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Original papers

Virulence of geographically different *Cryptosporidium parvum* isolates in experimental animal model

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ABSTRACT. *Cryptosporidium parvum* is a coccidian parasite which causes gastrointestinal disease in humans and a variety of other mammalian species. Several studies have reported different degrees of pathogenicity and virulence among *Cryptosporidium* species and isolates of the same species as well as evidence of variation in host susceptibility to infection. The study aimed to investigate infectivity and virulence of two *Cryptosporidium parvum* "Iowa isolate" (CpI) and a "local water isolate" (CpW).Thirty-three Swiss albino mice have been divided into three groups: Negative control Group (C), the CpI group infected with "Iowa isolate "and the CpW group infected with *C. parvum* oocysts isolated from a local water supply. Infectivity and virulence have been measured by evaluating clinical, parasitological and histological aspects of infection. Significant differences were detected regarding oocysts shedding rate, clinical outcomes, and the histopathological picture of the intestine, lung, and brain. It was concluded that the local water isolate is significantly more virulent than the exported one.

Key words: *Cryptosporidium parvum*, isolates, infectivity, virulence

Introduction

Currently, 26 species of *Cryptosporidium* were recognized; based on morphological criteria, host specificity and DNA-based studies [1,2]. *C. parvum* is an obligate intracellular parasite that infects the epithelial lining of luminal surfaces of gastrointestinal and respiratory tracts in a wide variety of hosts [3]. It has different strains and isolates identified according to genotyping and geographical characters respectively. The geographically diverse isolates differ in their infectivity and virulence [4].

The pathology of the parasite is mainly reflected by watery diarrhea and malabsorption. Diarrhea can have osmotic, inflammatory or secretory components [5]**.** The watery nature of diarrhea has suggested the presence of an enterotoxin; however, there is no evidence for a toxin-mediated secretory diarrhea despite efforts to identify such a toxin [6]**.** The outcome of infection is more severe among young children and immunocompromised individuals due to prolonged diarrhea with major alterations of the intestinal epithelium and malnutrition [7]. Most signs of cryptosporidiosis arise from the organism's ability to invade, encyst and prosper within the microvillus portion of the host enterocyte, resulting in atrophy and fusion of affected intestinal microvilli [8]. Consequently, epithelial cells are damaged by one of two models: The first is cell death; as a direct result of parasite invasion, multiplication, and extrusion. The second is cell damage; which occurs through T cell-mediated inflammation, producing villus atrophy and crypt hyperplasia. Both finally produce distortion of villus architecture [9]. What is noticeable is the association between an augmented severity of disease and certain types of immune suppression [10]. The severity of illness depends on the degree of the immuno-compromised status. The disease is self-limited in immunocompetent hosts, showing a major role for host defense factors in controlling the infection. [11–15].

It was found that *Cryptosporidium* does not only attack small intestine but also other organs. There are a rapidly growing number of case reports of respiratory tract infection by *Cryptosporidium* spp. The symptoms include cough, shortness of breath, wheezing, croup, and hoarseness. Diarrhea has not been reported in all of these patients [10]. *Cryptosporidium* spp. has also been documented as the cause of acute laryngotracheitis in measles infected infants [16], as the acute phase of measles causes transient immunosuppression [10]. Gall bladder disease, primarily acalculous cholecystitis and less frequently, sclerosing cholangitis has been reported in HIV-*Cryptosporidium*-infected patients [10,17,18]. Several cases of symptomatic pancreatitis with concurrent cryptosporidiosis have been reported [18]. *Cryptosporidium* oocysts were found at necropsy in the pancreatic ducts of a child with severe combined immune deficiency disseminated cryptosporidiosis is often found at autopsy, resulting in severe disease [4].

Virulence is the ability of the pathogen to induce host damage as defined by Woolhouse et al. [19]. Virulence and infectivity of the parasite appeared to be variable with different strain and isolate, or even within the same isolate. The majority of the assumed *Cryptosporidium* virulence factors which facilitate sporozoite invasion and attachment to intestinal epithelial cells and the inactivation of *in vitro* and *in vivo* infection are common to all *Cryptosporidium* spp. These virulence factors were identified mostly in *C. parvum* [2]. Putative virulence factors for *Cryptosporidium* are represented by genes, involved in the initial interaction processes of *Cryptosporidium* oocysts and sporozoites with host epithelial cells, representing; excystation, gliding motility, attachment, invasion, parasitophorous vacuole formation, intracellular maintenance, and host cell damage [20,21]. These virulence factors may well prove important as potential drug targets and vaccine candidates [22,23].

Several human and animals isolates have been previously subjected to in vivo and in-vitro testing [24]. Progress in the capability to recognize and track virulent strains, mainly during outbreaks, will afford opportunities for intervention [2]. Therefore, the study aimed to experimentally investigate infectivity and virulence of a locally collected isolate against *C. parvum* Iowa reference strain; thus, permitting to clarify the clinical, histopathological difference in virulence effect of the local strain.

Materials and Methods

Inoculum. *C. parvum* "Iowa isolate" oocysts (CpI): Iowa isolate of bovine source provided by Waterborne ^{TM} (P102M, Waterborne^{TM}, USA), passed one-time through experimentally infected mice. The parasite was suspended in PBS, antibiotics (Penicillin, Streptomycin, Gentamicin and Amphotericin B) and 0.01% Tween 20 and stored at 4°C until used. They were termed as "CpI" isolate.

C. parvum "**Water isolate"** oocysts (CpW): The oocysts were isolated from water samples at "Assiut University Hospitals" drinking water system. The oocysts were purified through discontinuous sucrose gradient flotation according to Suresh and Regh [25]. Part of the isolate was stored at –20°C to identify the *Cryptosporidium* species, oocysts were subjected to nested PCR technique using primers and probes described by Pedraza-Díaz et al. [26] and Spano et al. [27]. The separated oocysts were titled as "CpW" isolate after being molecularly characterized.

Nested-PCR Technique (nPCR). The stored oocysts were processed for Genomic DNA extraction using Favor Prep stool DNA isolation Mini Kit (Favorgen Biotech corporation ping-Tung 908, Taiwan) according to manufacturer`s guidelines after thermal shock of the samples (5 cycles of deep freezing and boiling in water bath each for 5 min.), with continuation of incubation for one hour at 95°C after 56°C at 10 minutes. The extracted DNA was amplified by nPCR targeting COWP gene, using two sets of primers (Table 1). According to Spano et al. [27], the reaction mixture and condition were completed in a total volume of 25µl. The amplified products were visualized using 1.5% agarose gel electrophoresis after being stained with ethidium bromide. PCR products were digested by *Rsa*I (Fermentas UAB, V. Graiciuno 8,LT-02241Vilnius, Lithuania) digestion of N-COWP fragments was determined by electrophoresis in 3.2% typing-grade agarose gels containing ethidium bromide, visualization of fragments were seen by UV light to determine *Cryptosporidium* genotype.

Experimental design. Laboratory-bred, Swiss albino male mice, eight weeks old with an average weight of 30 grams each were maintained under optimal laboratory conditions. Feed and water were provided by oral consumption. They were all proved to be free from any parasitic infection on three succeeding days, by examining their stools using the

Table 1. Primers sets used for nPCR targeting COWP gene

Primers	Sequences	Expected product size (bp)	Annealing temp. C°	References
	1st PCR (E-COWP = Extended COWP)			
BCOWPF	5'-ACCGCTTCTCAACAACCATCTTGTCCTC-3'	769	65	[26]
BCOWPR	5'-CGCACCTGTTCCCACTCAATGTAAACCC-3'			
	2nd PCR (N-COWP = Nested COWP)			
cry-15	5'-GTA GAT AAT GGA AGA GAT TGT G-3'	553	54	[27]
cry-9	5'-GGA CTG AAA TAC AGG CAT TAT CTT G-3'			

formol-ether concentration and modified Ziehl-Neelsen techniques [28,29]. They were divided into three experimental groups:

1. Control negative group: It included six non-infected mice;

2. *C. parvum* Iowa isolate "CpI" group: included six orally infected mice with the prepared inoculums of CpI isolate through tuberculin syringe connected to a polyethylene tube after water overnight deprivation [30]. The amount given per mouse was adjusted to contain approximately 1.5×10^5 oocysts at the 1st infection day, according to standard recommendation of Suresh and Rehg [25];

3. *C. parvum* Water isolate "CpW" group: included 21 mice, the oocysts quantity were adjusted by oral inoculation according to mice responses in order to determine the appropriate dose; as there is no previous work or knowledge about this local isolate infectious dose. At the commencement, the dose was adjusted to contain approximately 2000 oocysts counted by hemocytometer and were given initially to 6 mice. Oocysts count was readjusted several times till reached the non-lethal infectious dose at 600 oocysts in which mice survived until the end of the experiment at the 14th PID (post-infection day).

Assessment of infectivity. Infectivity of CpI and CpW isolates were evaluated in their respective groups at the following levels:

Incidence of infection

Feces from each mouse in the study groups including control were collected separately every day along the 14 days of the experiment, according to the group to which they were assigned. Each sample was divided into 2 parts: 1st preserved in Sodium Acetate Formalin (SAF) solution and homogenized for staining and the 2nd preserved in 2.5% Potassium dichromate solution and homogenized for oocysts counting.

Staining techniques. a) Kinyoun's with methylene blue counterstain "KMb": was used according to Cole [31] instructions. Samples were microscopically studied using ×400 and ×1000 magnification; b) Direct Fluorescent Antibody Staining (DFA): An Aqua-Glo™ kit (A100FLK, Waterborne, USA) was used according to the manufacturer instruction. The kit was provided with a Fluorescein isothiocyanate-conjugated anti-*C. parvum* monoclonal antibody (FITC-C-mAb) which recognizes surface epitopes on oocysts using Fluorescent microscope [32,33].

***Oocyst shedding count* [25]**

Starting from the second day post-infection, the number of oocysts/gm in mice stool discharged by all experimentally infected groups was counted by hemocytometer slide under bright-field microscopy. Number of oocysts were calculated according to the following equation (Total no. of oocysts counted-×dilution factor)/(tested stool volume in gm) until mice were sacrificed on the 14th PID recorded and tabulated.

***Evaluation of clinical outcome* [15]**

a) Body Weight – the body weight of mice of all groups was measured in gram and documented daily throughout the period of the experiment;

b) Clinical parameters – from day 1 PI, each mouse in each experimental groups was observed for the appearance of clinical signs through

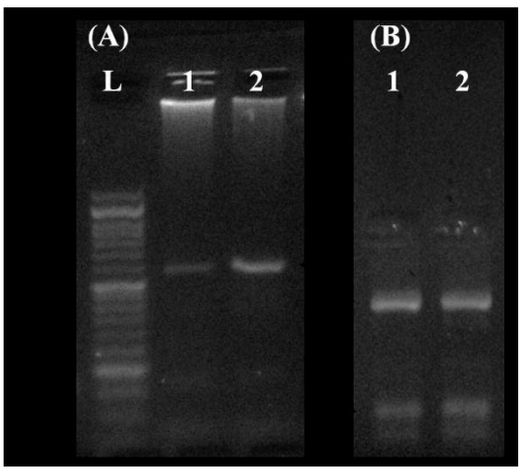

Fig. 1. Agarose gel electrophoresis showing (A) the products of the nPCR targeting COWP gene of *Cryptosporidium* at 553bp. (B) RFLP products after digestion with *Rsa*I endonuclease.
A: Lane L: 50 bp DNA molecular weight marker. Lanes 1–2: positive samples for *Cryptosporidium*; B: Lanes 1–2: *C. parvum* genotype 2 digestion products at 34, 106 and 410 bp (34 band is faint and difficult to see).

macroscopic examination of stool according to Pérez-Cano et al. [34] instructions. Calculation and documentation were done for these parameters; prepatent period, incubation period, patent period and duration of diarrhea.

The severity of diarrhea was evaluated according to clinical macroscopic scores of Pérez Cano et al. [34].

Assessment of virulence

Post-mortem specimens from CpW group before reaching the non-lethal dose

The terminal ileum, lung, and brain were collected from mice died in CpW group while trying to reach the non-lethal infectious dose for histopathological examination.

Post-mortem specimens at the end of the experiment

Animals of all groups (CpI, CpW and C groups) were followed up throughout the 14 days period of experiment and all survivors were euthanized by neck dislocation under anesthesia. Tissue samples were collected from the terminal ileum of mice of all groups. They were labeled, fixed in 10% formalin and processed for histopathological sections. They were stained with hematoxylin & eosin [35] and acid-fast stain with Malachite green counterstain [36].

Statistical analysis. Data were collected, tabulated and statistically analyzed using SPSS program version11. Chi-square test was used to compare qualitative variables while independent T-test was used in comparisons between quantitative variables. Data was expressed as mean±standard deviation (SD), *P* value equal to or less than 0.05 was considered significant and $P \leq 0.001$ was considered highly significant [35].

Ethics. The experimental animal studies were directed in agreement with the international valid rules and were upheld under appropriate conditions at the Animal House of Assiut Faculty of Medicine, Assiut University, Egypt.

Results

Adjustment of CpW minimum non-lethal infective dose (pre-experimental phase)

When the 2000 oocysts were given initially to 6 mice, they died within 24 hrs PI. The dose was readjusted to 1500 oocysts given to 5 mice, resulting in their death within 24 to 48 hr. Another 1000 oocysts dose was given to 4 mice, leading to their death within 72 hr. The non-lethal infective dose was reached at 600 oocysts and was given to 6 mice.

Nested-PCR Technique (nPCR) (Fig. 1)

The isolate CpW has been proved to be *Cryptosporidium parvum* by the appearance of diagnostic bands at 34, 106 and 410 bp.

Table 2. Incidence of infection throughout the experiment

Groups	No. samples	-ve samples	+ve samples	Percentage of infection %
Control	46	46	-	-
CpI	49	15	34	69.38 % [ab]
CpW	47	12	35	74.46 % [ab]

[b] $P \leq 0.001$ comparison between the incidences of infection from the originally infected mice

[a] $P \leq 0.05$ comparison between the incidence of infection in CpI and CpW group

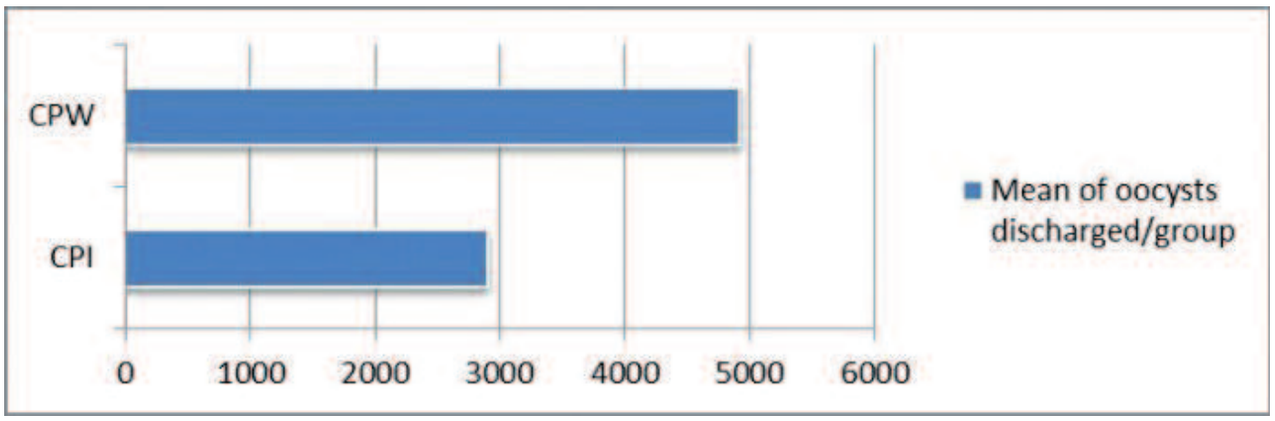

Fig. 2. Means of oocysts/gm discharged in stool by the infected groups

Assessment of infectivity (experimental phase)

Incidence of infection

CpW isolate has a significant ability to infect mice than the Iowa isolate (Table 2).

Oocyst shedding count

Maximum shedding of *C. parvum* oocysts was observed on the 14th PID in both groups, with a mean of 2900.00 and 4920.00 oocysts/gm of stools in CpI and CpW groups respectively. CpW group witnessed a significantly higher oocysts discharge (P=0.011) (Fig. 2.)

Table 3. Mean of BW in gram for all studied groups

Infection	Mean of BW in gram ± SD among studied group		
	C^{b}	CpI	CpW
1st	30±0.21	28 ± 0.22	30 ± 0.25
2nd	30 ±0.22	28 ± 0.23	30 ± 0.23
3rd	30.21 ± 0.22	28.1 ± 0.22	30 ± 0.25
4th	30.1 ± 0.21	28.2 ± 0.23	30.1 ± 0.25
5th	30.1 ± 0.22	28.2 ± 0.22	30.1 ± 0.25
6th	30.12 ± 0.21	28.1 ± 0.23	30.1 ± 0.24
7th	30.12 ± 0.23	28.1 ± 0.21	29.9 ± 0.24
8th	30.2 ± 0.23	28 ± 0.21	29.8 ± 0.25
9th	30.3 ± 0.22	28 ± 0.22	29.7 ± 0.25^{a}
10th	30.4 ± 0.22	27.8 ± 0.23	29.7 ± 0.23^{a}
11th	30.5 ± 0.23	27.7 ±0.24^{a}	29.7 ± 0.23^{a}
12th	30.5 ± 0.24	27.7 ± 0.23^{a}	29.6 ± 0.23^{a}
13th	30.5 ± 0.24	27.6 ± 0.22^{a}	29.5 ± 0.24^{a}
14th	30.6 ± 0.24	27.5 ± 0.21^{a}	29.3 ± 0.24^{a}

.^{a} $P \leq 0.05$; C^{b}: Control group

Evaluation of clinical outcome

a) Body weight (B.W): at the beginning of the study, there was no significant difference in body weights of mice in both groups. Statistically, a significant reduction in B.W of CpI and CpW mice were observed from their original weight, this reduction was nearly equal between the isolates by the end of the study (Table 3).

b) Clinical parameters (Table 4). The prepatent and patent period were nearly equal in studied groups with an average of 2 and 12 days respectively without any significant difference between the CpI and CpW groups;

Incubation period: Significant longer incubation period of 6 days was detected in CpI group, compared to CpW group which had a shorter incubation period of 3 days ($P \leq 0.001$);

High asymptomatic oocysts shedder was recorded in CpI group (33.3%), while none was recorded in CpW group (0.02%) ($P \leq 0.001$);

Duration of diarrhea: Significantly longer diarrhea duration of 6 days was recorded in CpI group;

The severity of diarrhea was significantly higher in CpW group (6 time/day) than in CpI group as the frequency of diarrhea was 2 time/day ($P \leq 0.001$).

Assessment of virulence

Post-mortem specimens from CpW group before reaching the non-lethal dose

a) Lung – grossly the lung appeared as an inflated oozing organ. While, by light microscopic examination showed emphysematous lung with distorted dilated airspaces and hyper distension of alveolar ducts associated with rupture of alveolar septa, acute inflammatory cells around alveolar blood vessels and inter-alveolar congestion (Fig. 3).

b) Brain – grossly the brain was congested and

Table 4. Means and standard deviations of determinant parameters among studied groups

Groups	Pre-patent period in days		Asymptomatic oocyst shedder %		Incubation period in days		Patent period in days		Duration of diarrhea		Severity diarrhea	
	Mean	SD	M	SD	M	SD	M	SD	M	SD	M	SD
CpI	2.000	±1.000	33.333	±.13663	6.000	±1.0000	12.000	±1.0000	6.000	±.31623	2.000	±.31623
CpW	2.000	±0.7071	0.0233[b]	±0.0571	3.000[b]	±0.31623	12.000	±0.70711	5.166 [a]	±0.75277	6.083 [b]	±0.66458

[a] $P \leq 0.05$; [b] $P \leq 0.001$

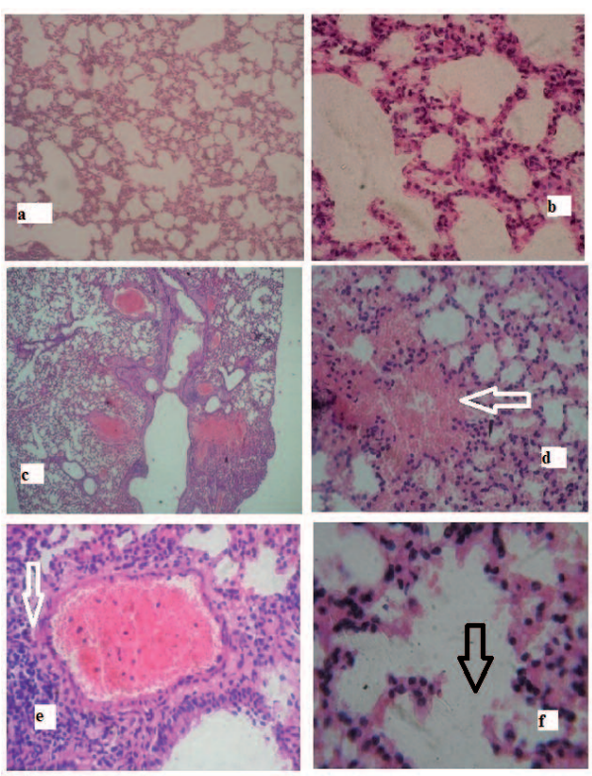

Fig. 3. Post-mortem section in lung alveolar tissue of mice stained with H&E at different magnification. a,b: normal alveolar tissue at ×40 and ×100 magnification, respectively from control mice group; c: lung section from CpW group using 2000, 1500, 1000 oocysts before reaching the non-lethal dose, showing distorted, dilated airspaces with hyper-distension of alveolar spaces associated with the rupture of alveolar septa at ×40; d: RBCs invading the lung inter-alveolar space (arrow), thickening of alveolar walls at ×100; e: inter-alveolar congestion and acute inflammatory cells infiltration (arrow) at ×400; f: ruptured alveolar septa (arrow) at ×1000.

oedematous with petechial hemorrhage on their surface. With light microscopic, Brain parenchyma showed hypercellularity, acute inflammatory cell infiltration. The small blood vessel was congested and brain parenchyma was necrotic indicating the

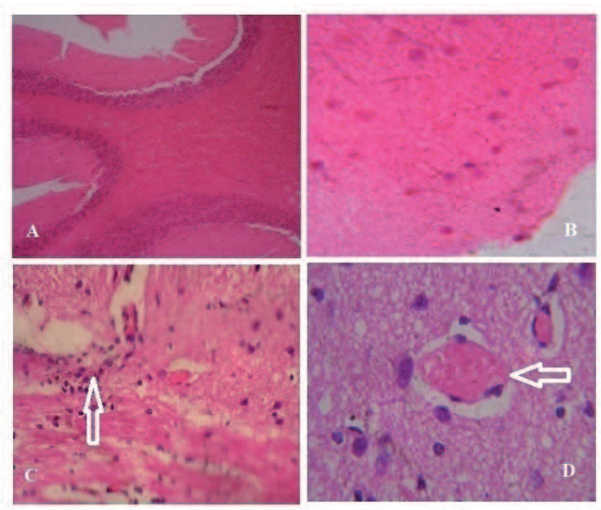

Fig. 4. Post-mortem section in brain parenchyma of tested mice stained with H&E at different magnification. a,b: normal brain parenchyma in control group from the cerebellum at ×100 and ×400, respectively; c,d: brain section from CpW group before reaching the non-lethal dose; c: brain parenchyma showing hyper cellularity and acute inflammatory cell infiltrations at ×400; d: necrotic brain parenchyma with congested vascular channels at ×1000.

presence of degenerative changes and vasculitis (Fig. 4).

c) Small intestine – grossly the small intestines looked congested. Light microscopic examination showed no obvious pathological changes could be detected except for *Cryptosporidium* stages on the brush borders.

Post-mortem picture of all groups at the end of experiment (14th PID)

a) Small intestine – grossly**,** small intestines obtained from CpI and CpW groups after the 14th PID appeared congested compared to control group. Using Haematoxylin & Eosin staining, small intestine sections of the control group showed

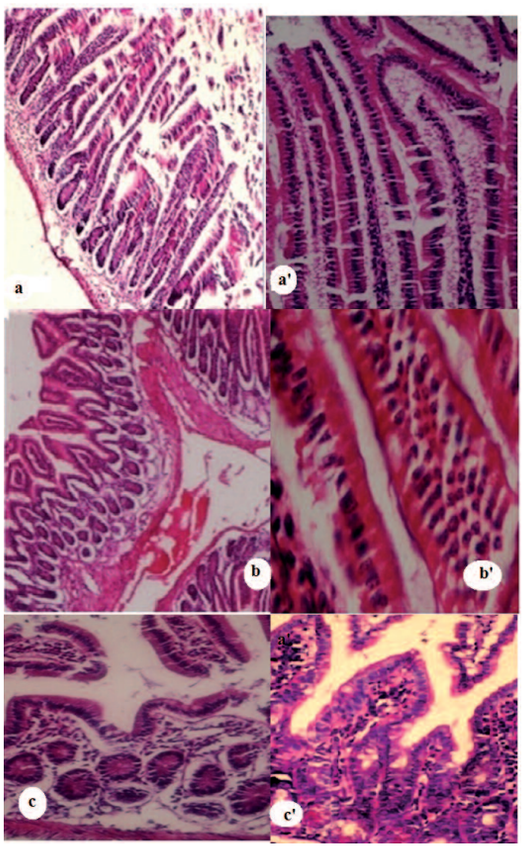

Fig. 5. Sections in small intestines of tested mice stained with (H & E) at magnification ×100 and ×400. a,a': normal villi finger with normal crypt appearance and no cellular infiltrations (control group); b,b': mild blunting and shortening of villi with moderate mononuclear cells infiltrations in lamina propria in CpI group; c,c': remarkable blunting and shortening of villi associated with severe infiltration of mononuclear and neutrophil cells in lamina propria in CpW group.

normal epithelium; villi finger-like projections without blunting, normal crypt appearance, the lamina propria was showing low mononuclear cell infiltration (1 lymphocyte per 5 enterocytes) and very few or absent neutrophil infiltration (Fig. 5a). While the intestinal epithelium of CpI group showed loss of single epithelial cells, mild blunting and shortening of villi, inflammatory cell infiltration of crypts, mild to moderate mononuclear cells in lamina propria and few neutrophils infiltrate (Fig. 5b). Sections of intestinal epithelium of CpW group showed loss of single epithelial cells, remarkable blunting and shortening of villi, inflammatory cell infiltration of crypts, moderate to severe mononuclear cells in lamina propria and neutrophils infiltrate (Fig. 5c).

Small intestine sections stained with modified acid-fast with Malachite green and methylene blue had revealed *Cryptosporidium* parasite within the brush border in CpI and CpW groups (Fig. 6b).

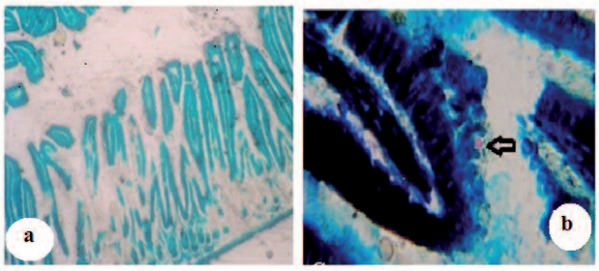

Fig. 6. Sections in small intestines of tested mice stained with modified acid fast at different magnification. In control group (a) no parasites stages could be detected (×100); b: *Cryptosporidium* stages (arrows) detected on the brush borders in both CpI and CpW groups (×400).

Discussion

Cryptosporidium oocysts isolated from local water samples (CpW) were identified by molecular characterization as *C. parvum* but not yet subtyped. We aimed to study infectivity and virulence of the local CpW isolate in comparison to the imported previously studied *C. parvum* Iowa (CpI) isolates on animal models on the clinical, parasitological and histopathological level.

As the infectious dose of *C. parvum* varies according to different isolate [24,37], the infectious dose of CpI (150.000 oocysts/mouse) was given as maintenance dose to CpI group according to standard recommendation of Suresh and Rehg [25]. On the other hand, CpW was not previously recognized as *C. parvum* isolate or subjected to genotyping so, its infective dose not yet known. Other workers as Okhuysen et al. [37] and Teunis et al. [38] had maintained the infection of different strains in patients with doses ranging between 10 oocysts for TAMU isolate reaching to >10,000 oocysts / patients in case of Iowa isolate. According to the availability of the CpW oocysts collected from local water, we had started with 2000 oocysts, followed by repeated trials till we reached the non-lethal maintenance dose which was 600 oocysts/mouse. At a dose of 2.000, 1.500, and 1.000 oocysts/mouse, mice died after 24, 24-48 and 72 hours post-infection respectively, which reflects the one of the aspects of virulence of the *C. parvum* local strain.

The duration of the experimental study was 14 days, which is considered by many authors ideal for

evaluating the course of infection in all groups subjected to the experiment. According to HSE [39], the infectious cycle of *Cryptosporidium* is up to 3 weeks. Abdou et al. [30] found that the duration of oocysts shedding was about 2–3weeks. During this period, both isolates infective dose achieved 100% infection in all experienced mice. Infection was insured by the detection of oocysts in the mice feces. Oocysts shedding characterize the parasite ability to multiply inside the animal hosts. The natural shedding period of *Cryptosporidium* infection in mice begins 36 hrs. PI and could be continued for another 21-24 hrs. [30,40]. The present oocysts shedding started at the 3rd PID, which correlates with what Medema et al. [4] reported. Maximum oocysts' shedding was observed in all infected animals in both CpI and CpW groups at the end of the 14 days, in agreement with Certad et al. [24]; Miller et al. [41] and Abdou et al. [30] in which the maximum shedding of oocysts was on the 13th–15th PID. This shedding was statistically significantly higher in CpW infected mice than CpI group in spite the great discrepancy in the initial given infective dose; 600 oocysts to CpW group in comparison to 150,000 oocysts to CpI group. Data reflects the progressive multiplication nature of the CpW inside the animal hosts compared to that of CpI.

Presently, although the pre-patent, patent periods and body weight reduction of CpI and CpW group were nearly equal, the clinical outcomes of CpW group were statistically sharper than CpI ones, in the form of shorter incubation period, higher diarrhea frequency. Okhuysen and Chappell [42] had reported different duration of prepatent and patent periods in different *C. parvum* isolates, even within the same isolate, in correlation to the infectious dose in contrast to the current study results. The incubation period was longer in CpI group reaching 6 days compared to 3 days in CpW group. Short incubation period was also observed in the work done by Tarazona et al. [43] due to immunosuppression of its experimental animals and in Okhuysen et al. [37] due virulence of TAMU isolate, while in the Surl and Kim [44], Garza et al. [45] studies, they have increased the infective dose in immunocompetent models. So, we can assume that the local isolate has a greater ability to induce host changes in a lesser time than the imported one, taking into consideration the lower infective oocysts dose.

It is true that both current groups recorded short pre-patent period as "CpI and CpW" oocysts appeared as soon as the 2nd PID that should not be considered as equality as the CpI given infective dose was 25 fold higher than CpW dose. It could be assumed that the higher number of oocysts infective dose had been overcome by the presumed virulence of the local isolate. Similar shortness was observed in the experimental work of Surl and Kim [44] and Garza et al. [45] where it was attributed to the high infective dose uptake. In contrast to Del Coco et al. [46], who reported a longer prepatent period of 6 days in their experimental model without clarifying the possible cause in spite the animals were immunosuppressed. Oocysts sustained shedding along the entire experiment duration without cessation. The patent period duration of oocysts shedding was equal in both isolates infected groups. The asymptomatic oocysts shedding was not detected in CpW infected mice in spite of low infective dose intake but was manifested in CpI mice, reflecting the softer effect of Iowa isolate on its host in contrast to the aggressive nature of the local isolate, as the more virulent is the strain, the greatly it manifests [4]. The less virulent nature of Iowa isolates could lead to a greater public health importance, due to the possibility of asymptomatic oocysts shedder to spread the infection.

The predominant sign appeared among the infected CpW mice was the highly statistically significant frequency of passage of unformed stool, which could be certified by the severe epithelial dysplasia which had been perceived during the course of infection. *Cryptosporidium* induces tissue/cell damage to the intestinal epithelium, as well as an increase of enterocytes permeability [47]. Abdou et al. [30] reported that *C. parvum* is one of the infectious agents that may induce intestinal dysplasia, even of high-grade category, and is highly affected by elevated endogenous parasite loads. This fact explains the positive correlation in the current study between the high number of oocysts shedding and severe epithelial changes in the small intestine of water isolate infected mice.

Although the duration of diarrhea was longer in CpI infected animals compared to CpW, but the number of unformed stool passage/day was higher in CpW mice, the result could postulate the positive correlation between the intensity of diarrhea and intensity of oocysts shedding. This is consistent with Current and Garcia [10] who said that the

severity of the cryptosporidiosis clinical outcomes shines and vanish in the infected host in correspondence to the intensity of oocysts shedding. BW reduction in our study occurred in both group but was significantly marked from their original weight by the end of the 14thDPI in CpW infected mice. Approving with Del Coco et al. [46] findings which accredited the BW reduction of their mice to the intense parasite burden and the associated pathology in the intestinal epithelium, which holds the nutrient absorptive function. The variable pathogenicity detected between the two geographically different *Cryptosporidium parvum* isolates (CpI and CpW) could be attributed to different patterns of colonization along the gastrointestinal tract reported in different species and strains of the parasite [24,37,48]. Even though Iowa isolate, has shown different patterns of colonization along the gut in experimentally infected animal models [49]. *C. parvum* usually establishes itself in a membrane-bound compartment on the apical surface of the intestinal epithelium, leading to significant abnormalities in the epithelial absorptive surface as well as lowering local mucosal immunity through the effects of inflammatory cells and cytokines recruited at the site of infection [42].

According to British medical dictionary, infectivity is the ability of an agent to infect, enter, survive and multiply in a susceptible host and cause disease [50], while virulence indicates the degree of pathogenicity, where pathogenicity is used solely to describe the ability of a parasite to inflict damage to the host [2]. Virulent nature of CpW isolate had been manifested by the fast and 100% animal death in the course of the pre-experimental phase. Severe histopathological picture justified these rapid deaths. In spite short duration elapsed after water isolate, different infective doses were inoculated, postmortem picture revealed degenerative changes, vasculitis in brain parenchyma and emphysematous deviations in lungs. Cerebral condition alone, or together with the pulmonary pathology may explain the rapid acute death of tested mice. The pathogenesis of pulmonary cryptosporidiosis has not yet been fully clarified. Vohra et al. [51] imposed the possibility of the pulmonary involvement due to inhalation of oocysts during an episode of vomiting or results from hematogenous dissemination. Albuquerque et al. [52] suggested the ability of extraintestinal spread of *C. parvum* via circulating phagocytes in immunocompromised people, a hypothesis supported by the presence of the parasite within the blood vessels in the intestinal and pulmonary sub-mucosa, as revealed by autopsy studies. We also had found oocysts on the brush border of the small intestine as early as 36 hrs. post infection likewise seen by Medema et al. [4] and the cerebral pathology seen in CpW mice support the idea of hematogenous dissemination of *Cryptosporidium* spp.

Okhuysen et al. [37] and Teunis et al. [38] have reached that TAMU isolate had higher virulence in comparison to other *C. parvum* isolates (Iowa and UCP). TAMU isolate is characterized by perpetrating pathological changes with the least infective dose. TAMU virulence was characterized by shortened incubation period, longer duration of diarrhea. Most of these criteria meet the local isolate (CpW) effects. They had attributed TAMU virulence for 2 reasons; first, its different host sources from which isolate was obtained; second is the less passage of TAMU isolate in animals host for oocysts propagation since repeated passage of the parasite may result in some attenuation of the parasite virulence.

Similarly infectivity and virulence differences detected among the two tested isolates (Cpi and CpW) may be attributed to the diverse genetic structures (CpW not yet reached), age of oocysts; vehicle of previous transmission (stool and water), exposure to environmental conditions and number of animal passage which is unknown for our local isolate [4,37].

It is concluded from the previous observations that the local water *C. parvum* (CpW) isolate is more virulent than the imported *C. parvum* Iowa (CpI) isolate. The given virulence could affect negatively persons at risk, drinking from such water from which the isolate originates; therefore, there is tremendous need to be further identified and studied.

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