Diagnosis of human fascioliasis in Arusha region, northern Tanzania by microscopy and clinical manifestations

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SUMMARY

Human fascioliasis (HF) is a zoonotic disease that has been reported in many countries. Fascioliasis is included in the list of WHO's Neglected Tropical Diseases as a Food -Borne Zoonosis which is acquired from infected livestock as the primary host. Human fascioliasis has not been described before in the East Africa region, including Tanzania. We enrolled 1460 patients presenting at a primary healthcare centre in Arusha Region, northern Tanzania. The patients provided fresh stool samples for routine ova and parasite screening using saline and iodine preparations. Subsequent stool samples were preserved in 5% formalin in saline and subjected to ether sedimentation for microscopic examination and identification. Of 1460 patients, 305 (20.9 %) were diagnosed positive for fascioliasis based on the demonstration of brownish, oval eggs with inconspicuous opercula. Two distinct egg sizes were identified: large 170–212.5 by 115–150 μm (mean 194.5 by 130.5 μm) and smaller eggs 120–150 by 87.5 – 112.5 μm (mean 138.8 by 101 μm). Clinically, patients presented with fever and abdominal pain. They were treated with Nitazoxanide (302) or Triclabendazole (3) and 122 (40.0 %) of them were cleared of infection with a single course. Snails of the genus *Lymnaea* were found in the surroundings. This report serves to remind medical professionals in East Africa that HF is a probable differential diagnosis in patients presenting with similar symptoms. It is possible to diagnose fascioliasis by light microscopy although specific antigen tests are required for confirmation. Human fascioliasis however, has not been described or reported in Tanzania before and begs further investigation.

Keywords: "Human fascioliasis", diagnosis, treatment, Tanzania

INTRODUCTION

Fasciola hepatica and *F. gigantica* are trematodes commonly referred to as the liver flukes in sheep, goats and cattle where they cause serious disease with significant economic losses to farmers. Humans become infected with the *Fasciola* by accidental ingestion of metacercaria encysted on leaves of vegetables including watercress, radishes, corn, spinach, alfalfa juice and broccoli (Valero et al., 2009). Humans can also be infected with *Fasciola* metacercaria when they drink contaminated water. Adult liver flukes are found in the bile ducts and liver parenchyma of infected human and animal hosts. *Fasciola* cannot be passed directly from one person to another (Mas-Coma *et al.*, 1999). Eggs passed in stool of infected hosts need *Lymnaea* snail species to develop to sporocysts, rediae and cercariae which leave the snail intermediate host and encyst on leaves of vegetation as the infective metacercariae (Chandler and Read, 1968; Mas-Coma *et al.*, 1999; Valero *et al.*, 2009). Human fascioliasis (HF) is usually asymptomatic, though symptoms may occur early on in the infection when immature flukes migrate from the intestine through the abdominal cavity and liver. Symptoms associated with HF are usually non-specific and can be categorized as acute and chronic. Symptoms of the acute disease can start 4 to 7 days after infection characterized by fever, nausea, malaise and abdominal pain. Less often patients may present with urticaria as well as subcutaneous nodules (Chandler and Read, 1968; Acha and Szyfres, 2003). The chronic phase of the infection occurs much later, usually 8 to 14 weeks after infection, when adult flukes have settled in the bile ducts. On clinical examination a tender or non-tender hepatomegaly may be found. The symptoms of chronic disease may last for months to years after exposure, usually resulting from inflammation and blockage of bile ducts. This is usually complicated by biliary colic, cholestasis or cholangitis in untreated patients (Esteban *et al.*, 2003; Cuomo *et al.*, 2009.

The rather distinct and rare ova in stool samples of various patients visiting TotalCare Medical Centre was first noticed in April 2012. At the time, it was a rather common finding to have high infection rates

with *Ancylostoma duodenale, Giardia lamblia as* well as *Entamoeba histolytica* in many stool samples examined for parasitic infections. By mid-September 2012, many patients presented with clinical symptoms suggestive of typhoid fever or intestinal worm infections. When stool specimens from these patients were examined microscopically, eggs with unfamiliar brownish, oval appearance and inconspicuous operculum were observed; this was usually accompanied by marked eosinophilia on blood film examination and where tested for, elevated serum transaminases and urine bilirubin.

Studies conducted within the northern Africa region showed the epidemiological picture of human fascioliasis has changed in recent years. The number of reports of humans infected with human fascioliasis has increased significantly since the 1980s and several geographical areas have been described as endemic for the disease in humans (Mas-Coma *et al.,* 1999; O'Neil *et al.,* 1999Esteban *et al.,* 2003; Acha and Szyfres, 2003; Mas-Coma *et al.,* 2005; Curtale *et al.,* 2007; Curtale *et al.,* 2008; Mas-Coma *et al.,* 2008; Cuomo *et al.,* 2009; Fentie *et al.,* 2013; Zumaquero-Rı´os *et al.,* 2013).

Literature cited on HF in Africa showed that both the prevalence and intensity of infection were significantly higher among girls than boys, with the highest prevalence and intensity of infection being reported in the 9-11 years age group. Women were more affected than men, but not at a significant level (Curtale *et al.,* 2007; Curtale *et al.,* 2008; Fentie *et al.,* 2013). Many *Fasciola* infected children were co-infected with other parasites (Curtale *et al.,* 2008; Fentie *et al.,* 2013; Zumaquero-Rı´os *et al.,* 2013). The clinical diagnosis of fascioliasis in most primary health centers depends largely on the microscopic examination of stool samples for ova by finding eggs with an inconspicuous operculum (Valero *et al.,* 2009; Chandler and Read, 1968; Cuomo *et al.,* 2009). In human infection the drug of choice for treating HF is Triclabendazole as it has the highest efficacy. In many areas with scarce supply of Triclabendazole, Nitazoxanide has also been used with differing success rates (Fairweather and Boray, 2009).

Laboratory Diagnosis

Patients presenting at the outpatient department (OPD) were asked to provide a stool sample on day 1, 3 and 6 for the diagnosis of parasite infection. Sample collection and processing protocol was as recommended by Cheesbrough (2011). Briefly, for fresh samples stool in a clear transparent container

This paper reports clinical and parasitological findings of cases of HF at TotalCare Medical centre, a privately owned primary healthcare facility located in Themi Hill, Arusha region, Tanzania. These cases were evenly spread demographically including, age, sex, social-economic background and race of people living in the Themi Hill surroundings, which has a notably high, seasonal population of various snail species, particularly of the family *Lymnaeidea*. Many local inhabitants of the area use man-made deep water wells and natural streams as a source of water for domestic consumption. During the seasonal rains, the area is subject to flooding and soggy, marshy soils especially towards the lower lying residential areas.

MATERIALS AND METHODS

Ethical approval

This study was approved in July of 2012 by the health management team of TotalCare Medical Centre in consultation with the Regional and National health authorities. In the conduct of this study, compliance to good clinical practice as defined by the Helsinki Declaration was observed. All subjects involved provided written informed consent. Children's parents offered the consent on behalf of their children.

Study patients

Most patients presenting at the clinic complained of high grade fever (39°-40°C), abdominal pain at the upper right quadrant area and malaise. No abnormality was detected on abdominal examination. Few of these patients also presented with complaints of pruritis around the mouth with swelling of the lips. Jaundice was also observed in some cases. In clinical practice such presentation would range from malaria, typhoid fever to urinary tract infection (UTI). Laboratory investigations commonly requested were blood smear examination for malaria parasites, Widal test for typhoid fever and urinalysis. Patients were provided with stool containers containing 5% formalin diluted in saline as an ova preservative in stool samples.
was processed within 30 min of collection. A plastic scoop provided with the stool container was used to sample the edges and centre of the specimen, collecting approximately 0.5 gm of feces. This was mixed to homogeneity in 10 microlitre saline on a glass slide. A drop of 1% iodine was placed on the homogenized fecal preparation and a cover slip was applied on the specimen. Iodized preparations were quickly examined under the light microscope for

identification of parasite eggs/ova at x10 and x40 magnification.

Subsequently patients who received anthelminthic treatment were provided with 50 ml stool containers containing 5 ml of 5% formalin diluted in saline as an ova preservative. They were instructed to collect the stool sample, typically a scoop full or approximately 5gm to be mixed with the preservative and the cover sealed tight. The sample was then brought to the OPD the following day. This protocol was routinely followed and proved to be useful when patients were treated with anthelminthics to assess effect of treatment.

Briefly, the stool was mixed to homogeneity in the preservative (5% formalin in saline). Ether (2 ml, 5%) was then added to the homogenate and mixed thoroughly. The mixture was then left to stand for 20 minutes before a sample of the sediment and the supernatant was collected for examination. Approximately 0.02 ml sample was placed at the centre of a microscope slide and a drop of iodine added. A cover slip was placed over the mixture, and the slide examined at x10 and x40 magnification.

A graduated reticle inserted in the objective piece was first calibrated against the stage micrometer for standardization. Egg size estimations were then made at x100 magnification, and pictures taken at x400 magnification for clarity of features. Repeat stool samples were taken day 3, 6, 14 and 21 post-therapy to screen for the presence of ova or parasites. When a diagnosis of fascioliasis was tentatively reached, all samples suspected to have eggs were stored at 2-8°C and transported to the reference laboratory at the Kilimanjaro Christian Medical University College for confirmation of the diagnosis. In cases of mixed infections, patients were first managed for other infections. Examination of stools for earlier suspected *Fasciola* eggs was started after confirmation of treatment for co-infections. This served as a further quality control method for the diagnosis. The data was then analyzed using descriptive statistics.

RESULTS

The majority of patients attending the clinic (96%) were from the Themi-Njiro area (living within 2-10km of the clinic), in Arusha municipality, Tanzania. A few patients also came from other municipalities outside Arusha. Children below 12 years were most often diagnosed with fascioliasis (45%).

Stool examination showed presence of oval *Fasciola* eggs along with *Ancylostoma duodenale* or *Strongyloides stercoralis*. Most of these were treated with Albendazole and effect of treatment was observed for 2 weeks after completion of medication. Oval *Fasciola* eggs (Figure 1) were demonstrated in 305 out of 1460 stool specimens. We reviewed 50 samples fixed in 5% formalin in saline and stored for 1 week at 2-8°C. Two distinct egg size patterns were noted; large ovoid eggs 170 - 212.5 by 115 - 150 µm (mean 194.5 by 130.5 µm) and a smaller egg range of 120 - 150 by 87.5 – 112.5 µm (mean 138.8 by 101µm). Snail of the genus *Lymnaea*, found in a natural water source around the study area are presented in Figure 2.

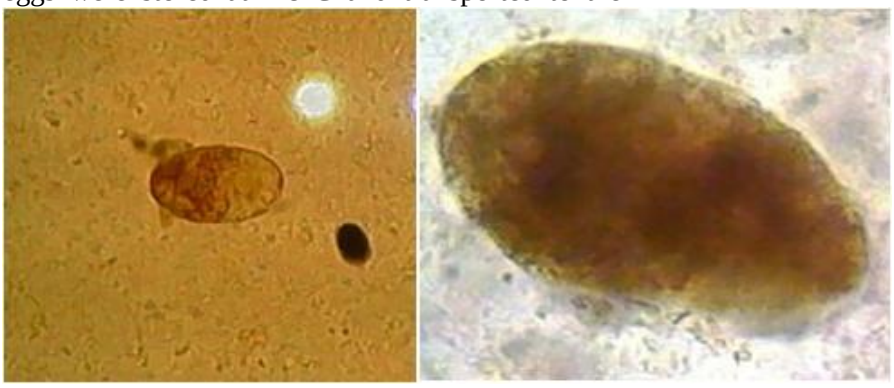

Figure 1. A (a, small size; 130.5 by 102 µm) and **B** (large 205 by 148 µm) as seen in an iodine stained stool sample after formol-ether sedimentation x400.

Figure 2. Snail of the genus *Lymnaea*, found in a natural water source around the study area.

Sex and age distribution of the patients diagnosed with fascioliasis and the annual distribution of cases diagnosed at TotalCare Medical Centre are shown in Fig 3 and 4 respectively.

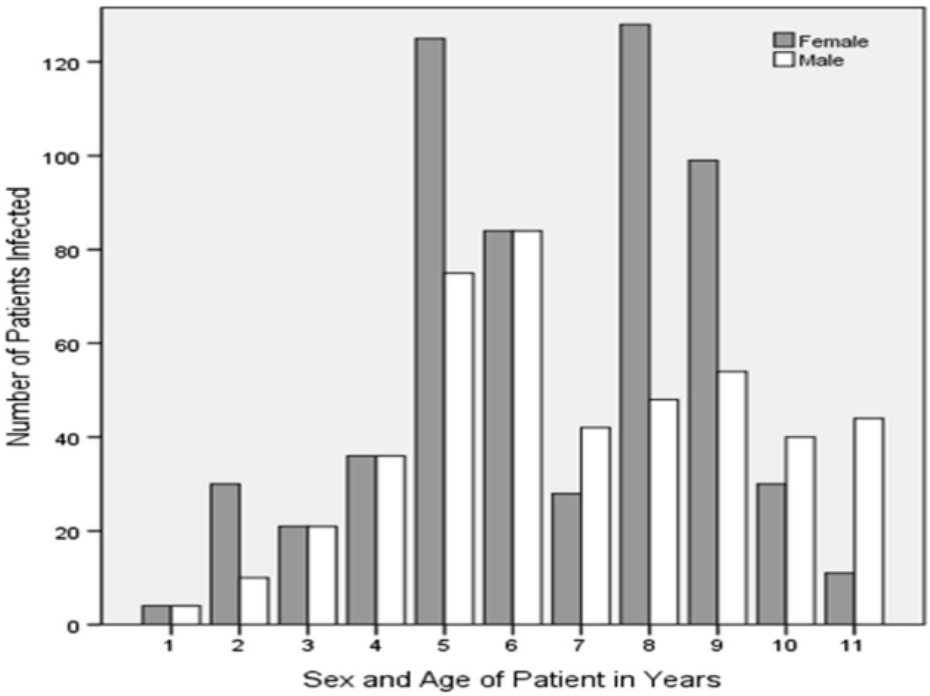

Figure 3. Frequency of occurrence of fascioliasis in males and females stratified from 1 to 11years based on stool examination.

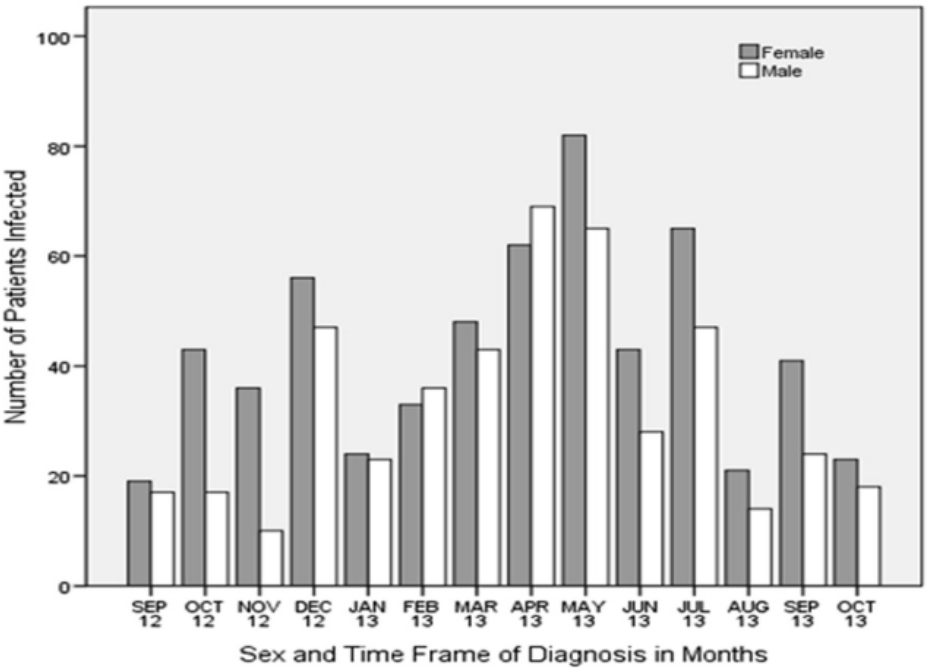

Figure 4. Frequency of diagnosis of fascioliasis in males and females of all age groups from September 2012 to October 2013.

DISCUSSION

Animal fascioliasis is a liver disease of ruminants which has been described in many parts of the world including a few African countries (Valero *et al.*, 2009; Mas-Coma *et al.*, 1999; Chandler and Read, 1968; Acha and Szyfres 2003; Mas-Coma, 2005; Curtale et al,. 2007; Mas-Coma *et al.*, 2008; Curtale et al,. 2008; Cuomo *et al.*, 2009; Fentie et al,. 2013; Zumaquero-Rı´os et al., 2013). Recently, human fascioliasis has also been described as a zoonosis acquired from domestic animals. Human fascioliasis is acquired by the ingestion of *Fasciola* metacercariae encysted on vegetables or in drinking water. The distribution of human fascioliasis has recently been reported in four African countries including; Egypt, Ethiopia, Cameroon and South Africa. In most rural African settings, domestic animals including cattle, sheep and goats are part of agricultural activities, for provision of meat, milk and hide. Similarly, in these settings, vegetables including lettuce, radish, corncob, alfalfa, and amaranths are cultivated to provide vegetable food source. Rural and semi - rural African settings readily provide the environment suitable for the completion of the life cycle of animal fascioliasis, which includes infected animals that would release infective eggs into the environment and the subsequent development of cercariae within *Lymnaea* snails, up to development of metacercaria which encyst on vegetable leaves. When human and animal fascioliasis occur concurrently the transmission cycle of fascioliasis is amplified many fold.

The suburbs of Arusha city, where this study was conducted, provide a scenario described above for efficient transmission of animal fascioliasis to humans, i.e. the agricultural system in this area includes cattle, sheep and goats and vegetables are cultivated in abundance as an additional food source. Additionally some of the inhabitants drink untreated water from natural streams. In our case series, which involved 1460 persons over a 12 month period, 305 (21%) were found to be infected with fascioliasis. Because *Fasciola* eggs are rarely detected during the acute infection, and because the disease has not been described before in Tanzania, it is likely that laboratory investigations intended to detect intestinal worm infections in stool would have missed the recognition of *Fasciola* eggs in the stool samples.

We also observed two distinct egg sizes on microscopic examination: large ovoid eggs mean 194.5 by 130.5 µm and smaller oval eggs mean 138.8 by 101µm, which correspond to reported egg size ranges for *Fasciola gigantica* and *F. hepatica* respectively (Valero *et al.,* 2009; Cuomo *et al.,* 2009). Although we were unable to speciate the causative parasite as being *Fasciola hepatica* or *F. gigantica* in this particular clinical study, other

veterinary and malacology studies conducted in the region and East African in general (Keyyu *et al.*, 2009; Howell *et al.*, 2012; Swai and Ulicky, 2009) have shown the presence of both species. Because *Fasciola* eggs have an inconspicuous operculum, it is difficult for the unsuspecting investigator to identify them with certainty, even in well documented endemic regions (Chandler and Read, 1968; Mas-Coma *et al.*, 1999; Esteban *et al*,. 2003; Mas-Coma, 2005; Mas-Coma *et al.*, 2008; Valero *et al.*, 2009; Cuomo *et al.*, 2009). Triclabendazole is the drug of choice for the treatment of both animal and human fascioliasis as noted in other studies (Fairweather and Boray, 2009; Zumaquero-Rı´os *et al.*, 2013). In this study, three patients were cleared of HF by a single dose of this drug. Use of Nitazoxanide did not clear all patients treated. This finding was consistent with some earlier reports.

This study has shown that more females than males are infected with HF and that fascioliasis is quite often associated with children below 12 years in both sexes. This finding is in agreement with what has been previously reported about the epidemiology of HF in some African and tropical countries (Esteban *et al.*, 2003; Acha and Szyfres, 2003; Mas-Coma, 2005; Curtale *et al.*,2007; Curtale *et al.*, 2008; Fentie *et al.*, 2013). The reasons for sex and age bias for HF could be because, at least in our settings, young girls and boys are culturally engaged as drawers of domestic water for house hold use. This practice would expose them more frequently to infection with HF than would adults. At TotalCare Medical Centre, records also indicated that there were more women and young children who visited the health centre than adult males.

The challenges associated with a definitive diagnosis of human fascioliasis, especially at the clinical level are many: First, eggs are never passed in stool during the acute phase of the disease, therefore patients presenting clinically are likely to be missed on routine stool screening especially if only a single sample is examined without concentration. Clinical symptoms are also usually less pronounced in subsequent infections (Chandler and Read, 1968; Cuomo et al., 2009; Hakyemez et al., 2012). Much of the symptomatology presented in this study was further confounded by other infections diagnosed and cannot definitively be attributed to fascioliasis, even where eggs were found in patients' stools.

Secondly, although we examined at least 2 samples per patient, we never used any concentration method as these screenings were not prejudiced towards the detection of *Fasciola* eggs alone. Other intestinal parasites were often diagnosed and consequently managed before specific screening for fascioliasis was started. Lastly, the definitive diagnosis of fascioliasis by species is usually based on the use of specific coprologic antigen tests or molecular analysis. This study was conducted in-house in a primary health centre where neither of these diagnostic options were available, nor had they been approved for use in the country and region to date.

It is concluded that human fascioliasis has not been hitherto reported from Tanzania or any other East African country. Subsequent studies could focus on this distinction and make further clinical associations once the disease has been recognized as a significant public health problem. This study serves as a reminder to the medical profession in East Africa that HF, a food borne trematode infection, is probably of more common occurrence than has hitherto been realized.

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