

Isolation and Identification of Some Intestinal Parasites Eggs, Cysts and Oocysts From two Species of Diptera: Calliphoridae in Baghdad

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Abstract

This study documented the role of blowfly *Chrysomya albiceps* and *Chrysomya megacephala* as carriers of 15 species of intestinal parasites eggs, cysts and Oocysts as a primary effort of Iraq, by external and internal techniques: 10 species of different parasites were reported in this study that transmitted mechanically by Calliphoridae fly ,eight of them are nematode eggs (*Ancylostoma duodenal*, *Ascaridia sp.*, *Ascaris lumbricoides*, *Parascaris equirum*, *Strongyloides stercoralis* , *Strongylus sp.*, *Trichostrongylus sp.* and *Toxocara canis*)and cysts of two species of protozoa (*Entamoeba sp.*and *Iodomaba butschlii*). Internal technique by stained the fluid gut of flies with Zael Nelson stain, was applied for 257 fly, three species of nematode eggs (*Ascaris lumbricoides*, *Strongyloides stercoralis* and *Trichuris sp.*); one species of cestode eggs (*Taenia sp.*) and three species of protozoa Oocysts (*Cryptosporidium parvum*, *C. muris*, and *Cyclospora cayetanensis*), were showed internally. This variety of parasites referred to wide distribution of these flies in Baghdad that risk for public health.

Key words: Calliphoridae, *Chrysomya albiceps*, *Chrysomya megacephala* ,intestinal Parasites, protozoa.

Introduction

Non-biting cyclorrhaphan flies, such as house flies, blow flies and flesh flies, are known to carry human intestinal parasites [1,2], human protozoa [3] and animal metazoan parasites [4]. The Calliphoridae family comprises around 150 genera and more than 1000 species distributed worldwide [5, 6, 7]. *Chrysomya albiceps* is a species belonging to the blow fly family, Calliphoridae. It is of great medical and sanitary importance, being associate with Myiasis in Africa and America although it plays a more significant role as a predator of other larvae [8]. The species is also of importance in forensic science and forensic entomology because it is the first insect to come in contact with carrion due to their ability to smell dead animal matter from up to ten miles 16 km away [9,10]. *Chrysomya megacephala* is reported in the same locations of other calliphorid species [11]. This survey is to reveal the role of blowfly *Chrysomya albiceps* and *Chrysomya megacephala* as carriers of pathogen as a primary effort of Iraq.

Material and Methods

A total of 257 fly was chosen and diagnosed as *Chrysomya albiceps* 235(91.4%) and *Chrysomya megacephala* 22(8.5%) by Iraqi Natural History Research Center and Museum from 1123 different flies were collected from slaughter markets of ruminants and fishes in Baghdad in addition of traps were put in zoo and slaughter of equine in Al-Zawra Park of Baghdad, from March to October 2011, so as to identify the eggs, larvae and cysts of protozoa.

Two methods applied:

- 1-Washing technique: all the flies put in distal water for 3 hours and centrifuge the supernatant to identify the parasites that transmitted mechanically [12].
- 2-Staining technique: The procedure to be used with gut contents of blowfly after deposit the abdomen; to identify the eggs, larvae and cyst of protozoa that transmitted internally by Zeal Nelson stain . Color Photographs of eggs, larvae and cysts were taken after Ocular micrometer calibration [13]. This work is diagnosis in Iraqi Natural History Research Center and Museum, University of Baghdad. Key of diagnosis [14, 15, 16,17].

Results and Discussion

Washing technique: 10 species of different parasite stages were reported in this study that transmitted mechanically by *Chrysomya albiceps* and *Chrysoma megacephala* ,eight of them are nematode eggs (*Ancylostoma duodenal*, *Ascaridia sp.*, *Ascaris lumbricoides*, *Parascaris equirum*, *Strongyloides stericoralis* , *Strongylus sp.*, *Trichostrongylus sp.* and *Toxocara canis*) and cysts of two species of protozoa (*Entamoba sp.* and *Iodomaba butschlii*). (Table 1), (fig. 1,2,3,4,5,6,7,8,9,10) respectively. Zael Nelson stain technique: three species of nematode eggs (*Ascaris lumbricoides*, *Strongyloides stericoralis* and *Trichuris sp.*); one species of cestode eggs (*Taenia sp.*) and three species of protozoa Oocysts (*Cryptosporidium parvum*, *C. muris*, and *Cyclospora cayetanensis*), from tested of 257 fly of *Chrysomya albiceps* and *Chrysoma megacephala* after stained with Zael Nelson stain, (Table 2) (fig. 3,5,11,12,13,14,15) respectively.

The current study showed eleven species of different parasites that transmitted mechanically by *Chrysomya albiceps* and *Chrysoma megacephala* ,this result may be explained by Sukontason [18] who claim that the ultrastructure of adhesive device or the pulvilli, pad-like structure between the tarsal claws of the legs, is presented in the blowfly (Calliphoridae), housefly and relatives (Muscidae), and flesh fly (Sarcophagidae) through scanning electron microscopy. These results provided anatomical information that allow us to not only understand the successful attachment of flies to smooth surfaces but also clarify their role as a mechanical carrier of microorganisms.

Flies from several families are attracted to human and animal feces for both feeding and breeding. Blowflies (Calliphoridae) require protein for the development of their eggs [19].

Eggs of several parasites which presented in stool were appeared in this survey like: *Ascaris lumbricoides*, *Ancylostoma duodenal*, *Strongyloides stercoralis*, *Taenia* sp. and *Trichuris* sp. These adult parasites are hosted in the human intestine and releases large numbers of eggs with the feces, so fresh moist feces attract flies more readily than old dry material.

The current study showed cysts of two species of protozoa : *Entamoeba* sp. and *Iodomaba butschlii* that similar to Pipken[20] who had studied the transmission of *Entamoeba histolytica* by filth fly experimentally.

In Kenya Round[21] experimented with three species of filth flies: *Chrysomia albiceps*, *Chrysomia chloropyga* and *Sarcophaga* sp. after giving the flies access to ova of *Taeniarhynchus saginatum* in dilute sugar solution, on raw meat or in an emulsion of human feces, the flies were washed (without killing) and put into a fresh cage. After different periods the fecal spots were examined for ova, he found that *Taeniarhynchus saginatum* eggs could be passed by these flies for periods of up 11 days after ingestion. However the majority was passed within three days and during that time the eggs were viable. He concluded that filth flies in Kenya may play an important role in the transmission of *Taeniarhynchus saginatum* eggs. The presence of intestinal eggs that transmitted by dogs feces like Tapeworm and *Toxocara canis* in this survey noted that the Calliphoridae fed readily on the excrement of canine animals, that similar to Schiller [22]. In other hand, Heniz [23] observed that two different fly populations were associated with dog feces; one visits feces to feed only, whereas the other feeds and reproduces within them. Lawson [24] made a similar observation and noted that the species associated with dog feces.

Three species of intestinal parasites of equine appeared in this survey (*Trichostrongylus* sp., *Strongylus* sp. and *Parascaris equurium*) that mean, Calliphoridae fly were feeding on intestinal content and feces of equine slaughter. In addition, the role and importance of horses for parasitic transmission and their significance in the continuity of some zoonotic diseases can be investigated [25]. Three species of protozoa were reveled in this survey that similar to [26]. The highest rate of this study is to *Cryptosporidium parvum* and *C. muris* (protozoa) 26%, 15% subsequently.

Cryptosporidium parvum is prevalent in preweaned cattle[26] in which the infections can produce high oocyst output sometimes exceeding 1010 oocysts per day.[27] Cattle manure is recognized source of *C. parvum* oocysts,[28,29] and is also a favorite breeding place, food source, and descending site of flies.[30] Although flies come into direct contact with manure, and it has been demonstrated that small particles readily adhere to the exterior surfaces of the fly,[31,32]. Farther than Psychodidae[33] found the larvae and adult stages of flies in the dung.

It was observed that more parasites were isolated from the external surfaces than gut contents of the flies examined ($P < 0.001$), that may be due to the variety of surfaces which these flies stopping on like: feces, fresh and died flesh, carcasses...etc.

This study documented the role of Calliphoridae flies *Chrisoma albiceps* and *Chrisoma megacephala* transmission of different intestinal parasites and protozoa, this variety of parasites referred to wide distribution of these flies in Baghdad that is a risk for Public health.

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References

1. Fetene, T and Worku, N. (2009). Public health importance of non-biting cyclorrhaphan flies. *Trans. R. Soc. Trop. Med. Hyg.*, 103:187-91
2. Gatechew, S; Teshome, G.M ; Erko, B; Balkew, M and Medhin, G. (2007). Non-biting cyclorrhaphan flies (Diptera) as carriers of intestinal human parasites in slum areas of Addis Ababa, Ethiopia. *Acta. Tropica.*, 103:186-94
3. 3- Graczyk, T.K; Knight, R and Tamang, L. (2005) Mechanical transmission of human protozoan parasites by insects. *Clin. Microbiol. Rev.*, 18:128-32
4. 4-Forster, M; Klimpel, S and Sievert, K.(2009). The house fly (*Musca domestica*) as a potential vector of metazoan parasites caught in a pig-pen in Germany. *Vet. Parasitol.*, 160:163-7
5. 5-Hennig, W. (1973). Diptera (Zweiflügler). *Handbuch der Zoologie, Berl.*, 31(4) : 1-337
6. 6-Pont, A. C. (1980). Family Calliphoridae, Catalogue of the Diptera of
7. the Afrotropical Region, British Museum (Natural History) .Londres, 779-800
8. 7-Shewell, G. E. (1987). Calliphoridae, Manual of Nearctic Diptera, Research Branch, Agriculture Canada, Ottawa, 1133-1145.
9. Margareth, M. (1996). Temperature Requirements of *Chrysomya albiceps* (Wiedemann, 1819) (Diptera, Calliphoridae) under Laboratory Conditions". *Memórias do Instituto Oswaldo Cruz (Brazil)*, 91 (6): 785–788
10. Martin, G.; Elisabeth, F. and Christian, R. (2003). The blowfly *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae) as a new forensic indicator in Central Europe. *Int. J. Legal Med.*, 117:75–81
11. Krzysztof, S.; Szymon, M.; Daria, B. and Szymon, K.(2008). *Chrysomya albiceps* (WIEDEMANN, 1819), a forensically important blowfly (Diptera: Calliphoridae) new for the Polish fauna. *J. Entomol.*, 77: 351-355
12. Mara, S. O.; Mara, J. ; Dantur, J. and Nstor, C.(2011). First Report of *CHRYSOMYA MEGACEPHALA*(DIPTERA: CALLIPHORIDAE) in northwestern Argentina. *Florida Entomologist*, 94(2):345-346
13. Gregor, F.; Rozkosny, R.; Bartak, M. and Vanhara, J. (2002). The *Muscidae* (Diptera) of Central Europe. *Scientiarum Naturalium Universitatis Masarykianae Brunensis*. 107. Masaryk.: Masaryk University, 280
14. Robert, A. S. and Robert, D.(2002). Grey Flesh Fly (*Wohlfahrtia vigil*) Parasitism of a Preble's Meadow Jumping Mouse (*Zapus hudsonius preblei*). *J. of Wildlife Diseases*, 38(3): 604–606
15. Thienpont, E.; Rochette, F. and Vanparijs, O. (1986). Diagnosing helminthiasis by coprological examination. Turnhoutsebaan 30, 2340 Beerse, Belgium). Available from: Vet lab Services, UNIT 11. Station Road, South water, Sussex RH 13 7HQ.
16. Edward, K. and Marietta, V. (1959). *Diagnostic Medical parasitology* . philadelphia and London. Library of Congress Catalog Card No .58-7955 . 276 pp.
17. Joobori, T.I.(2002). *Medical parasitology laboratory manual medical helminthology*. Department of micr.med .coll. Al- Nahreen Univ., 159pp.
18. World Health Organization (2004). *Integrated Guide to Sanitary Parasitology*. Regional office for the Eastern Mediterranean Amman – Jordan, 120pp.
19. Sukontason, K. L.; Bunchu, N. ; Methanitikon, R. ; Chaiwong, T. ; Kuntalue B. and Sukontason, K. (2006). Ultrastructure of adhesive device in fly in families calliphoridae, muscidae and sarcophagidae, and their implication as mechanical carriers of pathogens. *Parasitol. Research*, 98 (5): 477 – 481
20. Waterhouse, D. F. (1974). The biological control of dung. *Scientific American*, 230(4):100 – 109

21. Pipken, A. C. (1949). Experimental studies on the role of filth flies in the transmission of *Entamoeba histolytica*. American J. Hygiene, 49: 255 -275
22. Round, M. C.(1961). Observations on the possible role of filth flies in the epizootiology of bovine cysticercosis in Kenya. J. Hyg. Cambridge, 59:505 – 513
23. Schiller, E. L.(1954).Studies on the helminth fauna of Alaska XIX. an experimental study on blowfly (Phormia) region transmission of hydatid disease. Experimental Parasitology, 3:161.
24. Heinz, H. J. and Brauns, W. (1955). The ability of flies to transmit ova of *Echinococcus granulosus* to human food. South African J. Med. Sci., 20:131 – 132
25. Lawson, J. R. and Gemmell, M.A. (1983).Hydatidosis and Cysticercosis: The Dynamics of Transmission .Advances in Parasitology, 22 : 261-308
26. Ram Chandra, S. (2009). A Report on Prevalence of Helminthes Parasites in Mules of Brick Kiln of Lalitpur District. WWW.animalnepal.org/documents/donkey/
27. Hadi, A. M. (2011).A study of prevalence of some parasites and protozoa from
28. *Musca domestica* in Baghdad. Al- Anbar J. Vet. Sci., 4(2):88- 92
29. Anderson, B.C,(1981). Patterns of shedding cryptosporidial oocysts in Idaho calves. J. Am. Vet. Med. Assoc., 178: 982–984
30. Hiepe, T.and Buchwalder, R.(1991). Livestock manure as a vector for parasites a report of experiences. *Deutsche Tierärztliche Wochenschrift*, 98: 268–272
31. Pell, A.N. (1997). Manure and microbes: public and animal health problem? J. Dairy Sci., 80: 2673–2681
32. Hedges, A. (1980). Flies, gnats and midges. Mallis A, ed. *Handbook of Pest Control*. Cleveland, OH: Franzak & Foster Co., 621–685.
33. Ebeling, W.(1978). *Urban Entomology*. Davis, CA: University of California Press. 20. Bennett GW, Owens JM, Corrigan MS, Duluth, MN: Advanstar Communications.
34. Iwasa, M. ; Makino, S.I; Asakura, H. ; Kobori, H. and Morimoto, Y, (1999).
35. Detection of *Escherichia coli* O157:H7 from *Musca domestica* (Diptera: Muscidae) at a cattle farm in Japan. J. Med. Entomol., 36: 108–112
36. Greenberg, B. (1973). Flies and Diseases, Biology and Disease Transmission. Princeton, N J: Princeton University Press.
37. Heo Chong, C.; Nazni, W. A.; Chew, W. K.; Hiromu, K.; John, J. ; Heah Sock, K. and Baharudin, O.(2010). A Study of Cow Dung Diptera in Sentul Timur, Kuala Lumpur, Malaysia. J. Trop. Med. Parasitol., 33:53-61

Table (1): Stages of parasites are transmitted by external surface of two species of Calliphoridae fly.

Species of parasites	Class	Stage	Final host	No. of stage	%
<i>Ancylostoma duodenal</i>	Nematode	Egg	Man	7	2.7
<i>Ascaridea sp.</i>	Nematode	Egg	Avian	7	2.7
<i>Ascaris lumbricoides</i>	Nematode	Egg	Man	9	3.5
<i>Parascaris equirum</i>	Nematode	Egg	Equine	11	4.2
<i>Strongyloides stercoralis</i>	Nematode	Egg	Man	11	4.2
<i>Strongylus sp.</i>	Nematode	Egg	Equine	7	2.7
<i>Trichostrongylus sp.</i>	Nematode	Egg	Equine	8	3.1
<i>Toxocara canis</i>	Nematode	Egg	Dog	13	5
<i>Entamoeba sp.</i>	Protozoa	Cyst	Man	23	9.8
<i>Iodamoeba butschli</i>	Protozoa	Cyst	Man	9	3.5

Significant differences $P < 0.01$ between two methods.

Significant differences $P < 0.05$ among the species of parasites.

Table (2): Stages of parasites are transmitted by gut contents of two species of Calliphoridae fly stained with zeal nelson stain.

Species of parasites	Class	Stage	No. of stages	%
<i>Ascaris lumbricoides</i>	Nematode	Egg	11	4.2
<i>Strongyloides stercoralis</i>	Nematode	Egg	13	5
<i>Trichuris sp.</i>	Nematode	Egg	27	10.5
<i>Taenia sp.</i>	Cestode	Egg	8	3.1
<i>C. parvum</i>	Protozoa	Oocyst	67	26
<i>C. muris</i>	Protozoa	Oocyst	39	15
<i>Cyclospora cayetanensis</i>	Protozoa	Oocyst	21	8.1



Fig.1: *Ancylostoma duodenal* egg external transmitted by Calliphoridae fly.



Fig.2: *Ascaridea* sp. egg external transmitted by Calliphoridae fly.

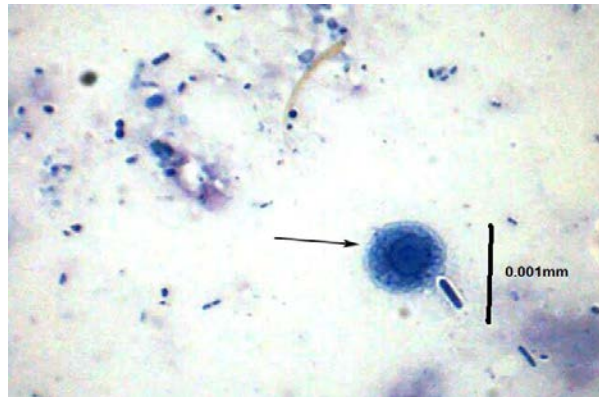


Fig.3: *Ascaris lumbriciodes* unfertilized egg, zeal nelson stain.



Fig.4: *Parascaris equium* egg external transmitted by Calliphoridae fly.

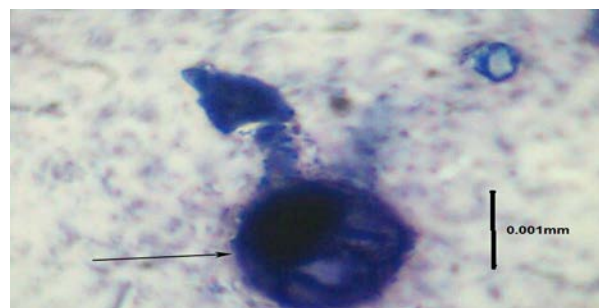


Fig.5: *Strongyloides stercoralis* egg, zeal nelson stain.



Fig.6:*Strongylus* sp. egg external transmitted by Calliphoridae fly.



Fig.7:*Trichostrongylus* sp. egg external transmitted by Calliphoridae fly.



Fig.8: *Toxocara canis* egg external transmitted by Calliphoridae fly.



Fig.9:*Entamoeba* sp. cyst external transmitted by Calliphoridae fly.



Fig.10:*Iodomaba butschlii* external transmitted by Calliphoridae fly.



Fig.11:*Trichuris* sp. egg zeal nelson stain.

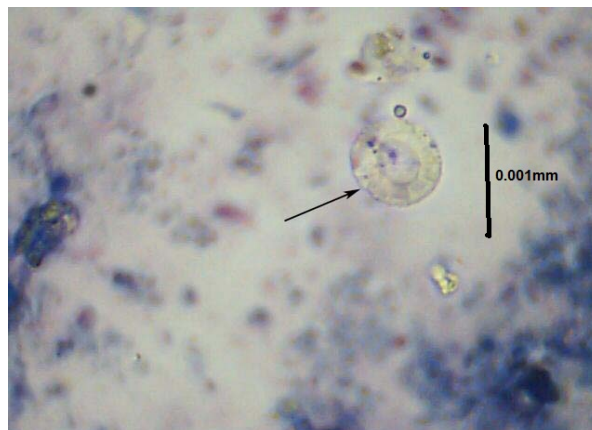


Fig.12:*Taenia* sp. egg zeal nelson stain.

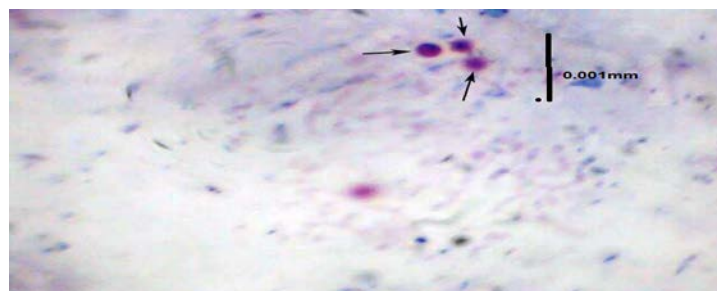


Fig.13:*Cryptosporidium parvum* Oocyst, zeal nelson stain.

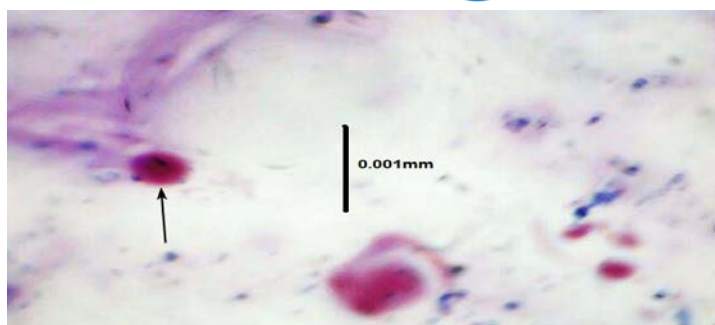


Fig.14:*Cryptosporidium muris* Oocyst, zeal nelson stain.

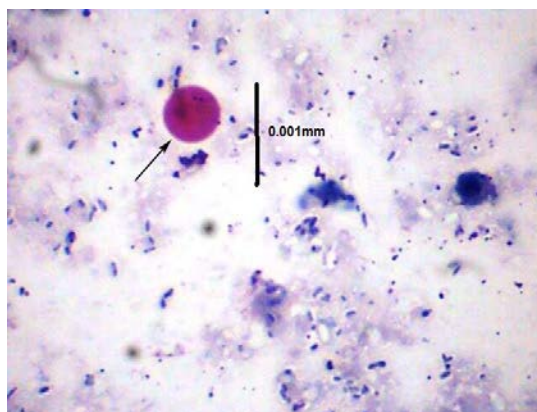


Fig.15:*Cyclospora cayteniasis* Oocyst, zeal nelson stain.

عزل وتشخيص بيوض ،أكياس وأكياس بيض بعض الطفيليات المعوية من نوعين من عائلة ذباب الكاليفوريدي في بغداد

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مركز بحوث ومتحف التاريخ الطبيعي / جامعة بغداد

استلم البحث في: 20 شباط 2012 قبل البحث في: 20 تشرين الثاني 2012

الخلاصة

بينت الدراسة دور نوعين من الذباب هما :

Chrysomya albiceps, *Chrysomya megacephala*

في نقل 15 نوعا من بيوض وأكياس بيض بعض الطفيليات المعوية خارجيا وداخليا ولأول مرة في العراق. أظهرت الدراسة أكياس وبيوض 10 أنواع من الطفيليات التي ينقلها هذين النوعين من الذباب ميكانيكيا، ثمانية منها هي بيوض الديدان الخيطية، وأكياس نوعين من الحيوانات الابتدائية. كما أظهر الفحص الداخلي لصبغ سوائل القناة الهضمية بصبغة الزيل نلسن ل 257 ذبابة للنوعين قيد الدراسة، أكياس بيض ثلاثة أنواع من الحيوانات الابتدائية، وبيوض ثلاثة أنواع من الديدان الخيطية وبيوض نوعا واحدا من الديدان الشريطية. هذا التنوع في ظهور الطفيليات يشير الى الانتشار الواسع لهذه الذبابة في بغداد وهذا يشكل خطرا على الصحة العامة.

الكلمات المفتاحية : ، الطفيليات المعوية، الاوالي المعوية، *Calliphoridae*, *Chrysomya albiceps*, *Chrysomya megacephala*