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Microscopical and immunological diagnosis of some Enteric protozoal parasites at Menoufiya governorate, Egypt

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Abstract

Enteric protozoal parasites are important enteropathogens of wild, domestic animals, and humans; and they are responsible for important zoonotic diseases. Currently, the diagnosis of parasitic infections rely on several laboratory methods, as microscopy that plays a prominent role in the identification of these parasites, also a new immunological methods are continually evolving, most of these tests depending on the detection of an antigen in fecal samples or antibody in serum samples, in the present study, fecal samples were collected from calves, lambs, dogs, and cats, aged from 1 day to 30 in the Menoufiya province. All samples were examined directly by floatation centrifugation technique by using zinc sulphate solution(sp. g:1.18), for *Giardia spp, and Entamoeba histolytica, and* Modified Sheather's Solution (SG 1.27) for *Cryptosporidium spp. oocysts* then stain with the Modified Ziehl-Neelsen method, and were preserved with PBS at -20˚C for detection of copro antigen using antigen capture sandwich ELISA, and immunochromatographic assay, and the aim from this study was to evaluate the sensitivity and specificity of immunodiagnosis in relation to microscopy. Results revealed that, by microscopic exam, a three main protozoa were found in all examined animals with different percentage *(Cryptosporidium spp. oocysts, Giardia spp, and Entamoeba histolytica).* Direct Eliza test was done on *Cryptosporidium spp* suspected fecal samples, and immunochromatographic assay was done on *Giardia spp, and Entamoeba histolytica* positive samples by microscopy, then the sensitivity and specificity and statistical analysis were calculated.

Key words: *Cryptosporidium spp, lamb, calves, immunodiagnosis, Giardia spp.*

1. INTRODUCTION

Enteric protozoal parasites are important enteropathogens of the intestinal tracts of a broad range of wild, domestic animals, and humans and Companion animals; some of these parasites are responsible for important zoonotic diseases such as Entamoeba histolytica, Giardia spp and Cryptosporidium spp **(Martinez-Moreno *et al*., 2007).**

Currently, the diagnosis of parasitic infections relies on several laboratory methods. Microscopy plays a prominent role in the identification of these enteric protozoal parasites, most of the current tests cannot distinguish between past, latent, acute, and reactivated infections and are not useful for following response to therapy or for prognosis , also with the advent of PCR and its trailing technologies, new methods are continually evolving (**Pappas, 1988)**

Other techniques such as ELISA, and rapid immunochromatographic assay, have also emerged as valuable detection tools. Thus, an evaluation of detection methods should include both the microscopic methods

currently in use, as well as immunological methods in development **(Momar Ndao, 2009).**

2. MATERIALS AND METHODS

2.1. Study area

This study was carried out from different localities in some rural areas Menoufia Governorates throughout a period of one year. stool samples were collected from preweaned animals, (70) from calves, (50) from lambs, (35) from dogs, and (35) from cats, then samples were identified.

2.2. Microscopic exam

In the laboratory, all samples were examined directly by floatation centrifugation technique by using zinc sulphate solution(sp. g:1.18), for *Giardia spp, and Entamoeba histolytica, and* Modified Sheather's Solution (SG 1.27) for *Cryptosporidium spp. oocysts* **(Sheather, 1923)** then stain with the Modified Ziehl-Neelsen method, then samples were visualized under oil immersion lens and photographed, and 5 gm of each fecal sample was preserved with PBS and stored at -20˚C for Further immunological techniques, (**Henrikson and Pohlenz, 1981)**.

2.3. Immunodiagnosis

detection of copro antigen to preserved samples using direct antigen capture sandwich ELISA was done on positive samples for *Cryptosporidium spp*. (27) samples from calves, (25) from lambs, (10) from dogs, and (14) from cats**,** but the rapid immunoassay kit was done on *Giardia spp*, and *Entamoeba histolytica*, then the sensitivity and specificity of these tests was done and microscopical exam was the reference test after **(Joseph *et al*., 1995)**.

2.4. Statistical analysis

Computed using Chisquare (SPSS program).

3. RESULTS

The results for this study revealed three species of enteric pathogenic protozoa, *Cryptosporidium parvum oocysts, Giardia duedenalis (cyst – trophozoite), and Entamoeba histolytica (cyst- trophozoites),* (Fig, 1-2-3) by microscopy, the results for, *C. parvum* sporulated oocyst, in this study was shown in table(1), fig(1), indicating that, it have been detected in 22 (73.3 %) out of 30 calves, and in 6 (24 %) out of 25 examined lambs. Among examined dog, oocysts have been detected in 6 (30%) out of 20 samples, in cats, it was 3(15%) out of 20 examined cats. Concerning the direct ELISA test, for the qualitative determination of *Cryptosporidium parvum* in faecal samples, it was performed on 86 suspected samples from different examined animals, as the results shown in (Table 2, Histogram 2), Among infected calves, positive samples have been detected in 20 (74 %) out of 27, by examining infected lambs, the percent of infection was in 5 (25 %) out of 20 samples, in samples from kids, 12 (48 %) out of 25 samples. Among dog, *C. parvum*. antigen have been detected in 3(30%) out of 10 dogs, in cats the infection rate was 3 (21.4%) out of 14 examined cats. And by comparison the results obtained by staining and microscopy, we found that the ELISA test has a high specificity (79.1 %) and sensitivity(81.1%) (Table: 4 , Histogram: 4). For, *G. duedenalis* cyst, in this study by microscopy was shown in table(2), fig(2),), indicating that; it have been detected in 8 (40%) out of 20 calves, and in 6 (40 %) out of 15 examined lambs. Among examined dog, cysts have been detected in 5 (50%) out of 10 samples, in cats, it was 4 (40%) out of 10 examined cats. Concerning ICT test, Among the infected calves, positive samples have been detected in 5 (33.3 %) out of 15, by examining infected lambs, the percent of infection was in 4 (40 %) out of 10 examined samples. Among examined dog, fecal antigen have been detected in 4(30.7%) out of 13 examined dogs, in cats the infection rate was 4 (66.6%) out of 6 examined cats. And by comparison the results obtained by staining and microscopy, we found that the ICT test for *Giardia duedenalis* has a specificity (73.8 %) and sensitivity (76.8 %). Table (4), histogram(4). For, *E.histolytica* cyst, in this study by microscopy was shown in table(3), indicating that; it have been detected in 6 (20%) out of 20 calves, and in 4 (40 %) out of 10 examined lambs. Among examined dog, cysts have been detected in 3 (60%) out of 5 samples, in cats, it was 2 (40%) out of 5 examined cats. Concerning ICT test, Among

the infected calves, positive samples have been detected in 3 (30 %) out of 10, by examining infected lambs, the percent of infection was in 4 (40 %) out of 10 examined samples. Among examined dog, and cats it was negative, by comparison between the results obtained by staining and microscopy, we found that the ICT test for *E.histolytica* has a specificity (73.8 %) and sensitivity (76.8 %). Table(4), histogram(4). By calculating the specificity and sensitivity of the different immunological method that used in this work, we observed that; for ELISA test of *Cryptosporium parvum*, the specificity was (79.1 %), and sensitivity was(81.1%). For Immunochromatographic test (ICT test) for diagnosis of *Giardia duedenalis* the specificity was (73.8 %) and the sensitivity was (67.8%), also in case of ICT test for *Entamoeba histolytica* specificity was (52.7 %) and the sensitivity was (42.8%), as seen from the above results, we can observed that, the highest specificity and sensitivity for immunological diagnostic method from above mentioned is the ELISA test was higher than (ICT test) in specificity and sensitivity.

Table (1): Prevalence of infection among examined animals by microscopy and ELIZA

C. parvum	**Calves**			**Lambs**			**Dogs**			**Cats**		
	Ex.	+ ve	%	Ex	+ ve	%	Ex.	+ ve	%	Ex.	+ ve	%
Microscopy	30	22	73.3	25	6	24	20	6	30	20	6	30
ELIZA	27	20	74	20	5	25	10	3	30	14	3	21.4

Table 2: Rate of infection for G. duedenalis by microscopy and ICT test.

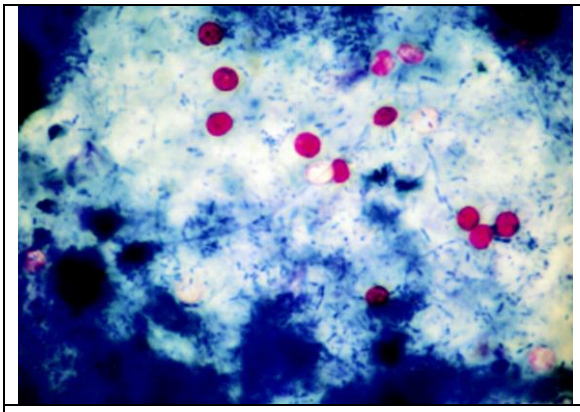
G.duedenalis	**Calves**			**Lambs**			**Dogs**			**Cats**		
	Ex.	+ ve	%	Ex	+ ve	%	Ex.	+ ve	%	Ex.	+ ve	%
Microscopy	20	8	40	15	6	40	10	5	50	10	4	40
ICT	15	5	33.3	10	4	40	13	4	30.7	6	4	66.6

Table 3: Rate of infection for E. histolytica by microscopy and ICT test

E.histolytica	**Calves**			**Lambs**			**Dogs**			**Cats**		
	Ex.	+ ve	%	Ex	+ ve	%	Ex.	+ ve	%	Ex.	+ ve	%
Microscopy	20	6	30	10	4	40	5	3	60	5	2	40
ICT	10	3	30	5	2	40	3	0	0	2	0	0

Table (4): Comparison between Sensitivity and specify of different immunological methods

Test	**+ve**	**-ve**	**Total**	**Microscopy**		**Sensitivity (%)**	**Specificity (%)**	**Total**
				+ve	-ve			
ELISA *C. parvum*	43	53	96	53	67	81.1	79.1	120
(ICT) *G. duedenalis*	19	31	50	28	42	67.8	73.8	70
(ICT) *E. histolytica*	6	19	25	14	36	42.8	52.7	50



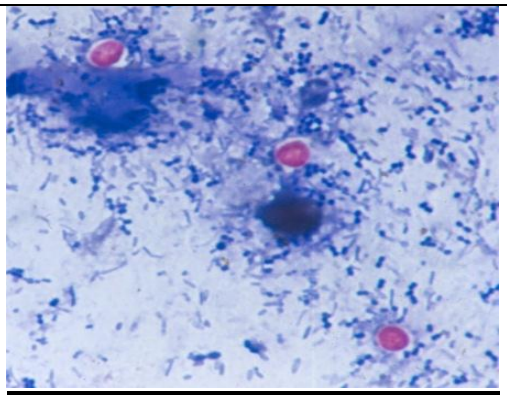


Fig:1: *Cryptosporidium parvum* oocyst(4-6µl) stained with modified ziehl Nelseen stain after floatation centrifugation technique by sheather sugar solution(specific ravity:1.27)

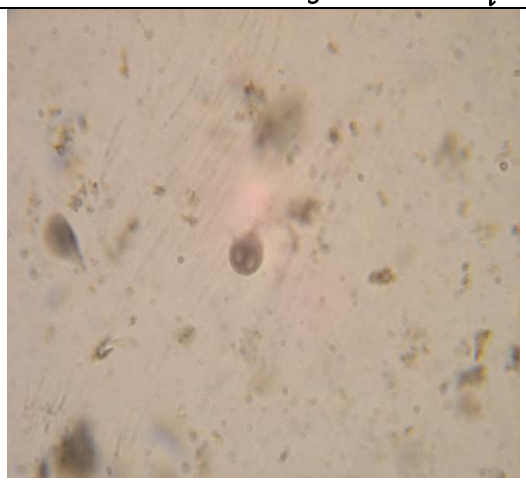


Fig:2a: Giardia duodenalis trophozoites (1000x OIL).

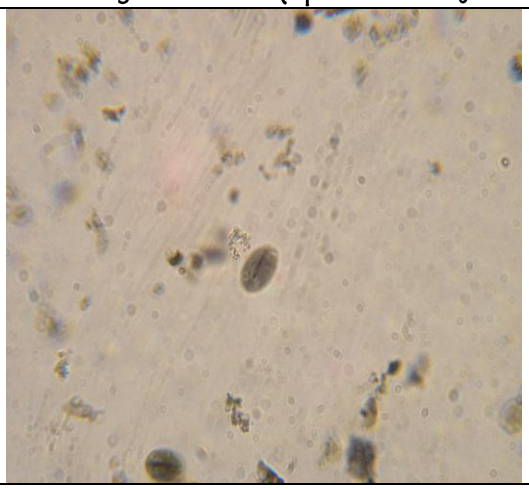


Fig:2b: Giardia duodenalis cyst (1000x OIL).

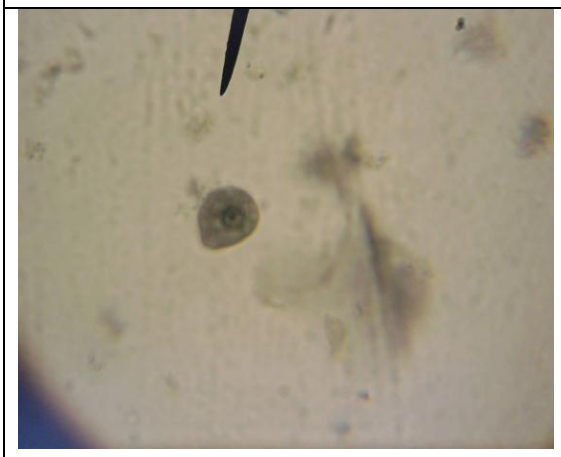


Fig:3a: Entamoeba histolytica trophozoite (1000x)

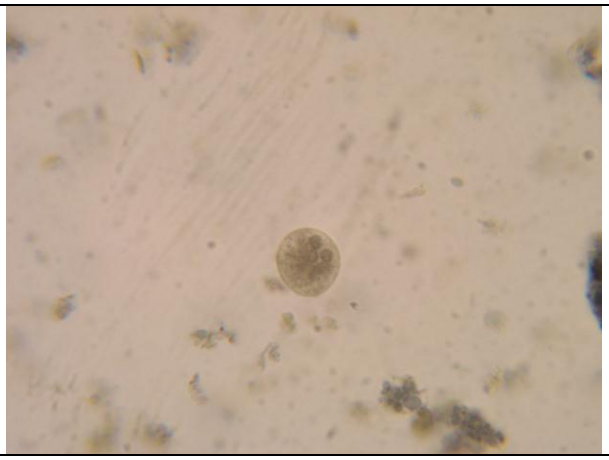


Fig:3b: Entamoeba histolytica cyst.(1000x)

4. DISCUSSION

Enteric diseases caused by animal protozoal agents are common in many places especially the rural areas of Egypt, and the role of animals harboring *C. parvum, Giardia lambalia and Entamoeba histolytica* in transmission of infection to human in different localities of Menoufia Governorates was studied. **(Hunt *et al.* (2000)** and **Isaacs *et al.* (1985)** stated that cryptosporidiosis is a worldwide emerging zoonotic disease affecting the gastrointestinal tract of. Persons at greatest risk are immunocompromised adults and children, especially those with AIDS, children in day care, travelers to endemic regions, dairy or cattle farm workers

or contacts, household contacts of cases or carriers and possibly owners of dogs or cats (Hall, et al., 1992) . These findings results are supported by other workers **(Keusch *et al.*, 1992),** and **(Das *et al.*, 1993)** who observed that the highest detection rate of *C. parvum* was in the first two years of life in both diarrheic and control children. The high cost of reagents and instruments together with the need to experience which is not available in many clinical laboratories render MZN staining technique was a reliable method for screening and detection of the cryptosporidial oocysts in stool and fecal samples from human and **animals (Uga *et al.*, 2000; Stantic-Pavlinic *et al.*, 2003)**. MZN staining technique has been widely used as a reliable method for detection of *Cryptosporidium spp*. oocysts in fecal samples since it allows observation of the protozoan oocysts at lower magnification power and solves the problem of differential diagnosis related to the presence of yeasts. Nearly similar results were recorded by other workers **(Majewska *et al.*, 2000; Uga *et al.*, 2000).** In the present study, it was found that, the highest percent of infection with *Cryptosporidium parvum* was in calves (**73.3%)**, and the lowest in lambs (24%). It was reported that clinical infections with *C. parvu*m in cattle are largely confined to new born calves aging (7-21 days old) **(McCluskey *et al.*, 1995)**. In addition, other workers **(Villacorta *et al.*, 1991; Garber *et al.*, 1994; Scott *et al.*, 1994)** indicated that excretion of oocysts has been found in apparently healthy cows and lambs. These results are supported by those of other workers **(Shehata, 1997)**. *Giardia spp.* cysts are highly infectious for humans and animals, infections can be established by ingestion of as few as 10 viable cysts. In the present study, *Giardia spp.* cysts have been detected in 8 (40%) out of 20 calves, and in 6 (40 %) out of 15 examined lambs. Among examined dog, cysts have been detected in 5 (50%) out of 10 samples, in cats, it was 4 (40%) out of 10 examined cats. Domestic, and pet animals living in close contact with man in rural areas may have a great opportunity to ingest cysts of *E. histolytica* . On the contrary, some workers in Egypt **(Abo-Shady *et al.*, 1983)** detect *E. histolytica* in 112 cows, 85 buffaloes, 57 sheep and 46 goats' samples in Dakahlia Governorate, and the detection of *E. histolytica* in fecal samples from dogs was supported by the findings of other workers **(Grewal *et al.*, 1970)**, and **(Omar *et al.*, 1978)**. These results emphasis the role of dogs as a companion animal in transmission of *Giardia lamalia* and *E. histolytica* to man. For *E.histolytica* cyst, in this study by microscopy was shown in table(3), indicating that; it have been detected in 6 (20%) out of 20 calves, and in 4 (40 %) out of 10 examined lambs. Among examined dog, cysts have been detected in 3 (60%) out of 5 samples, in cats, it was 2(40%) out of 5 examined cats. The obtained positive samples by microscopy was confirmed by ELIZA test and the ICT test, to show the specificity and sensitivity of these immunological tests. By calculating the specificity and sensitivity of the different immunological method that used in this work, we observed that; for ELISA test of *Cryptosporium parvum*, the specificity was (79.1 %), and sensitivity was(81.1%). For ImmunoChromoTographic test (ICT test) for diagnosis of *Giardia duedenalis* the specificity was (73.8 %) and the sensitivity was (67.8%), also in case of ICT test for *Entamoeba histolytica* specificity was (52.7 %) and the sensitivity was (42.8%), as seen from the above results, we can observed that, the highest specificity and sensitivity for immunological diagnostic method from above mentioned is the ELISA test was higher than (ICT test) in specificity and sensitivity. **Wade *et al.* (2000)** indicated moderate agreement between two diagnostic methods, with the ELISA being the more sensitive, other workers **(Uga *et al.*, 2000)** showed that both methods had the same sensitivity

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الملخص العربي

التشخيص الميكروسكوبي والمناعي لبعض الاوليات المعوية في محافظة المنوفية - مصر

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تمت الدراسة على اربعة أنواع من الحيوانات(العجول الرضيعة – الحملان – الكلاب – القطط) فى محافظه المنوفية وكان الهدف منها معرفه نسب الإصابة بمختلف الأوليات المعوية التي تصيب هذه الحيوانات بالطرق التقليدية للتشخيص بالفحص الميكروسكوبي بعد صبغها بصبغه الزيل نيلسين المعدلة ثم اعاده فحص العينات

الايجابيه بطرق التشخيص المناعيه الحديثه مثل اختبار الاليزا المباشر من خلال اكتشاف الانتيجين فى البراز للحيوانات المصابه ثم عقد مقارنه بين نتائج هذه الاختبارات بنتائج الفحص الميكروسكوبى وتقييمها من خلال معرفه الحساسيه والتخصصيه لهذه الاختبارات. وقد تم التوصل ميكروسكوبيا الى معرفه ثلاث انواع من هذه الاوليات فى براز جميع انواع الحيوانات المصابه (كريبتوسبوريديم بارفام – جيارديا انتستينياليس – انتاميبا هستوليتيكا) وكانت نسب الاصابه متفاوته حيث كانت اعلى نسبه اصابه بكريبتوسبوريديم بارفام فى العجول الرضيعه(73.3 %) واقل نسبه فى الحملان (24%) ينما كانت نسبه الاصابه ب جياردیا انتستينياليس (50 %) فى الكلاب واقلها (20 %) فى العجول وكانت فى انتاميبا هستوليتيكا (60 %) فى الكلاب واقلها (20 %) فى العجول. ثم تم عمل اختبار الاليزا على العينات الايجابيه للكريبوسبوريديم وتم مقارنته بالفحص العادى لكنه اعطى نسبه عاليه من الحساسيه (81.1 %) وكذلك التخصصيه (79.1 %). وتم عمل اختبار الشرائط السريع على العينات الايجابيه فى (جياردیا انتستينياليس – انتاميبا هستوليتيكا) لكنه لم يكن بنفس الكفاءة لاختبار الاليزا وكانت نسبه عاليه من الحساسيه (76.8 %) وكذلك التخصصيه (73.8 %). مما سبق نستنتج انه لاغنى عن التشخيص الميكروسكوبى باستخدام الصبغه فى حالات الكريبتوسبوريديم وكذلك نجد ان اختبار الاليزا له نسبه عاليه من التخصصيه والحساسيه عن الاختبارات السريعه ولكن تكمن عيوب الفحص الميكروسكوبى فى حالات ندره العدوى او وجود الطفيل باعداد قليله فى البراز وكذلك يكون افراز الطفيل فى البراز مرتبط بالحاله المناعيه للحيوان لان الكريبتوسبوريديم من الطفيليات الانتهازيه ومن ثم كان اللجوء الى طرق التشخيص الحديثه والتى تعتمد على وجود الانتيجين فى البراز.