

Domestic Pigeons as A Potential Hazzard for Transmission of Some Human Protozoan Parasites

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Abstract

Domestic pigeons (*Columba livia domestica*) of the order Columbiformes are ubiquitous birds and can be found in virtually every town and city around the globe. Their interaction with humans and domestic animals and wild birds makes them a potential carrier of zoonotic parasites. The present study aimed to detect the prevalence of different zoonotic protozoans that affect different-aged domestic pigeons in different localities in Assiut Governorate, Egypt. A total of 50 fecal samples from 20 young and 30 adult pigeons were collected and examined for identification and estimation of prevalence of *Cryptosporidium* sp. and *Microsporidium* sp. using modified Kinyoun acid-fast stain. For detection of the prevalence of *Toxoplasma gondii*, serum samples from 50 pigeons were examined serologically for the presence of *Toxoplasma* antibodies by using Latex Agglutination test. The prevalence of *Cryptosporidium* sp. infection was 20%; 6.7% in adult pigeons and 40 % in young pigeons while that of *Microsporidium* sp. was 40% both in adult and young pigeons. Mixed infection was detected in only two young pigeons (10%). Regarding *Toxoplasma gondii* detection, the number of seropositive cases detected by LAT was 29 out of 50 (58%). The positive agglutination titers, among 14 (48.27%) seropositive pigeons ranged between 1:2 -1:128. It was concluded that domestic pigeons may be considered as a reservoir host for *Cryptosporidium*, *Microsporidium*, and *Toxoplasma gondii* human infection which represents a serious human public health problem especially for high risk groups of population living in the same dwellings with pigeons. Moreover, the present pilot results provide a baseline data for planning future researches and control strategies against domestic pigeon's parasites.

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Introduction

Pigeons occur worldwide and are reared as a symbol of peace, love, gentleness and spirit messengers and nowadays also for meat, ornamental pet bird. Unfortunately, they may suffer from many zoonotic protozoan infections [10].

Domestic pigeons (*Columba livia domestica*) of the order columbiformes are ubiquitous birds and can be found in virtually every town and city around the globe. *Columba livia* is a species that descended from wild rock pigeon [25].

Poultry industry is the most effective and economical source of animal protein in shortest possible time. Poultry producers are looking for some substitute of chicken meat, which in the future will come in the form of pigeon meat to contribute towards its increase in gross domestic production through livestock sector [4].

Among the birds, importance of domestic pigeons cannot be ignored, as they act as reservoir hosts or carriers and important source of parasitic infections for other avian hosts which share the common parasitic fauna and sometimes harbor zoonotic parasites. [26] Moreover domestic pigeons are part of subsistence farming done by most poor families [9].

Aim of the Present Study

The aim of the present work is to do a pilot study of the prevalence of different zoonotic protozoans affecting different-aged domestic pigeons in different localities in Assiut Governorate, Egypt including *Cryptosporidium* sp. and *Microsporidium* sp. through examination of fecal samples by modified kinyoun acid fast stain and *Toxoplasma gondii* through serological estimation of *Toxoplasma gondii* antibodies by Latex Agglutination test.

Material and Methods

The present study was conducted from October through December 2018 in the Department of Medical Parasitology, Faculty of Medicine, Assiut University and Animal Health Research Institute (Assiut branch), Assiut, Egypt.

In this study, a total of 50 fecal samples, were collected from domestic pigeons (*Columba livia domestica*) of different ages (20 young and 30 adult) from different localities of Assiut Governorate and

examined for identification and estimation of prevalence of *Cryptosporidium* sp. and *Microsporidium* sp. The specimens, consisted of concentrated sediment of fresh stool, prepared and stained using Modified Kinyoun acid-fast stain according to [16].

For serological examination, a total of 50 serum samples were examined for the presence of *Toxoplasma* antibodies by Latex Agglutination test according to [41]. All samples were examined qualitatively, 14 of the positive samples were examined quantitatively. The presence of agglutination indicated an antibody concentration equal or more than 4 IU/ml and the titer, in the quantitative method were defined as the highest dilution showing a positive result.

Statistical analysis was carried out using [25] program, version 8.2 for differences between young and adult domestic pigeons in numbers of infected samples. The significance differences between young and adult pigeon means were tested by Duncan Multiple Range Test [37]. The data was presented in mean \pm Standard error of means (S.E.M). Level of significance was set at $P < 0.05$.

Results

The examination of 50 (20 young and 30 adult) pigeon's fecal samples by Kinyoun's acid fast stain showed that the total prevalence of *Cryptosporidium* sp. infection was 20 %. It was found in 6.7% in adult pigeons and 40 % in young pigeons (Table 1). On the other hand the total prevalence of *Microsporidium* sp. infection was 40 % in both adult and young pigeons (Table 1). Mixed infection of *Microsporidium* sp. with *Cryptosporidium* sp. was detected in only two young pigeons (10%). The difference was not statistically significant; p value = 0.347.

Using Latex Agglutination serological test for toxoplasmosis, the number of seropositive cases in domestic pigeons was 29 out of 50 (58%). Quantitatively, among seropositive pigeons; 14 (48.27%) were positive at titers ranging from 1/2 to 1/128. Four samples (28.57%) were detected positive at a titer of 1/16 as well as at a titer of 1/32. Three samples (21.42%) were detected positive at a titer of 1/64. One sample (7.14%) was detected positive at a titer of 1/2, 1/4, and 1/128. On the other hand, no samples were detected positive at a titer of 1/8 (Table 2)

Table 1. Examination of fecal samples of pigeons by modified Kinyoun acid-fast stain.

Parasites	Samples					
	Fecal N=50					
	young n=20		adult n=30		total	
	Inf.	%	Inf.	%	I n f .	
<i>C. Wtosporidium sp.</i>	8	40	2	6.7	10	20
<i>Microsporidium sp.</i>	8	40	12	40	20	40
Mixed infection	2	10	0	0	2	4

Table 2. Distribution of Latex titers for 14 positive serum samples of pigeons.

Titer	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Positive samples	1	1	0	4	4	3	1
	7.14	7.14	0	28.57	28.57	21.42	7.14

Discussion

Endoparasitic infections are of great importance in poultry industry especially in backyard pigeon worldwide [8].

In the present study the prevalence of *Cryptosporidium* sp. in young domestic pigeons was 40%, which was higher than adult pigeons (6.7%); this agreed with [12] who stated that the development of the disease in young pigeons might indicate that the immunologic status could have played an important role in the spread of the infection. The parasite was reported from pigeons in Turkey, Spain, Iran, Thailand, Malaysia and Brazil [5,21,22,28,29,32,33,34].

From Egypt, Ahmed *et al.* [2] recorded for the first time the occurrence of *Cryptosporidium* sp. in 46.2% out of 322 examined domestic pigeons from Gharbia Governorate.

In all previously mentioned works, *Cryptosporidium* oocysts were identified only to the generic level. For the first time, [1] reported the occurrence of the human species *C. hominis* in 5.9% of 34 domestic pigeons from Canary Islands based on DNA findings. Later on,

zoonotic crypto-sporidiosis; particularly *C. meleagridis* was detected in pigeons in Thailand through molecular identification [21]. Moreover, [15] detected cryptosporidial oocysts in droppings in 40% out of 120 pigeons collected from house roofs in Baghdad, Iraq and identified them according to their size to be small-sized (4x5.2µ) *C. meleagridis*, medium-sized (6.2x4.5µ) *C. baileyi* and large-sized (8x6.5µ) *C. galli*. Recently, [23] characterized pigeon's cryptosporidia in Southern China by DNA sequencing of small subunit ribosomal RNA (SSU rRNA) gene, and detected them in 0.82% out of 244 domestic pigeons. They added that they were morphologically and genetically belonging to *C. meleagridis* (5.15x4.6µ) and *C. baileyi* (6.11x5.16µ). As the present cryptosporidial oocysts were small-sized (4.5-5µ), they belong to the zoonotic *C. meleagridis*.

It is worth mentioning that during the present study, cryptosporidian oocysts were reported from pigeons in Assiut for the first time. As this parasite is one of the most serious human protozoan parasites, it is recommended that domestic pigeons in all Egyptian Governorates should be examined genetically to find out if the cryptosporidian oocysts found in their droppings

are actually of human origin as this will indicate that these pigeons act as reservoir hosts to human beings in the surrounding dwellings.

In the present study, the total prevalence of *Microsporidium* sp. in fecal samples of fifty examined droppings of domestic birds was 40 % and this disagreed with [36] who showed that there was 8.6% microsporidial spores found in droppings from urban feral pigeons in Poland. This may be attributed to environmental pollution with microsporidial spores in Assiut and may be potentially infectious to domestic animals and birds by direct contact with them.

Although these spores were reported frequently from fecal droppings of pigeons [18,19] from Spain, [6] from the Netherlands, [12] from Brazil, [36] from Poland, [3] from Iraq, [31] from Iran) it had been not reported from Egyptian pigeons before; denoting that our record is for the first time from Egypt.

The discovery of zoonotic strains of microsporidiosis that can infect man even by inhalation of pigeon's droppings [6,17,36] highlights the extreme risk of human infection particularly pigeon fanciers living in association with domestic pigeons.

Hence, future studies are recommended to examine domestic pigeons' droppings not only by acid fast stain but also by genotyping techniques in order to explore their exact role as reservoir hosts for human microsporidiosis.

Regarding *Toxoplasma gondii*, detection of the parasite in tissue lesions was very difficult because the disease is usually manifested by mild clinical symptoms and developed as an unapparent infection, accompanied by low parasitaemia [27]. Therefore; the detection of anti-*Toxoplasma* antibody response appeared to be the suitable tool for early and accurate diagnosis [24].

In the present study, the prevalence of toxoplasmosis in fifty domestic pigeon's sera was estimated by latex agglutination test which showed that the seroprevalence of infection was 58%. The advantages of using Latex Agglutination test agreed with [30] in that it detects total antibody level. Moreover, it is simple to perform, and required no expensive capital equipment. Toxoplasmosis seroprevalence studies conducted with pigeons from different continents exhibited different prevalence rates. In Portugal; rates ranging from 4.0% to 5.9% [38], in Colorado in United

States was 6–10% of domestic pigeons tested by the Sabin–Feldman dye test [13].

The levels of pigeon's antibodies to *T. gondii* in southern China was evaluated by Modified Agglutination Test (MAT) mainly because of its high specificity, sensitivity and cost-effectiveness; the cut-off titer of MAT for positive infection in pigeon was 1:5 which was considered indicative of *T.gondii* exposure [39]

Seven (58.3%) out of 12 inoculated pigeons in southeastern Brazil were tested positive for anti-*T. gondii* antibodies by MAT (modified agglutination test) and IFAT (Indirect Fluorescent Antibody Test), Titers ranged from 1:40 to 1:5120 by MAT and 1:512 to 1:4096 by IFAT [14].

[40] found that the seropositivity of 275 domestic pigeon serum samples was 8.7% in southern China using Modified Agglutination Test (MAT), the difference in seropositivity of *T. gondii* in pigeons may be attributed to differences between feral and domestic pigeons, under different geographical conditions, serologic tests used, as well as bird husbandry practices and bird welfares. The high prevalence of *T. gondii* in the examined domestic pigeons is probably due to technical deficiencies in the management of pigeon breeding where the circulation of cats may be permitted. Sero-prevalence of *Toxoplasma* antibodies in northwest China was detected in 11.86% of examined domestic pigeons with titer 1:5-1:100 [11]. Moreover, *T. gondii* antibodies were with overall percentage of 62.5% was detected in sera of domestic pigeons in Nigeria [7].

From Egypt, [20] using serological, histopathological and immunohistochemical diagnostic methods, *Toxoplasma gondii* was detected in domestic pigeons from Giza, Fayoom, Beni-Suef and Minya Governorates (Egypt). Their study indicated soil contamination and observable infection in pigeons which could be considered a major danger of human infection.

The present study is the first study of toxoplasmosis detection in domestic pigeons in Assiut, Egypt. As domestic pigeons live in intimate association with human beings in their dwellings, they could be a very dangerous reservoir host in transmitting the disease to man. However, future studies are recommended to find out if pigeon's *Toxoplasma* strain is of the pathogenic human strain.

Conclusion

Microsporidium sp. was recorded from domestic pigeons in Egypt for the first time and the present study indicated that domestic pigeons may be considered as a reservoir host for *Toxoplasma*, *Cryptosporidium* and *Microsporidium* human infection which represents a serious human public health problem especially for high risk group of population living in the same dwellings with pigeons. Moreover our results provide a base line data for planning future researches and control strategies against possible zoonotic domestic pigeons' parasites.

Ethical Considerations

All applicable international, national and institutional guidelines for the care and use of animals were followed and the study and steps of practical experiments were approved by the Research Ethical Committee of the Faculty of Medicine, Assiut University, Egypt.

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Conflict of Authors

The authors declare that there are no conflicts of authors regarding the publication of this paper.

Author's Contribution

Refaat M.A. Khalifa; Planning the study design, supervising and revising the manuscript

Abdallah A.E. Hassan: Follow up of parasitological studies

Mohsen I. Arafa: Revising and writing the results

Hanan E. M. Eldeek: Follow up of serological studies

Wafaa G. Mahmoud: Undergoing the practical tests and writing the manuscript

References

1. Abreu-Acosta N., Forondo-Rodriguez P., López M., Valladare B. 2009. Occurrence of *Cryptosporidium hominis* in pigeons (*Columba livia*). *Acta Parasitologica*, 54 (1): 1-5.
2. Ahmed N.E., El-Akabawy, L.M., El-Madawy R.S., Toulan E.I. 2013. Studies on intestinal protozoa of poultry in Gharbia Governorate, Benha. *Veterinary Medical Journal*, 25 (2): 78-81.
3. Al-Sadi H.I., Al-Mahmood S.S. 2013. Microsporidial infection in some domestic and laboratory animals in Iraq. *International Journal of Basic and Applied Medical Science*, 3 (3): 78-91.
4. Bahrami A.M., Hosseini E., Razmjoo M. 2013. Important parasite in pigeon, its hematological parameter and pathology of intestine. *World Applied Science Journal*, 21 (9): 1361-1365
5. Bamaïyi PH, Redhuan NE 2016. Prevalence and risk factors for cryptosporidiosis: a global emerging neglected zoonosis. *Asian Biomedicine*, 10 (4): 309-325.
6. Bart A., Wentink-Bonnema E.M., Hendrema E.R., Buija J., van Gool T. 2008. Frequent occurrence of human-associated microsporidia in fecal droppings of urban pigeons in Amsterdam, The Netherlands. *Applied and Environmental Microbiology*, 74 (22): 7056-7058.
7. Bata S, Karshima NS, Oshada AH *et al.*, (2020): Detection of *Toxoplasma gondii* antibodies in domestic pigeons (*Columba livia domestica*) in Jos, north central Nigeria. *Parasitol Vector Biol.*, Open Access, Article in press.
8. Bhat S.A., Khajuria J.K., Katoch R., Wani M.Y., Dhama K. 2014. Prevalence of endoparasites in backyard poultry in North Indian Region: A performance based assessment study. *Asian Journal of Animal Veterinary Advances*, 9 (8): 479-488.
9. Borji H., Meghaddas E., Razmi G.R., Azad M. 2012. A survey of ecto- and endo-parasites of domestic pigeons (*Columba livia*) in Mashhad, Iran. *Iranian Journal of Veterinary Science and Technology*, 4 (2): 37-42.
10. Borkatiki B., Katoch P., Goswami P., Godara R., Khajuria J.K., Yadav A., Kour R., Mir I. 2013. Incidence of *Haemoproteus columbae* in pigeons of Jammu district. *Journal of Parasitic Diseases*, Doi: 10.1007/s 12639-013-0356-8.
11. Cong W., Huang S., Zhou D., Xu M., Wu S., Yan C., Zhao Q., Song H., Zhu X. 2012. First report of *Toxoplasma gondii* infection in market-sold adult chickens, ducks and pigeons in North West China. *Parasites and Vectors*, 5:110.
12. Current W.L., Blagburn B.L. 1990. Infections in man and domestic animals. In: Coccidiosis of man and domestic animals. P. L. Long, ed. CRC Press, Boca

- Raton, FL. 155-185.
13. Dubey J.P., Felix T.A., Kwok O.C. 2010. Serological and parasitological prevalence of *Toxoplasma Gondii* in wild bird from Colorado. *Journal of Parasitology*, 96(5), 937–939.
 14. Godoi F.S.L., Nishi S. M., Pena H. F., Gennari S. M. 2010. *Toxoplasma gondii*: diagnosis of experimental and natural infection in pigeons (*Columba livia*) by serological, biological and molecular techniques. *Revista Brasileira de Parasitologia Veterinaria*, 19, (4), 238-243.
 15. Faraj A.A. 2014. Distribution of *Cryptosporidium* spp infection in wild pigeons in Baghda city, Iraq. *Basrah Journal of Veterinary Research*, 1(2): 48-53.
 16. Gracia L.S. 2001. *Diagnostic Medical Parasitology*. ASM Press, Washington, DC.
 17. Graczyk T.K., Sunderland D., Rule A.M., Da Silva A.J., Moura I.N., Tamang L., Girouard A.S., Schwab K.J., Breyse P.N. 2007. Urban feral pigeons (*Columbia livia*) as a source for air and water borne contamination with *Enterocytozoon bieneusi* spores. *Applied and Environmental Microbiology*, 73: 4357-4358
 18. Haro M., Izquierdo F., Henrique-Gil N., Andres I., Alonso F., Fenoy S., del Aguila C. 2005. First detection and genotyping of human-associated microsporidia in pigeons from urban Parks. *Applied and Environmental Microbiology*, 71 (6): 3153- 3157.
 19. Haro, M.; Henriques-Gil, N.; Fenoy, S.; Izquierdo, F.; Alonso, F.; Águila, C. 2006. Detection and genotyping of *Enterocytozoon bieneusi* in pigeons. *Journal of Eukaryotic Microbiology*, 53 Suppl s1: S58-S60.
 20. Ibrahim HM, Osman GY, Mohamed AH *et al.*, (2018): *Toxoplasma gondii*: Prevalence of natural infection in pigeons and ducks from middle and upper Egypt using serological, histopathological and immunohistochemical diagnostic methods. *Vet. Parasitol. Reg. Stud. Reports*, 13: 45-49.
 21. Koompapong K., Mori H., Thammasonthijaren N., Prasertbun R., Pintong A.R., Popruk S., Rojekkittikhun W., Chaisiri K., Sukthana Y., Mahittikorn A. 2014. Molecular identification of *Cryptosporidium* spp. in seagulls, pigeons, dogs and cats in Thailand. *Parasite*, 21: 52, Doi.org/10.1051/parasite/2014053
 22. Lallo M.A., Calabria P., Milanelo L. 2012. *Encephalocytozoon* and *Enterocytozoon* (Microsporidia) spores in stool from pigeons and exotic birds: Microsporidia spores in birds. *Veterinary Parasitology-journal*, 190 (3-4): 418-422.
 23. Li J., Lin X., Zhang L., Qi N., Liao S., Lv M., Wu C., Sun M. 2015. Molecular characterization of *Cryptosporidium* spp. in domestic pigeons (*Columba livia domestica*) in Guangdong Province, Southern China. *The journal Parasitology Research*, 114 (6): 2237-2241.
 24. Marghany M.E. 2004. *Some studies on toxoplasmosis in poultry transmissible to man M.D.* Thesis, Faculty of Medicine, Assiut University, Egypt.
 25. Marques S.M., Quadros R.M., Silva C.J., Baldo M. 2007. Parasites of pigeons (*Columba livia*) in urban areas of Lages, Southern Brazil . *Parasitologia Latinoamericana*, Chile, 62: 183 – 187.
 26. Maslin W.R., Latimer K.S. 2009. Atxoplasmosis in canary fledglings: Severe lymphocytic enteritis with preferential parasitism of B lymphocytes. *Avian Diseases*, 53(3): 473–476.
 27. Masoud A.M., David W.D., Armah D.C. 1990. Toxoplasmosis in sheep in North Eastern United States. *Journal of the American Veterinary Medical Association*, 196: 263-265.
 28. Oliveira BCM, Ferran ED, da Cruz ME *et al.* (2017): First description of *Cryptosporidium parvum* in carrier pigeons (*Columba livia*). *Vet. Parasitol.*, 30 ! \$#: 148-150.
 29. Özkul I.A., Aydin Y. 1994. Small-intestinal cryptosporidiosis in a young pigeon. *Asian Pathology*, 23: 369-372.
 30. Parker S.P., Cubitt W.D. 1992. Modified latex agglutination test for antibodies to *Toxoplasma gondii* in eluates from Guthrie cards. *Clinical Pathology.*, 45:907-909.
 31. Pirestani M., Sadraei J., Forouzandeh M. 2013. Molecular characterization and genotyping of human related microsporidia in free-ranging and captive pigeons of Tahrán, Iran. *Infection, Genetics and Evolution*, 20: 495-9

32. Radfar M.H., Fathi S., Asl E.N., Dehaghi M.M., Seghinsara H.R. 2011. A survey of parasites of domestic pigeons (*Columba livia domestica*) in South Khorasan, *Iranian Veterinary Research*, 4 (1): 18-23.
33. Rodríguez F., Orós J., Rodríguez L., González J., Castro P., Fernández, A. 1997. Intestinal cryptosporidiosis in pigeons. *Avian Diseases*, 41, 748-750.
34. Sari B., Karatepe B., Karatepe M., Kara M. 2008. Parasites of domestic (*Columba livia domestica*) and wild (*Columba livia livia*) pigeons in Nigde, Turkey. *Bulletin of the Veterinary Institute in Pulawy*, 52: 551-554.
35. S.A.S 2001. SAS/ STAT Guide for personal computer (version 8.2 End). SAS. INST., Cary, N.C.Co., New York.
36. Slodkowiicz-Kowalska A., Graczyke T.K., Nowosad A., Majewska A. 2013. First detection of microsporidia in raised pigeons in Poland. *Annals of Agricultural and Environmental Medicine*.20 (1): 13-15.
37. Steel R.G., Torrie J.H. 1982. "Principles and Procedures of Statistics". A Biometrical Approach (2nd Ed) Mc Grow- Hill Book
38. Waap H., Vilares A., Rebelo E., Gomes S., Angelo H. 2008. Epidemiological and genetic characterization of *Toxoplasma gondii* in urban pigeons from the area of Lisbon (Portugal). *Veterinary Parasitology*, 157: 306–309.
39. Yan C., Yue C.L., Yuan Z.G., He Y., Yin C.C., Lin R.Q., Dubey J.P., Zhu X.Q. 2009. *Toxoplasma gondii* infection in domestic ducks, free-range and adult chickens in southern China. *Veterinary Parasitology*, 165: 337–340.
40. Yan C., Yue C.L., Qiu S.B., Li H.L., Zhang H., Song H.Q., Huang S Y., Zou F.C., Liao M., Zhu X.Q. 2011. Sero-prevalence of *Toxoplasma gondii* infection in domestic pigeon (*Columba livia*) in Guangdong Province of southern China. *Veterinary Parasitology*, 177:371–373.
41. Young D.S. 1995. *Effects of drugs on clinical laboratory tests*, 5th ed. AACC Press