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A study of parasitic infections in the luminal contents and tissue sections of appendix specimens

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Abstract. Appendicitis has a worldwide prevalence and affects all age groups. The aetiology of acute appendicitis is still much debated, many factors have been implicated. The pathology is likely to be due to obstruction of the lumen of the appendix. Parasites, both helminths and protozoa have been suggested to be the cause of acute appendicitis. Studies have demonstrated that parasites are present in the appendix specimens removed from surgery methods.

INTRODUCTION

Appendicitis or the inflammation of the vermiform appendix occurs in approximately 7% of the population (Mowlavi *et al.*, 2004). It is the most common acute abdominal condition, and one of the leading indications for surgery (Elezary *et al.*, 2005; Old *et al.*, 2005). The peak incidence occurs in the age range between 10 to 30 years old (Hardin, 1999). As appendicitis is common in the young, appendectomy is the most common surgical operation in children (Dorfman *et al.*, 2003).

The aetiology of acute appendicitis has not been established and is still much debated. Several factors have been suggested which include diet, lymphoid hyperplasia, faecolith, allergic reaction and infections due to bacteria and viruses (Andersson *et al.*, 1995; Guo *et al.*, 2004). Parasitic infection with helminth and protozoa has been suggested as a cause of appendicitis (Martin & Gustafson, 1985). Helminths that have been reported include *Enterobius vermicularis*, *Schistosoma* spp., *Trichuris trichiura*, *Ascaris lumbricoides*, *Strongyloides stercoralis* and *Taenia* spp. Protozoa that were reported were *Entamoeba histolytica*, *Balantidium coli* and *Cryptosporidium parvum* (Díaz & Fernández, 2001; Dorfman *et al.*, 2003).

The primary objective of the present study was to determine the prevalence of parasitic infections in the tissue sections and luminal contents in appendices removed during appendectomy in Batu Pahat District Hospital which serves both urban and rural areas. Since parasitic infections are more common in rural than in urban areas, it would be important to study the relationship between parasitic infections and appendicitis in patients from these areas.

We also studied the association between the histological changes in the appendix with the presence of parasites in the tissue, lumen or luminal content. It was hypothesized that the detection rate of parasitic infection would be improved, if both the tissue and luminal content were examined.

MATERIALS AND METHODS

A total of 100 samples were obtained between April and September 2006 from

Batu Pahat District Hospital in Batu Pahat district, Johor state. Tissue and the luminal content were taken from resected appendices from patients who had undergone appendectomy at the hospital. Personal details of all patients were obtained which included gender, age and ethnicity.

Tissues obtained were fixed in 10% neutral formalin. Two transverse sections from the base and the middle portion of the appendix and a longitudinal section from the tip of the appendix were taken. Sections of 4 µm thickness were prepared and stained with haematoxylin and eosin.

The luminal content of the appendix was divided into two portions. One portion was stored in 10% neutral formalin while the other was stored in PVA. The luminal content was fixed on glass slides and stained with both trichrome and modified Ziehl-Neelsen method.

Histopathological examination of appendix sections was carried out to detect helminths in the appendix. Components of histopathological analysis included the lumen of the appendix, the tissue layers of the appendix, the degree of lymphoid hyperplasia, degree of inflammation and the eosinophil response.

The degree of inflammation was divided generally into acute appendicitis and recurrent or chronic appendicitis. Acute appendicitis in general is diagnosed when there is presence of neutrophils infiltrating the mucosa and muscle layer (Ojo *et al.*, 1991). This can be divided into early acute appendicitis, acute appendicitis and acute suppurative appendicitis as follows.

Early acute appendicitis: presence of inflammatory cells, mild inflammation of the mucosa, muscularis mucosa or serosa (Petro & Minocha, 2005).

Acute appendicitis: neutrophils infiltrating the mucosa and muscularis mucosa, ulceration, transmural polymorphs infiltration or serosal inflammation (Herd *et al.*, 1992).

Acute suppurative appendicitis: purulent exudates in the lumen and the wall of the appendix (Floch *et al.*, 2005).

Chronic appendicitis or recurrent appendicitis is due to recurrent inflammation which has healed from an acute attack. There is fibrotic thickening of the appendiceal wall. Inflammatory infiltrate in the wall may be present from previous inflammation (Mohan, 2005).

Stained smears prepared from the luminal content were examined for helminths and protozoa.

RESULTS

In this study, there was a slight preponderance of males over females, with 58 (58%) and 42 (42%) cases respectively. The majority were Malays (69%), followed by Chinese (10%), Indians (6%), and a group of minority races and foreigners (15%). Most positives (80%) were in patients 30 year old or younger. The mean age was 22.5 years with a range between four and 81 years. 78% of the cases presented with acute appendicitis (early acute or acute or acute suppurative appendicitis). Twenty two percent presented as recurrent or chronic appendicitis. Of the 78 cases of acute appendicitis, 50 were males and 28 females. In chronic appendicitis 14 cases were females compared to 8 males. There was a significant association between acute appendicitis and male patients ($\chi^2 = 5.420$, $p<0.05$).

Of the 100 appendices 16 were positive for at least one parasite (Table 1). Only protozoa were detected; no helminth ova, larvae and adults were seen. Parasites detected were *Entamoeba* spp., *C. parvum*, microsporidia, *Blastocystis hominis* and *Isospora belli*. Parasites were detected in 9 male and 7 female patients. The infection rate in the age-group 0-30 years was 16.3% (13 out of 80) while in those over 30 years old, this was 15.0% (3 out of 20). This difference was not statistically significant ($p>0.05$). Parasitic infection rates in acute and chronic appendicitis were 16.7% (13 out of 78) and 13.6% (3 out of 22), and this difference was again not statistically significant ($p>0.05$).

Table 1. Patients' detail of cases that were positive for parasites

Gender	Age	Race	Parasite	Inflammation
Male	25	Malay	*E. coli*	Acute
Male	33	Others	*E. coli*	Acute
Male	4	Malay	Microsporidia	Suppurative
Male	10	Chinese	*B. hominis*	Acute
Female	14	Malay	*C. parvum*	Suppurative
Female	13	Malay	Microsporidia, *C. parvum*	Chronic
Male	14	Malay	*B. hominis*, *C. parvum*, *I. belli*	Acute
Female	19	Chinese	*E. hartmanni*	Suppurative
Male	7	Malay	*E. histolytica*, Microsporidia	Acute
Male	9	Malay	*E. coli*, *B. hominis*	Acute
Female	16	Indian	*C. parvum*	Chronic
Female	14	Malay	Microsporidia	Chronic
Female	42	Malay	*E. histolytica*	Suppurative
Male	10	Malay	*E. coli*	Acute
Male	22	Chinese	*B. hominis*	Acute
Female	81	Chinese	*E. hartmanni*, *I. butschlii*	Suppurative

DISCUSSION

Our study has shown that parasite infection is a possible cause of acute appendicitis. Although the number of positive cases was not large enough to make a significant association, an incidence of 16% is higher than most previous studies.

In this study appendicitis is more common in males (58%), and this is similar to a previous study in Kuala Lumpur by Thanaletchimy (1986) where male and female cases were 55.8% and 44.2% respectively. The same study also demonstrated that there was no racial difference in rates.

In this study 80% of the appendicitis patients were in those 30 years and below and this is in agreement with other studies. Chang (1981) in New Zealand found 82% of cases in those 30 years of age or below. Similarly, Thanaletchimy (1986) found that 77.9% of such patients in Malaysia were in this group. It was also interesting to find that the youngest (4 years) and the oldest (81 years) patients had parasite infection and presented with acute suppurative appendicitis.

Acute appendicitis made up 78% of the cases in our study, being similar to Thanaletchimy (1986) where the number was 77.5%. In the study by Thanaletchimy (1986), out of 225 (22.5%) cases that were considered non-inflamed, only 57 (5.7%) were normal while the remainder showed some pathology. Our study was different as all 22 (22%) cases that were not acutely inflamed showed features of chronic appendicitis; normal appendices were not seen.

Parasites were found in 16% of the cases in our study, and this infection rate is higher than that of previous studies. Chan & Fu (1987) did a study of over 10 000 cases of appendectomy in Hong Kong and found parasites to be present in only 0.51% of cases. A more recent study by Dorfman (2003) in Venezuela found parasites to be present in 7.47% of the total cases. In the study by Thanaletchimy (1986), the positive rate was 8%.

Our study reported only protozoa parasites. Other previous study had shown helminths to be more predominant, *E. vermicularis* was the most common. Chan and Fu (1987) only found helminths to be present, *E. vermicularis* alone made up 26 out of 58 cases that had shown positivity for parasites. The study by Thanaletchimy (1986) had also shown *E. vermicularis* to be the most common parasite. *Entamoeba histolytica* was also present in six cases out of 80 (7.5%) cases that were positive for parasites. A number of parasites found in our study have not been previously reported, especially *B. hominis*, *I. belli* and microsporidia.

In our study, parasites were found mainly in the luminal content on microscopic examination. Similarly, Chan & Fu (1987) reported that the majority of parasites were detected under the microscope, few grossly. As the ova of helminths can be present, detecting the parasites by examining only the gross specimen would miss out on this important finding. This proves the importance of microscopic examination of appendectomy specimens.

Acute appendicitis was found in 13 out of 16 (81.3%) cases where parasites were positive. This was not in common with other findings as the study by Chang (1981) had found acute appendicitis in only 13 out of 89 (14.6%) cases that were positive for parasites. Thanaletchimy (1986) also found acute appendicitis in 34 out of 80 (42.5%) cases that were positive for parasites.

Our study did not find a significant association between parasitic infection and acute appendicitis, but the parasite positive rate of 16% is relatively high. A limitation of our study is the small sample size number. It is possible that helminth parasites were not detected due to the limited number of cases studied. The extraction of the luminal content had proven to be an important method as most of the parasites were detected there. In conclusion, the current method of examining both the tissue sections and also the luminal contents in appendices removed during appendectomy is of immense value, especially for the detection of protozoa.

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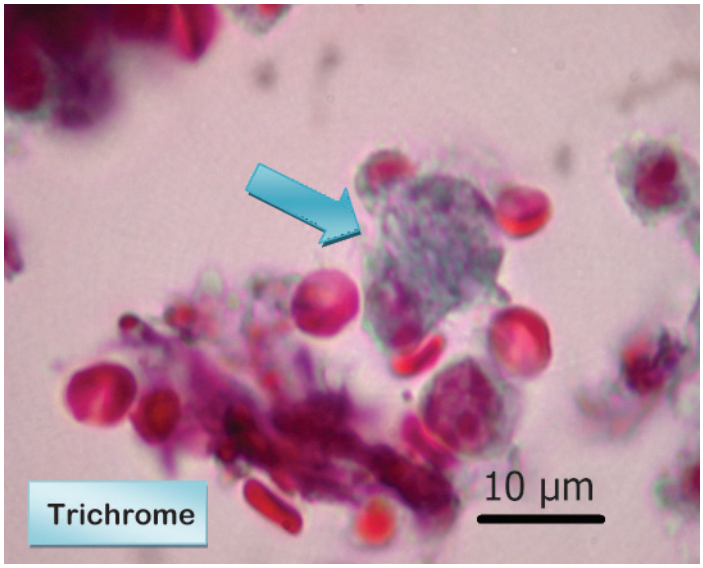

Figure 1. Photomicrograph of a trichrome stained luminal content showing an *Entamoeba histolytica* trophozoite ingesting red blood cells (arrow). Scale = 10 µm.

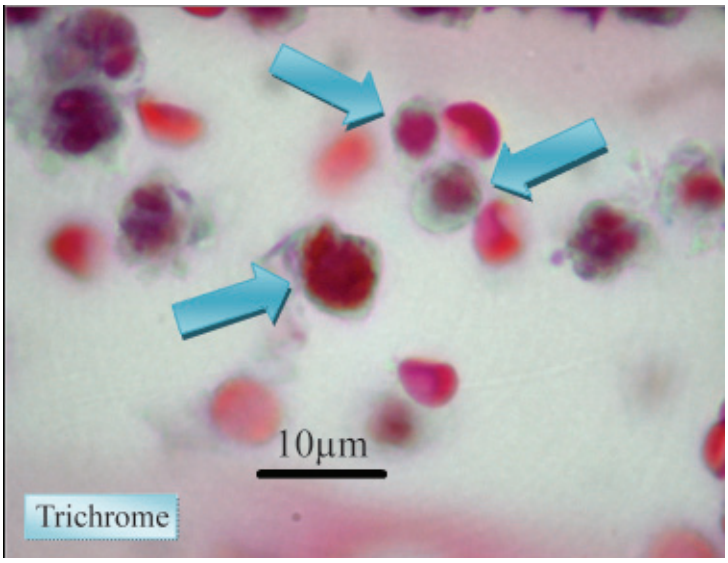

Figure 2. Photomicrograph of a trichrome stained luminal content showing a number of *Blastocystis hominis* (arrows). Scale = 10µm.

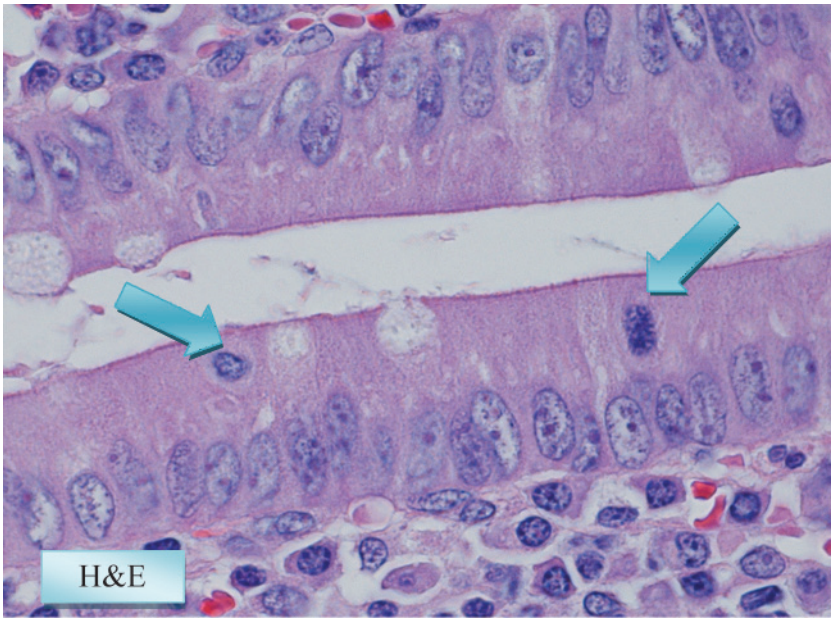

Figure 3. Photomicrograph of a haematoxylin & eosin stained transverse section of the appendix tissue showing Microsporidia in the epithelial cells (arrows). Magnification: 1000X.

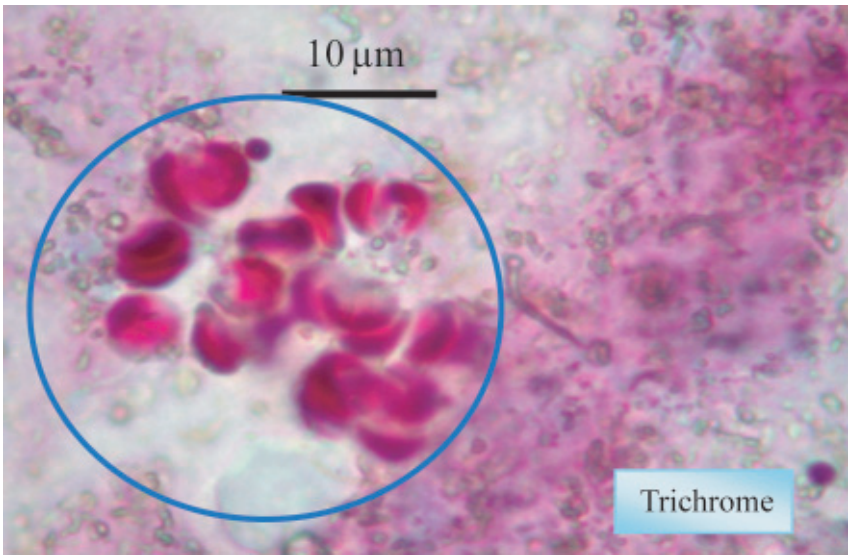

Figure 4. Photomicrograph of the stained luminal content showing a group of *Cryptosporidium parvum* (oval).

REFERENCES

Andersson, R., Hugander, A., Thulin. A., Nystrom, P.O. & Olaison, G. (1995). Cluster of acute appendicitis: further evidence for an infectious aetiology. *International Journal of Epidemiology* **24**: 829-33.

Chan, W. & Fu, K.H. (1987). Value of routine histopathological examination of appendices in Hong Kong. *Journal of Clinical Pathology* **40**: 429-33.

Chang, A.R. (1981). An analysis of the pathology of 3003 appendices. *The Australian and New Zealand Journal of Surgery* Apr **51**: 169-78.

Díaz, O.M.G. & Fernández, F.A.N. (2001). Apendicitis parasitarias. *Revista Mexicana de Patologica Clinica* **48**: 42-5.

Dorfman, S., Cardozo, J., Dorfman, D. & Villar, A.D. (2003). The role of parasites in acute appendicitis of pediatric patients. *Investigacion Clinica* **44**: 337-40.

Elezary, R., Maly, A., Khalaileh, A., Rubinstein, C., Olstain-Pops, K., Almogy, G., Rivkind, A.I. & Mintz, Y. (2005). Schistosomiasis and acute appendicitis. *The Israel Medical Association Journal* **7**: 533-4.

Floch, M.H. Diseases of the Appendix: Inflammation, Mucocele, and Tumors (2005). In: Netter's Gastroenterology (Editors, Floch, M.H., Kowdley, K., Pitchumoni, C.S., Floch, N.R., Rosenthal, R. & Scolapio, J.) pp. 452-4. Elsevier, Amsterdam.

Guo, Y., Xiao, S.Y., Yan, H., Sun, N.D., Jiang, M.S. & Liu, D.Y. (2004). Cluster of acute hemorrhagic appendicitis among high school students in Wuhan, China. *American Journal of Surgery* **188**: 115-21.

Hardin, D.M. (1999). Acute appendicitis: review and update. *American Family Physician* **60**: 2027-34.

Herd, M.E., Cross, P.A. & Dutt, S. (1992). Histological audit of acute appendicitis. *Journal of Clinical Pathology* **45**: 456-8.

Martin, D.L. & Gustafson, T.L. (1985). A cluster of true appendicitis cases. *American Journal of Surgery* **150**: 554-7.

Mohan, M. (2005). In: Textbook of pathology. 5th ed. pp. 593-94. Jaypee Brothers, New Delhi.

Mowlavi, G., Massoud, J., Mobedi, I., Rezaian, M. & Mohammadi, S.S. (2004). *Enterobius vermicularis*: a controversial cause of appendicitis. *Iranian Journal of Public Health* **33**: 27-31.

Ojo, O.S., Udeh, S.C. & Odesanmi, W.O. (1991). Review of the histopathological findings in appendices removed for acute appendicitis in Nigerians. *Journal of the Royal College of Surgeons of Edinburgh* **36**: 245-8.

Old, J.L., Dusing, R.W., Yap, W. & Dirks, J. (2005). Imaging for suspected appendicitis. *American Family Physician* **71**: 71-8.

Petro, M. & Minocha, A. (2005). Asymptomatic early acute appendicitis initiated and diagnosed during colonoscopy: a case report. *World Journal of Gastroenterology* **11**: 5398-400.

Thanaletchimy, N. (1986). Acute appendicitis: pathology of 1,000 cases. *Medical Journal of Malaysia* **41**: 336-42.