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Incidence and Detection of Parasite Ova in Appendix from Patients with Appendicitis in South-eastern, Nigeria

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Abstract: This study reports the incidence and detection of cysts or ova of parasites in appendix from patients with appendicitis in Southeastern, Nigeria. The mucosa or mucous membrane of the appendix can be injured by worms and other intestinal parasites, which occasionally block the lumen of the organ leading to appendicitis. The appendix specimens were prepared into histological slides and examined. The ova of five parasitic worms: *Enterobius vermicularis, Trichuris trichiura, Ascaris lumbricoides, Schistosoma mansoni, Schistosoma haematobium and cyst of Entamoeba histolytica* were detected in 21 of the 28 appendix specimens examined. There were cases of polyparasitism as seen in *S. haematobium* and *T. trichiura* 4(19.1%), *E. vermicularis* and *E. histolytica* 3(14.3%), *A. lumbricoides* with *E. vermicularis* 3(14.3%), *S. mansoni* and *T. trichiura* 2(9.5%) and *T. trichura with E. histolytica* 2(9.5%) and also 7 (25%) cases of absence of parasites ova or cysts. There was high incidence of the cyst of *E. histolytica* with 19.1% and ova of *E. vermicularis* with 14.3% signifying their monoparasitism observed in this study and a low incidence of co-infection of *S. mansoni* with *T. trichiura* and *T. trichiura* with *E. histolytica* with 9.5%. From the result, most of the inflamed appendix revealed presence of one parasite ova or the other pointing to the fact that ova or cysts of parasites in the appendix can lead to inflammation of the organ namely appendicitis. These striking observations should re-enforce the need for an urgent effort to be made to check the unnecessary and avoidable heavy parasitic worms load before the body becomes laden with these parasites and their ova/cysts which may result to health and life-threatening infestation leading to the painful disease process- appendicitis. If deworming is carried out to its logical conclusion, heavy parasitisation leading to appendicitis will be avoided. This way, people will be saved from the pains and agony of undergoing appendicectomy. Although, the findings of this study may have minor discrepancies with others from different countries, it still boils down to the fact that parasites and their ova/cysts in the appendix can provoke appendicitis. Therefore, measures should be adopted to checkmate, control or prevent this tendency for parasites and/or their ova to invade the appendix to cause appendicitis.

Key words: Appendix % Appendicitis % Appendicectomy % Cysts % Inflammation % Ova % Parasite % polyparasitism

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INTRODUCTION

The appendix is a hollow tube lined with mucous membrane, with a muscle similar to that of the caecum with which it communicates. It varies greatly in size, the average length being 3 inches (7.5cm). It may turn upwards behind the caecum, pointing out towards the groin-the retro-caecal position or it may lie on the front of the caecum immediately under the anterior abdominal wall [1].

The appendix is sometimes referred to as the 'abdominal tonsil' because it is composed largely of the lymphoid tissues and is very susceptible to infection [1]. The vermiform (worm-shaped) appendix is a blind-ended tube, which opens into the wall of the caecum 2cm below the ileocaecal valve [2]. The organ is a small tube-shaped bag of the tissue attached to the intestine. It is the undeveloped distal end of the large caecum found in many lower animals [3]. The vermiform appendix is present only in man, certain anthropoids, apes and the wombat- a nocturnal, burrowing animal [3]. However, the appendix is a blind sac which at times harbours some virulent organisms [4]. The appendix shares in abnormalities in position of the caecum, its base lies where the teniae of the caecum converge, thus enabling the surgeon to find it at operation [5].

Although it assumes varying positions, the appendix occupies a retrocaecal (posterior to caecum) position in approximately 65% of the population. The appendix has its own short mesentery, the mesoappendix, which is a triangular fold of peritoneum from the left (interior) layer of the mesentery of the terminal ileum. In children, the mesoappendix is so transparent that the contained blood-vessels can be seen but in many adults, it becomes laden with fat, which obscures these vessels. Its wall contains lymphoid tissues [6].

The diseases associated with the appendix include: 1). Tumours of the appendix which refers to the tumour forming a discrete, hard, rounded yellowish nodule, usually small and rarely exceeding the size of an almond. Tumours of the appendix are very common. 2). Appendicitis which refers to inflammation occurring in the vermiform appendix and it is very common. Appendicitis was the first disease in which the importance of early diagnosis and of prompt surgical intervention was emphasized as essential to reduction in mortality from a specific disease entity. It was also the first of the surgical diseases, in which the signs and symptoms of early diagnosis were widely published among the laity, leading to a lowering of mortality rates [7].

Acute appendicitis results from obstruction of the lumen followed by infection [8]. This applied to the distinct entity characterized by acute transmural inflammation with peritonitis and clinically by the shift by the acute onset of abdominal pain, initially periumbilical, but later shifting to the right iliac fossa where it is accompanied by classic sigh of rebound tenderness [9]. It accounts for more than half of all abdominal emergencies between the ages of 10 and 30 [10]. The menace of acute appendicitis lies in the frequency with which the peritoneal cavity is infected from the focus by perforation and transmigration of bacteria through the appendicular wall [11]. The most frequent cause (about 90%) of acute appendicitis is occlusion of the lumen of the appendix. The cause is usually a faecal impactation or concretion and rarely a foreign body such as a seed [12]. Although, appendicitis is seen in both the old and the young, it is seen most frequently in patients between the ages of 10 and 20 and it is probably still the most frequent inflammatory surgical condition arising in the right lower quadrant in any decade of life [12].

Appendicitis has hitherto been mainly a disease of the western world and particularly of Great Britain and North America and less common in Denmark, Spain, Italy, Asia and Africa, but now being recognized with increasing frequency in Africa and Asia because of their adaptation of Great Britain and American dietary [13]. It seems certain, however, that obstruction of the appendicular lumen is involved in many cases of appendicitis [14]. The obstructing agent is usually the presence of a concretion (Faecolith), a foreign body, or a round worm or thread-worms, or rarely a tumour in the lumen which may predispose it to perforation, because the swollen wall becomes stretched over the concretion [14]. Worms like o*xyuria*, *vermicularis* and other intestinal parasites can injure the appendicualr membrane and occasionally block its lumen [15].

Some intestinal nematodes such as *Ascaris lumbricoides*, *Trichuris trichuria*, *Enterobius vermicularis*, *Ancyclostomidae duodenale*, *Necator americanus*, *Strongyloides stercoralis* and some species of *Schistosoma* as well as *Entamoeba histolytica* have been incriminated in the cause of appendicitis. They are large intestinal worms of man and are by far known to be the most widespread and commonest parasites of man in tropical Africa [16-17]. Their eggs are resistant to adverse conditions of low temperature, desiccation and strong chemicals and can remain viable for several years [17]. These worms have been found to clogging the appendix which leads to complication "appendicitis" caused by migrating worms [16-18]. *Enterobius vermicularis* (pinworms or threadworms) is also refered to as one of the most common nematode parasites of man which flourish in the temperate zones where 'probably every child has

been infected not once, but many times in early childhood'. It has a worldwide distribution with children being more commonly infected than adults. Infection of the urinary and genital tract can occur and worms in the appendix can cause appendicitis [16, 19]. There are possibilities of autoinfection with *Strongyloides* which could convert a light infection into a heavy one or even hyperinfection with grave pathological consequences.

Infection with these parasites is by ingesting infective eggs in contaminated food or from contaminated fingers [16]. Most diseases in all cases associated with poor sanitary conditions, the possibility of their wide-scale prevalence in tropical Africa is no doubt high [20]. Unsafe water is a global public health threat, placing persons at risk for a host of diarrheal and other diseases as well as chemical intoxication [21]. Unsanitary water has particularly devastating effects on young children in the developing world [22-23]. Biological contaminants such as bacteria, viruses, fungi, protozoa and helminthes constitute the major cause of food-borne diseases with varying degrees of severity, ranging from mild indisposition to chronic or life-threatening illness, or both. In developing countries, such contaminants are responsible for food borne diseases such as cholera, campylobacteriosis, *E. coli* gastroenteritis, salmonellosis, shigellosis, typhoid fever, brucellosis, amoebiasis and poilomyletis [24-25]. The original source of any drinking water is rich in aquatic microbes, some of which could be dangerous if they enter the human body [26]. The presence of indicator and other organisms in water is of special concern and perhaps the greatest danger associated with water used for food processing, drinking purposes and for human consumption is contamination by human excrement [24, 27].

The signs and symptoms of appendicitis vary and may mimic many other acute abdominal conditions [28]. It may kill the patient especially the very young and the very old [29]. Treatment of appendicitis is appendicectomy i.e. excision of the appendix vermigormis, if the diagnosis is made at an early stage of a localized mass, the consent of the patient and his/her relatives is given. Conservative treatment with antibiotics and intravenous fluid may be used in the absence of anaesthesia or a competent surgeon or when the patient presents with an appendiceal mass. If the mass increase in size or the patient's general condition deteriorates, the appendiceal abscess must be drained [29, 30].

This study is in an attempt to determine the extent to which intestinal parasites are found in the appendix and to know whether they are aetiologically associated with the inflammation of the organ and their roles in the causation of appendicitis. This entailed examination of appendix specimens for parasites and/or their ova/cysts.

MATERIALS AND METHODS

Materials: November 9, 2008The materials used `in this study include: sterile specimen bottles, 10% formal-saline solution (FSS), glass slides, cover slips, dissecting knife, molten wax (paraffin wax for embedding and impregnate), microtome (cutting/slicing machine to section paraffin blocks), alcohol (75%, 90%, 95% and absolute alcohol), xylene (rapid clearing agent), wooden block for mounting wax block, haematoxylin stain, eosin solution (counter-stain), Dystrene phosphate xylene (DPX) mountant and appendix specimens.

Samples Collection: Appendix specimens (tissues) were collected from patients presenting with appendicitis at General Hospital, Oguta, Imo State, Southeastern, Nigeria. These specimens were collected after the surgical operation exercise has been performed by the surgeons. These specimens were collected in a sterile specimen bottles free of microbial contamination and 10% Formal-saline solution (FSS) was used as a fixative in the preservation of the specimens in order to prevent them from autolysing, post-mortem changes and spoilage which occur shortly after removal from the body. The samples were obtained by informed consent of the patients used for this study and the permission to that effect was obtained from the ethical committee.

Methodology: The appendix specimens were collected using the sterile specimen bottles. These were taken to the histopathology laboratory where the slides were prepared as described by the method of Sood [31] and Baker *et al.* [32]. The prepared slides were stained haematoxylin stain and counterstained with eosin stain. After the staining, the slides were mounted in DPX mountant and covered with cover slips. They were then viewed under the microscope at x10 to x40 objectives lens.

RESULTS

The ova and cysts of some parasites were detected and evaluated among patients with appendicitis. These parasites include ova of *Enterobius vermicularis, Trichuris trichiura, Schistosoma haematobium, S. mansoni* and *Ascaris lumbricoides* and cysts of *Entamoeba histolytica*. Table 1 shows the description of the different ova/cysts of the parasites detected.

Table 1: Description of different ova/cyst of parasites detected

Parasites	Description of Ova/cyst
Enterobius vermicularis	Ova are oval but asymmetrical (flattened on one side, rounded on the other). It has a smooth and thin shell but double line is visible.
Trichuris trichiura	Ova are oval or rounded with polar plugs
Ascaris lumbricoides	Ova are oval or rounded
Entamoeba histolytica	Trophozoites are seen in fresh smears and encyst to form cysts in adverse conditions. Cyst is round and has 1-4 nuceli and chromatoid bodies that are bar-like
Schistosoma haematobium	Ova are not operculate and is usually provided a characteristic spine which is terminal
Schistosoma mansoni	Ova are not operculate and is characterized by the large backward pointing lateral spine

Table 2: Frequency of occurrence of ova/cysts of parasites among patients with appendicitis

Parasites	No. (%)
Enterobius vermicularis	3(14.3)*
Entamoeba histolytica	4(19.1)*
Ascaris lumbricoides and *Enterobius vermicularis*	3(14.3)**
Enterobius vermicularis and *Entamoeba histolytica*	3(14.3)**
Schistosoma haematobium and *Trichuris trichiura*	4(19.1)**
Schistosoma mansoni and Trichuris trichiura	2(9.5)**
Trichuris trichiura and *Entamoeba histolytica*	2(9.5)**
Total	21(100.0)

* = monoparasitic** = polyparasitic

Table 3: Distribution of parasitic infections in relation to age (years) of patients with appendicitis

Age (years)	No. Tested	No. Positive (%)	No. Negative (%)
10-19	12	10(83.3)*	2(16.7)
20-29	5	4(80.0)*	1(20.0)
30-39	5	4(80.0)*	1(20.0)
40 and above	5	3(60.0)*	2(40.0)
Total	27	21(75.0)	6(25.0)

* = No significant difference (P >0.05)

Table 4: Distribution of parasitic infections in relation to gender (sex) of patients with appendicitis

Gender (sex)	No. Tested (%)	No. Positive (%)	No. Negative (%)
Male	12	9(75.0)**	3(25.0)
Female	15	12(80.0)**	3(20.0)
Total	27	21(75.0)	6(25.0)

** = No significant difference (P >0.05)

Table 2 shows the frequency of occurrence of the ova/cysts of parasites among patients with appendicitis. Ova of different worms and cysts of *Entamoeba histolytica* were detected. Some of the ova appeared alone, while some appeared in combination with others. Some appear with cysts of *Entamoeba histolytica*. In some cases, only the cysts appeared. *Entamoeba histolytica* and *Schistosoma haematobium* with *Trichuris trichiura* had the highest frequency of occurrence 4(19.1%), followed by *Enterobius vermicularis* 3(14.3%), *Ascaris lumbricoides* with *Enterobius vermicularis* 3(14.3%) and *Enterobius vermicularis* with *Entamoeba histolytica* 3(14.3%). *Schistosoma mansoni* with *Trichuris trichiura* 2(9.5%) and *Trichuris trichiura* with *Entamoeba histolytica* 2(9.5%) had the lowest incidence (Table 2).

Table 3 shows the distribution of parasitic infections in relation to age (years) of patients among patients with appendicitis. The highest number of patients with ova/cysts of parasites was mostly found amongst teenagers; age groups 10-19 years [10(83.3%)], followed by age groups 20-29 years [4(80%)] and 30-39 years [4(80%)] while the age group 40 and above [3(60%)] had the least number of patients positive for ova/cysts of parasites (Table 3).

Table 4 shows the Distribution of parasitic infections in relation to gender (sex) of patients among patients with appendicitis. Nine 9(75%) males and 12(75%) females had ova/cysts of parasites (Table 4).

DISCUSSION

In this study, among the appendix specimens studied from 27 patients, the ova of *Ascaris lumbricoides*, *Schistosoma mansoni Enterobius vermicularis*, *Trichuris trichiuria* and *Schistosoma haematobium* and cysts of *Entamoeba histolyitica* were identified in 21 specimens. In 6 appendix specimens, the ova of one parasite and cysts of *Entamoeba histolytica* were found to co-exist, while in 15 specimens, ova of 2 parasitic worms or ova and cysts of *Entamoeba histolytica* were also found to co-exist. In 6 specimens, no parasitic ova or cysts were found. This showed that the appendicitis (inflammation of the appendix organ) was precipitated by parasitic worms and protozoans.

The appendix can be infiltrated by a lot of materials including faecal material, microbes and parasites. Some of these transients' materials which find their way into the organ may remain there for a while without causing any disease. At times, few of them can initiate a disease process which may lead to inflammation of the appendix. If the disease process is serious and last for quite sometime, the condition known as appendicitis may result. This is the case with some parasites and worms especially when they stay too long in the lumen or when their ova/cysts block or are lodged within the wall of appendicular lumen.

Some of these parasites especially the worms or ova of *Ascaris lumbricoides, Schistosoma mansoni* and cysts of *Entamoeba histolyitica* (cysts) can accidentally enter the appendix but others like *Enterobius vermicularis, Trichuris trichiuria* and *Schistosoma haematobium*, for unknown reasons, sometimes nest in the appendicular lumen where they at times cause reactions that can result in appendicitis. The results and findings of this study are not different from the foregoing observation.

In a previous study, Onuigbo [33] noted that microscopically proven, acute appendicitis tends to be associated with clinically suspected acute appendicitis rather than with unrelated conditions. This was observed to be true in this present study because 6 of the appendix specimens had no ova or cysts parasites. Such unrelated diseases lead to the incidental removal of the appendix and to the inconsequential retrieval of an organ in which the ova are still, as it were, mere passengers. What makes the ova become an agent of suppurative inflammation is specifically the position of the ova in the organ.

In this study, the ova of worms and cysts of *Entamoeba histolytica* were detected in 21 cases of appendicitis. This finding compares favorably and correlates with previous studies by Cheesbrough [34] and Ukoli [35] which reported that migratory worms occasionally cause appendicitis. The ova were obviously deposited by migratory worms that wondered into the appendix lumen. Schmid [36] reported the pattern of ova distribution and noted that the passage of a high number of ova through the mucosa, granulomatous disruption of the muscularis and defigurement of the appendix by pseudotubercles of the serosa usually lead to acute appendicitis. In the 3 cases reported by El-Shenawy [37], suppurative appendicitis was present with "ova deposition in the different layers of the appendix".

In this study cases of polyparasitism was observed in which *Schistosoma mansoni* and *Trichuris trichuria*; *Ascaris lumbricoides* and *Enterobius vermicularis*; *Trichuris trichuria* and *Entamoeba histolytica*; *Schistosoma haematobium* and *trichuris trichuria*; *Entamoeba histolytica* and *Enterobius vermicularis*. This compares favourbly with previous studies. In most of the surveys in tropical Africa, it has been shown that multiple infection with intestinal nematodes is very common, such that cases of polyparasitism with nematodes (Ascaris, hookworms and Trichuris); flatworms (*Schistosoma mansoni* and *Taenia saginata*); and protozoa (*Entamoeba histolytica* and *Giardia duodenalis*) have been reported [38-40]. Kionti [41] reported that the combination of Ascaris and hookworm, Ascaris and Trichuris, Ascaris, hookworm and Trichuris accounted for more than 76% of all multiple infections in school children in the Kano plain in Kenya.

The detection of schistosomal ova in this study differs from what was written in Kenya by Ochola-Abila in 1979 [42] stated that the findings of schistosomal granulomata does seem to be a very uncommon finding in our appendicectomy specimens though Shija (Muhimbili Hospital, Tanzania) in a personal communication over two years ago indicated that this was a very uncommon finding in their appendicectomy specimens. In the same country, focal factors exist. Discrepancies observed in the findings of this study and previous studies by other researchers and could be attributed to differences in place of study i.e. geographical location and other conditions which can affect the research finding so that slight discrepancies could occur. The findings of this study also revealed that there is high incidence of *Enterobius vermicularis* and *Entamoeba histolytica* with 32% respectively and low incidence of Ascariasis of the appendix with 11%. It has also been suggested that the increasing parasite ova in the appendix can be traced back to heavy worm load in the body organs-intestine and other tissues [43-45].

In response to this high incidences, World Health Organization (WHO) have outlined strategies to combat the problem of parasitism and by extension its association with appendicitis. In this regards, the current deworming programme by some agencies and NGOs should be cost effectiveness and use of potent but safe anti-helminthic drugs. Measures directed at ensuring that heavy parasite infestations in humans are reduced include: 1.) maintaining high standards of personal and domestic hygiene. 2.) Avoiding contact with contaminated water, food and clothing. 3.) High standard of education and adequate health education and 4.) Presence of modern public restaurants hygienically kept and maintained [45].

The finding of this present study supports the view that parasites or their ova can cause appendicitis. There are a lot of literature on the reason for high prevalence of parasitic and worms (helminthic) infections in tropical Africa and none more succinct and eloquent than Cowper's [46] comment which states that a child born in a Nigerian village is almost bombarded by parasitic worms from soon after birth throughout life. In both rural and urban environments, the water used for drinking and domestic purposes is a source of schistosomaisis and guineaworm, the food both vegetables and meat, could be a source of tapeworm, roundworm and whipworm and also lung fluke where fresh water crustacean are consumed;

the soil on which children walks barefooted infects them with hookworm and strongyloides and the profusion of biting insects with onchocereciasis and filaririasis. Non-biting insects, dogs, cats, goats and poultry in the houses and yards assist the mechanical spread of intestinal helminthes and the use of human night soil as manure increases the risk. It is little wonder, therefore, that the prevalence of these intestinal helminthes and the average worm burdens have not shown any appreciable decrease from the estimates in the much quoted paper, 'This wormy world', by Stoll [47].

As evident in the findings of this study, 21 out of 28 surgically removed appendix specimen from patients with appendicitis revealed one parasite ova/cysts or the other and even two parasite ova/cysts. These striking observations should re-enforce the need for an urgent effort to be made to check the unnecessary and avoidable heavy worm load before the body becomes laden with these parasites and their ova/cysts which may result to health and life-threatening infestation leading to the painful disease process known as appendicitis. If deworming is carried out to its logical conclusion, heavy parasitisation will be avoided and thus parasite-caused appendicitis will also be avoided. This way, people will be saved from the pains and agony of undergoing appendicectomy.

The result of this study revealed that a higher percentage of the patient who had parasites ova/cysts in their appendix fall within the 10-19 years age group with 83.3%, followed by age groups 20-29 years and 30-39 years of age with 80% respectively. Age group 40 years and above had the least 60%. However, there is no significant difference ($P>0.05$) between the ages of the subjects. Also, this study revealed that both male and female subjects had the same parasite ova/cysts load in equal magnitude of 75% respectively. This suggests that both males and females are equally exposed to parasitic infection and gender may not necessarily be an important epidemiological determinant for appendicitis and parasites loads. There is no significant difference between males and females tested ($P>0.05$). This implies that the level of appendicitis and parasitic infection (parasite load among patients tested is the same regardless of gender or age.

Concluding from the forefoing pieces of information, it is clear that there is urgent need for de-worming i.e. reducing the worm load in the body system. This is because if one is de-wormed at intervals, there is the possibility of killing most worms present in the intestine before these worms wander and infect the appendix. Although, the findings of this study may have minor discrepancies with others from different countries, it still boils down to the fact that parasites and their ova in the appendix can provoke appendicitis. For this reason, measures should be adopted to checkmate, control or prevent this tendency for parasites and/or their ova to invade the appendix to cause appendicitis. The obvious preventive measures would include: the improvement of general standards of sanitation through the installation of suitable sewage treatment and disposal facilities and provision of pipe-borne water supply as pre-requisites for successful prevention and control. Generally, health authorities should make concerted efforts to ensure the prevention of these parasitic worms from infecting mans appendix. This would go a long way to eliminate the pains of appendicitis and the resultant risk of undergoing appendicectomy. This will also ensure the survival and continuity of the normal appendix in the body.

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