحافظة القليوبية من أكتوبر ٢٠١٠ إلى أكتوبر ٢٠١١ من أجل تحديد عوامل الخطر المرتبطة بها، أظهرت ٨% نتائج إيجابية للمتورقة (٦ المتورقة الكبدية و ٢ المتورقة جيجنتيكا)، من فحص ١٠٠ عينة برازية من الرجال المخالطين للماشية، كانت هناك ٢% أصابة ايجابية للمتورقة الكبدية فقط. أظهرت عينات الهستوباثولوجيا لكبد الماشية المصابة بالمتورقة وجود قنيات صفراوية شكلت حديثا مع تسلل خلايا التهابية وتليف مرتبط بتضخم في البطانة الظاهرة مع أشكال من الاورام الحميدة فضلا عن ان منطقة مدخل الكبد أظهرت تليف شديد مع تسلل خلايا التهابية في كل الأصابة لنوعى المتورقتين. أما بشأن تقنية PCR، وباستخدام انزيم التقييد من EaeI النووى كعلامة وراثية للمتورقة الكبدية فكانت فاعلية كبيرة إلى حد كبير عندما التجزئة الفريدة للجين SrRNA في شريطين . وهذا يعني أن PCR يمكن أن يساعد في تقييم الإمكانية النسبية المشتركة للمتورقة الكبدية وللمتورقة جيجنتيكا ، وخاصة في تلك المناطق التي يزيد فيها حدوث داء المتورقات للإنسان في السنوات الأخيرة.