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Immune profile of HIV+ with microsporidium and Hepatitis-B in Benue State, Nigeria

Yako AB[*1], Ajayi JA[2], Makpo JK[1], Yako JB[3], Auta KI[2], Nweze CC[1], Banyigyi HA[1].

[1]Department of Biological Sciences, PMB 1022, Nasarawa State University, Keffi, Nigeria
[2]Department of zoology, Applied Parasitology and Entomology unit, PMB 2084, University of Jos, Plateau State Nigeria
[3]Police Staff College Clinics, Jos Plateau State Nigeria

ABSTRACT

Survey was focused exclusively on human excreta and sera for microsporidium and hepatitis-B.The immune profile was determined of HIV+ individuals in Benue State. Intestinal epithelium was the most prevalent site of human microsporidium infection, 54 (4.2%) were infected in both sexes. Enterocytozoon bieneusi was most prevalent in Makurdi with no significant relevance in the spread of microsporidium species among a cohort population study (r=0.79 >0.60, df=9). Urinary tract microsporidiosis appears in 31(2.4%) patients with Encephalitozoon cuniculi although, many persons during these findings do not have symptoms referable to the urogenital route and 9(1.3%) were HBsAg+ with no significant difference since r=0.76>0.75, df=6. Stools were loose to watery and co- infected with laboratory evidence for intestinal microsporidium in 1(0.5%) with T-cells < 80 cell/µl. However, sexual transmission of microsporidiosis cannot be excluded. Therefore, the clinical spectrum of the disease should be tied to thorough laboratory investigation and strict hygienic condition should be maintained.

Keywords: Microsporidium. Hepatitis-B. Immune. Profile. HIV.

INTRODUCTION

Persons suffering from variety of microsporidial disease manifestations have been identified from all continents, predominantly from Europe recognition and North America. New interests in the organisms have however, improved diagnostic approaches, case recognition in the developing regions of the world is gathering momentum [7, 15]. Therefore, human infections are

now well documented in several African nations which include; Nigeria, Egypt and South Africa, as well to Southeast Asia, and South America.

Microsporidiosis is an illness caused by recently discovered protozoan parasites called microsporidia. The most known of the parasite that infect the small intestine is *Enterocytozoon bieneusi* and has also been reported as a cause of sinusitis, and cholangitis [6].

Serious illness due to *microsporidium* appears to occur predominantly in adults suffering from immunosuppression in Nigeria, especially that associated with human immunodeficiency syndrome (AIDS). Similarly, increasing recognition of human microsporidiosis with other forms of immunosuppression has been reported [16, 3]. Infections with *Enterocytozoon bieneusi* have been confirmed in HIV individuals with hepatitis B infection, as well with *Encephalitozoon cuniculi* identified in a genitourinary tract.

Although, most recognized cases of human Microsporidiosis are associated with some form of immunosuppression, reports describing microsporidial infections in HIV- negative, immunocompetent patients are also increasing. These reports have included instances of *Enterocytozoon bieneusi* infections in travelers as well as in adult and children residents of various tropical countries [4, 9, 2]. Transmission of microsporidia occurs by several potential mechanisms. Water borne transmission is increasingly possible and the well documented presence of viable, infective spores in multiple body fluids and excreta suggest that person-to-person transmission is possible for most forms of human microsporidiosis in extension of genitourinary tract involvement among HIV-positive individuals in Benue State, Nigeria.

MATERIALS AND METHODS

Study area

Benue is a state in east-central Nigeria located in the middle belt area which is bordered by Nasarawa state to the north, Taraba state to the east, Ebonyi and Cross-river to the south and Kogi state to the west. The population of Benue State is at 4,253,642 which is made up of 2,144,043 (males), and 2,109,598 (females) according to 2006 National Population Census. And this survey covers 18 communities, sampling a total of 2008 HIV+ individuals in Benue state, Nigeria.

Calcofluor White Staining Procedure

A thin smear of fecal matter was prepared and was fixed in methanol for 30 seconds, and was stain with 0.01% calcoflour or white reagent for 1 minute. Then, it was rinsed with distilled water and the smear was let to dry. The slide was mounted with a # 1 thickness cover slip and examined with an epifluorenscence microscope equipped with blue violet filter cube with a wavelength of 400nm or less.

Hepatitis B surface antigen (HBsAg) Diagnostic procedure.

Blood samples were collected into EDTA containers after informed consent and ethical clearance from a research ethics committee. The reagents were brought to room temperature and were mixed gently; it was sure that, the latex reagent was completely in suspension. The dilute serum was prepared into 1:40 by mixing one drop (50µl) of serum with physiological saline of 0.9%,

and one drop of dilute serum was appropriately dropped onto the slide cell. One drop of undiluted serum was placed onto another slide, and at this point, one of each positive and negative control were placed appropriately onto the slide cells and dilution of controls were highly/strongly prohibited. The latex reagents again were gently mixed for complete re-suspension of particles and one drop was added to all cells (i.e. controls, diluted, and undiluted sera) on the slide. Separate sticks were used to mixed and spread the fluid over the entire area of the cells. And the slides were tilted using an automated rotator at 100rpm for 5 minutes.

HIV 1 and 2 Q-spot

Assay Procedure

The Q-spot method was employed to purified recombinant proteins from HIV 1 and 2 envelop for detection of specific antibodies to HIV 1 and 2 viruses. Although, this was considered an animal screening test for HIV 1 and 2. Two of W& W buffer was dispensed onto the center of the device, and one drop was equally added (50w) of specimen to the center of the device for 1 minute after which the specimen was completely soaked into the membrane. At this point, three drops of W & W buffer were added of equal volume onto the center of the device and two drops of cold conjugate was thereafter added onto the center of the device which again, two drops of W & W buffer were added and the result immediately was read within 5 minutes.

Most importantly the interpretation of the result was observed with two pinks to red coloured spots in the inner circle of the device. And this obviously suggests strongly that, the specimen contains 1 and 2 antibodies. Irrespective of the coloured intensity, any spot, which was developed 10 minutes after completion of the assay, was considered invalid. Again, any one spot on the left of the inner circle marked ‘c’ was visible, and any test running that develop onto that one spot or on the overall surface of the membrane, the result was considered negative and was discarded.

RESULTS AND DISCUSSION

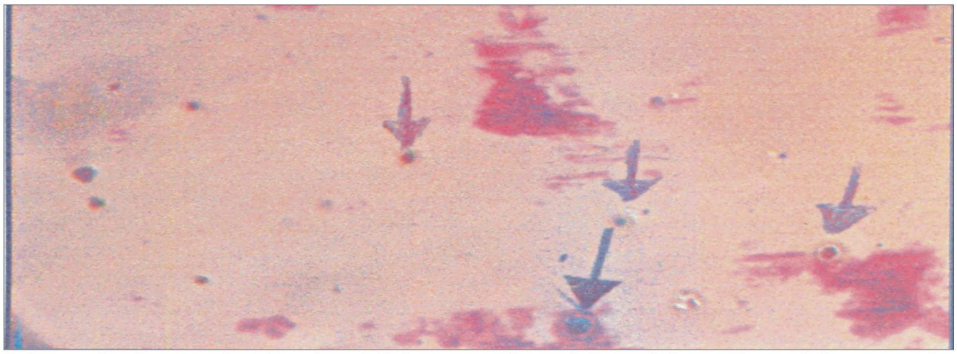


Plate 1: Parasitological Diagnosis of Positive *Ent. bieneusi* from human faeces. Magnification x 1000

Gastrointestinal tract

Intestinal epithelium was the most prevalent site of human microsporidium infection among individuals defined with HIV illness, and two microsporidial agents were isolated from the intestinal genitourinary tract-*Enterocytozoon bieneusi* and *Encephalitozoon cuniculi* (Plate I), detected also hepatitis B surface antigen (HBsAg). The common microsporidial intestinal infection described in this case study was *Ent. bieneusi.* The organism tends to be present

throughout the length of the small intestine, occurring within the cytoplasm of superficial lining enterocytes.

In table 1 of 1,289 HIV+ population sampled, 54(4.2%) were infected in both sexes considering that, *Ent. bieneusi* was most prevalent in Makurdi with 4(2.0%) occurring in the males, and 6(2.7%) in the females respectively. Other prevalent rates were 31(2.4%) for *Enc. cuniculi* in both sexes with the least isolated in Konshinsha 1(3.3%), and 1(2.2%) in the female category with none in Vandekya. There was however, no significant difference in the spread of microsporidium species among cohort population study, given that r=0.79 (calculated) >0.60 (tabulated), df= (n-2), 11-2=9. And HIV seropositive individuals were 16(1.2%) with hepatitis B.

Table 1: Prevelance of Microsporidium and Hepatitis B infections in HIV in Tiv land, Benue State, Nigeria.

Tiv communities	Sex	Total No. of HIV+ examined	No. positive *Ent. bieneusi* (%)	No. positive *Enc. cuniculi* for (%)	No. positive Hepatitis B (%)
makurdi	M	200	4 (2.0)	2 (1.0)	3 (1.5)
	F	220	6 (2.7)	3 (1.4)	1 (0.5)
Gboko	M	50	4 (8.0)	4 (8.0)	2 (4.0)
	F	64	3 (4.7)	1 (1.6)	0 (0.0)
Katsina-ala	M	60	3(5.0)	1 (1.7)	1 (1.7)
	F	40	2 (5.0)	3 (7.5)	1 (2.5)
Konshinsha	M	34	0 (0.0)	0 (0.0)	2 (5.9)
	F	30	1 (3.3)	1 (3.3)	0 (0.0)
Vandekya	M	47	2 (4.3)	0 (0.0)	0 (0.0)
	F	45	1 (2.2)	0 (0.0)	0 (0.0)
Ushongo	M	62	4 (6.5)	0 (0.0)	2 (3.2)
	F	40	2 (5.0)	2 (5.0)	0 (0.0)
Gwer-West	M	60	4 (6.7)	2 (3.3)	0 (0.0)
	F	60	2 (3.3)	2 (3.3)	0 (0.0)
Gwer-East	M	55	3 (5.5)	1 (1.8)	1 (1.8)
	F	53	2 (3.8)	1 (1.9)	0 (0.0)
Buruku	M	20	2 (10.0)	0 (0.0)	0 (0.0)
	F	22	3 (13.6)	1 (4.5)	1 (4.5)
Kwande	M	40	1 (2.5)	2 (5.0)	0 (0.0)
	F	30	2 (6.7)	3 (10.0)	1 (3.3)
Guma	M	20	1 (5.0)	1 (5.0)	1 (5.0)
	F	37	2 (5.4)	1 (2.2)	0 (0.0)
Total		1,289	54 (4.2)	31 (2.4)	16(1.2)

Ent. = *Enterocytozoon. Enc.* = *Encephalitozoon.* No. = Number. %=Percentage.

Clinical association with chronic diarrhea was observed to -persist in the immunocompromised. Other symptoms of weakness, fever, weight loss, anorexia were facts associated with HIV seropositivtivity of the individuals. Diarrhea illness was associated with enteric *Ent. bieneusi* infection which appears to be similar to that associated with *Enc. intestinalis*. Also similar as the ability of *Ent. bieneusi* to infect biliary epithelium. In addition to enteritis, clinical manifestations of ocular involvement due to *Enc. cuniculi* have severally been reported.

Genitourinary tract

In table 2, urinary tract Microsporidiosis appears in patients with *Enc. cuniculi,* although many persons during this finding do not have symptoms referable to the urogenital route. The

microsporidial infection in 11 (1.5%) was generally symptomatic with detects of *Enc. cuniculi*, and 9(1.3%) were HBsAg+ with no significant relationship among HIV positive (r=0.76>0.75, df= (n-2), 7-1=6). Genital tract involvement for *Encephalitozoon* spp. was observed in one patient following a discharged which suggested that, the spores infect the renal tubular epithelium which was carried in the direction of urine flow from the kidneys through the ureters and into the bladder, infecting the urothelial lining epithelium of those structures which eventually resulted in necrotizing the route of urine flow from the kidney.

Table 2: Prevalence of *microsporidium* and Hepatitis B infections in HIV positive individuals in Idoma land, Benue State, Nigeria.

	Idoma communities														
Communities	Otukpo		Oju		Ohimihi		Ogbadibo		Adoka		Okpokwu		Agatu		Total for both sexes
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
Total No. of Individuals Examined.	90	117	47	55	26	30	50	64	40	48	20	37	45	50	719
No. positive For *Ent.bieneusi*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
(%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
No. positive For *Enc.cuniculi*	1	2	0	2	0	1	2	0	0	1	0	2	0	0	11
(%)	1.1	1.7	o.o	3.6	0.0	3.3	4.0	0.0	0.0	2.1	0.0	5.4	0.0	0.0	1.5
No. positive For Hepatitis B.	0	3	0	1	0	0	0	2	0	3	0	0	0	0	09
(%)	0.0	2.6	0.0	1.8	0.0	0.0	0.0	3.1	0.0	6.3	0.0	0.0	0.0	0.0	1.3

Ent=Enterocytozoon bieneusi; Enc=Encephalitozoon cuniculi; %= Percentage.

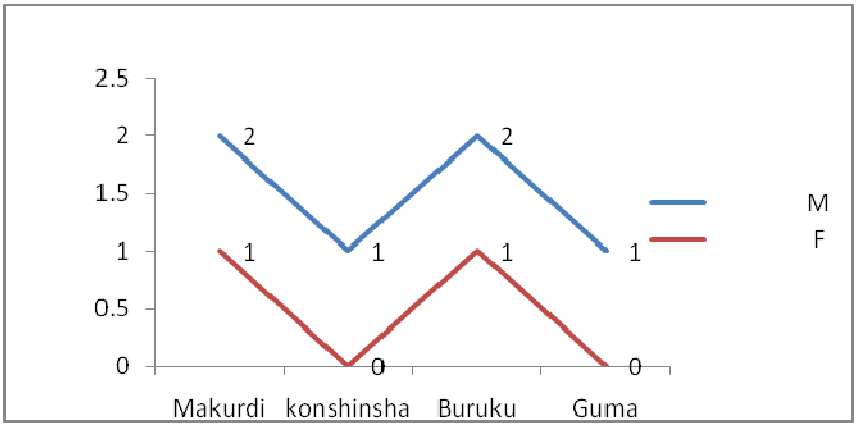
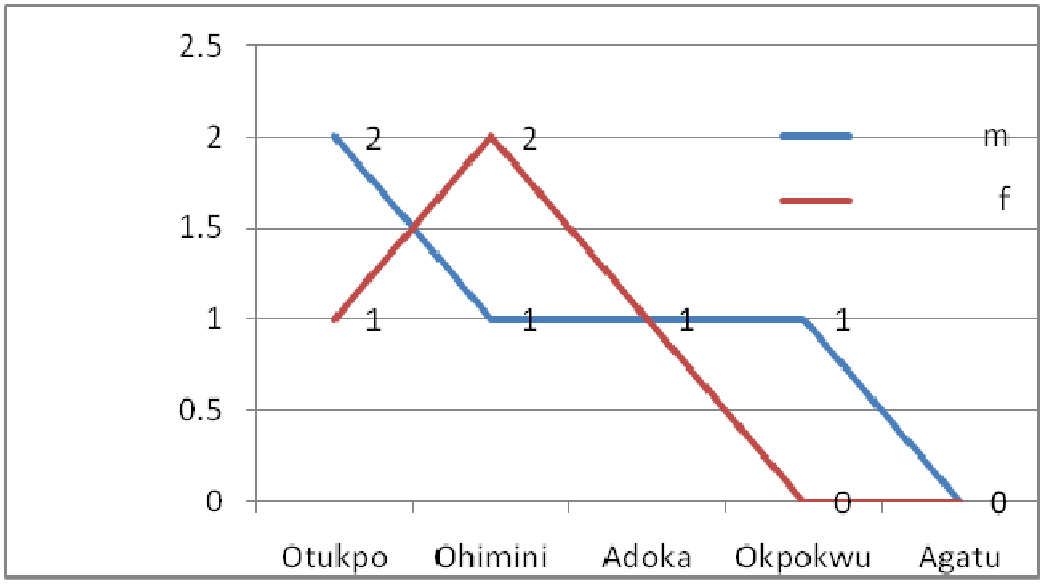


Figure I: An estimated frequency of HIV individuals with double infection in 4 communities of Tivs in Benue State, Nigeria.

It was certain in figure 1, where 4 communities in Tiv land had double infection establishing microsporidiosis and hepatitis B carriage rate. This indicates that chronic diarrhea, anorexia, and weight loss were the most common manifestations of *Ent. bieneusi* in HIV infected population. Laboratory evidence for intestinal malabsorbtion was common in 1(0.5%) of the male in koshinsha community with CD_4 counts <90cell/ µl, 2(1.0%) in Makurdi and Buruku were ≥50cell/ µl respectively. Moreso, 1(0.5%) in Guma had CD4 counts >80 cell/µl among hospital admitted patients with HIV/AIDS developed stage.

Findings in figure II, however represent concomitant biliary infection that typically produces clinical manifestations consistent with hepatitis B. *Ent. bieneusi* and *Enc.cuniculi* is in this pilot study recognized as at least one cause of AIDS-related opportunistic infection. Clinical jaundice was evident in 2(2.2%) in Otukpo community, 1(1.1%) in Ohimini, and Adoka with ≤50cell/µl respectively. In the female category 1(0.8%) in Otukpo showed a laboratory value of alkaline phosphatase elevated two times above the upper limit of (20-90iu/l), others were 2(1.7%) in Ohimini, and 1(0.8%) in Adoka. This shows that, infection in in some quota was bilateral which has greater impact in one patient in Otukpo having exhibited clinical manifestation of renal failure and it was suggested that, histopathology which usually appears to occur in kidneys most have set in to cause the patient renal function of *Enc. cuniculi*. Intestinobiliary co- infections was a fact establishing *Enc. Cuniculi* and hepatitis B which is being recognized with increasing frequency therefore, emphasizing the need to perform further thorough diagnostic evaluations for dual or multiple pathogenic infections in persons with HIV-related chronic diarrhea.



Figuere II: An estimated frequency of HIV individuals with double infection in 5 communities of Idoma in Benue State, Nigeria.

The intestines were recognized to be involved in cases of *Ent. bieneusi* infection. And that, this microsporidian does not produce any specific tissue abnormality though, *Ent. bieneusi* is invasive, infecting not only entorocytes but also the endothelial cells, and this pattern of intestinal infections correspond with the findings which described not only infecting the

enterocytes but also cells in the lamina propria including fibroblasts, macrophages, and endothelial cells [16].

The mode of transmission of microsporidia is unknown, though the oral faecal route is suspected [5]. In this prospective study, it was strongly suggested that, transmission of microsporidia has been linked to unprotected sexual activity considering high promiscuity at the community level. Eating food and drinking or swimming with water contaminated with the spores of microsporidia.

However, microsporidiosis was observed to be associated with heavy watery diarrhea, and because nutrients were not properly been absorbed from the gut, there was notice of weight loss. Other symptoms of cramping stomach pains, nausea, and irregular bowel movement (3-10times/daily) were observed. In some cases we have noticed undigested food or tablets in the faeces in those patients with less than <90 cell/µl, and ≤50 cell/ µl and these reports have included instances of *Ent. bieneusi* infections in adults and children born of HIV+ parents residents in various communities of Benue State, Nigeria.

[1] observed that, the pathologic features of renal infection due to *Enc. cuniculi* in one patient did not differ significantly from the histologic appearance of microspodial nephritis in patients with AIDS, which means that, the propensity for *Enc. cuniculi* to infect renal tubular epithelium most have affected the urothelial lining epithelium which resulted in (one patient in this case study) a renal failure and accompanied jaundice.

Seroprevalance of HBsAg specific serum antibody among HIV infected individuals in Benue state was lacking data to the bilateral endemicity of Microsporidium and hepatitis B carriage rate. 2.2% of HIV infected individuals were generally positive with HBsAg in the males with a closed range of pathogenesis of infection among the females (0.5%).

Contrary from the report [11], a cohort of mate factory workers was observed and 6% men had HBsAg+ with no significant result in the females. [13] Confirms higher carriage rates amongst females at 44.1% against their male counterparts (1.6%). Although, low rate of HBsAg infection was observed in the females with 0.5%[10,8].

A decompensated liver disease (Jaundice) or rather complications directly related to it, gastrointestinal bleeding, hepatorenal syndrome, and peritonitis was diagnosed and 8.6% hospital admission were positive of chronic viral liver disease with clinical jaundice which is in conformity with this present case study were in one patient co- infected suffers from jaundice, and in other, weight loss due to *Enc. cuniculi* which predisposes keratoconjuctivities in HIV+ infected which present dry eyes, ocular pain, and blurred vision which range from mild to severe [14]. Similarly, [12] confirmed also that cases with mild conjunctivitis have been reported.

However, data to support effective preventive measures are quite limited. The presence of infective spores in body fluids and faeces, body substances, precaution in health and other institutional settings, advice strictly to meticulous hand washing, and other personal hygiene measures be adhered and patients are warned that, sexual transmission of microsporidiosis

cannot be excluded and patients should be offered screening for microsporidiosis regardless of their HIV-status.

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