



Comparative Evaluation of Direct Smear and Culture Methods for Detection of *Trichomonas gallinae* Infection in Pigeon and Chicken of Assam

Munmi Saikia¹, Kanta Bhattacharjee², Prabhat Chandra Sarmah³, Dilip Kumar Deka⁴

^{1,2,3,4} Department of Veterinary Parasitology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781022, Assam

ABSTRACT: A study was carried out in order to compare the detection of *Trichomonas gallinae* by examination of smears of throat swab and culture of protozoa collected from both pigeon and chicken. In the present study, five culture media viz. Modified Diamond's Media, Nutrient broth, Medium 199, Minimum Essential Medium (MEM) and Roswell Park Memorial Institute-1640 (RPMI-1640) were used for culture of *T. gallinae*. Microscopic examination of wet mount/ Giemsa stained smear and culture of the *T. gallinae* revealed the latter method to be superior to wet mount preparation or staining methods.

KEYWORDS: Diamond's Media, *in vitro* culture, microscopy, MEM, RPMI-1640, *Trichomonas gallinae*.

INTRODUCTION

Among all the domestic birds duck, chicken, geese, pigeon, quail, turkey etc. are more popular throughout the world. Among the various types of parasites, digestive tract protozoan parasites mainly *Trichomonas* are known to be harmful to domestic birds in different countries including India ([1, 2, 3]). Avian trichomoniasis is caused by *Trichomonas gallinae*, a flagellate protozoan parasite belonging to the class Zoomastigophorea and order Trichomonadida. *T. gallinae* primarily infects the columbiformes birds (pigeon and mourning dove) although several other birds including the wild carnivorous ones may be infected ([4]). The parasite is transmitted from infected mother doves and pigeons to their nestlings through crop milk feeding while adult to adult transmission occurs through billing activities during courtship, feed and water contamination. The disease has been recognized as an emerging and potentially fatal disease of birds ([4]). Diagnosis of the disease is generally done on the basis of gross clinical symptoms exhibited by the affected birds such as raised areas of yellow like buttons mostly distributed in the throat, mouth and beak commissure, yellowish white focal caseated materials in the buccal cavity, esophagus, crop and proventriculus, off-feed, weight loss, open mouthed breathing, drooling of saliva from the mouth and nostrils etc. Infection due to *T. gallinae* organism can be confirmed in birds most commonly by microscopic examination of freshly prepared wet-mount of throat swabs/ oropharyngeal swabs and by staining the thin swab smears with Giemsa stain ([5,6,7,8]). However, in addition to microscopic detection, *in vitro* culture of the protozoa in different media is also carried out for accurate diagnosis and *in vitro* drug study. For the first time, *T. gallinae* was cultivated free from bacteria in Locke-egg-serum and liver bouillon media ([9]). *In vitro* cultivation was considered as the gold standard for detection of trichomonads due to easy interpretation even in poorly infected birds ([10]). Cell culture was adopted to differentiate between different strains of *T. gallinae* pathogenicity and it proved to be a sensitive tool ([11]). *T. gallinae* could be cultured in a variety of media including liquid, semi liquid types and semi-solid CPLM-agar medium ([12]). The incubation temperature had an influence in the growth rate of trichomonads wherein the number of live cells recorded for *T. gallinae* was higher at 37°C in comparison to 40°C of incubation ([13]). Medium 199, a general media for maintenance of eukaryotic cells, supplemented with rice starch and inactivated fetal bovine serum was a standard media for isolation of *Trichomonas gallinae* ([14]). The isolation, diagnosis and cultivation of *T. gallinae* from domestic Pigeon was studied and the parasites were confirmed by wet mount preparation, different staining techniques like Giemsa, Gram stain and Fields staining method and by culture method ([15]). Parasites were cultivated by In- tube and In-pouch culture systems using modified Diamond media (TYM), Cysteine Peptone Maltose media (CPLM) and *Trichomonas* CM0161 media (CM 161). Two commercially available cultured media, namely modified Diamond's medium and modified thioglycolate medium were used to identify the growth of *T. gallinae* ([16]).

As regards to *in vitro* culture of *T. gallinae* in India and Assam in particular, there is not much literature available. Owing to the importance of diagnosis of the flagellate by direct microscopy of throat swabs and the sensitivity of different culture methods



for early treatment of affected birds, the present study was carried out to compare the efficacy of direct smear and culture methods for detection of *T. gallinae* infection in pigeon and chicken of Assam. This is the first report of *in vitro* culture of *T. gallinae* from this part of India.

MATERIALS AND METHODS

1. Wet mount preparation

The throat swabs collected from 464 nos. of pigeon and 304 chickens, irrespective of clinical signs were squeezed thoroughly in the fluid contents of the tubes (Nutrient broth). One or 2 drops of well mixed fluid were taken on a clean glass slide pre warmed at 37°C, covered with a cover slip and immediately examined under low power (10X) and high power (40X) objectives of a compound microscope. The parasites if present were detected by their characteristic vigorous jerky movement ([17]).

2. Staining of smear/permanent mount

Two to three drops of well mixed culture medium were taken on a clean glass slides and spread to air dry thoroughly. These smears were fixed in methanol for 2 minutes and stained for 30-40 minutes with diluted Giemsa's stain as per routine procedure ([17]). The slides were washed thoroughly, air dried and examined under high power (40X) and oil immersion objective (100X). Similarly, the direct smears prepared in field condition during sample collection were fixed and stained as per the above procedure for examination

3. Preparation of Modified Diamond's Media

The Modified Diamond's media was prepared according to ([18]), adding ingredients like 20.0 g of trypticase, 10.0 g of yeast extract, 5.0 g of maltose, 1.0 g of L-cysteine hydrochloride, 0.2 g of ascorbic acid. Solution was brought up to 1,000 ml with distilled water and autoclaved for 15 min at 121°C under 15 lb/in pressure for sterilization. Antibiotic mixture (Sodium penicillin G100000IU/100ml, Streptomycin sulfate 0.1 gm/100 ml) and 10% fresh inactivated fetal calf serum (inactivated by boiling at 56°C for 30 minutes in water bath) were added to the solution. The pH of the culture media was adjusted to 6.5 with hydrochloric acid ([19]).

4. Commercially available media

In the present study, four commercially available media were purchased, i.e. Nutrient broth from HiMedia Laboratories, Mumbai, India; Medium 199 from Sigma Aldrich; Minimum Essential Medium (MEM) from Thermo Fisher Scientific, Invitrogen Bioservices India Pvt. Ltd. and Roswell Park Memorial Institute-1640 (RPMI-1640) from Thermo Fisher Scientific. Antibiotic mixture prepared for Modified Diamond's medium at same concentration was added to each media.

5. Counting of *Trichomonas gallinae*

For *in vitro* culture, oral swabs were taken from oropharynx and crop after visual inspection of birds as pigeon and chicken with a sterilized cotton swab. For each bird, swab was inoculated individually into tubes containing *Trichomonas* selective culture medium i.e. Medium 199. The initial inoculation in the media was 1×10^3 cell/ml (estimation of the number of trophozoites in culture was made in Neubauer chamber and counted at 400X magnification). Only motile *T. gallinae* organisms were counted as high (+++), moderate (++) , less (+), weakly motile (+/-) and dead (-). After *in vitro* culture of the parasites, the concentration of the organisms increased many fold along with their motility and it became difficult to make live count, so Trypan blue (0.3%) dye was used to charge into the chamber for detection of parasites as the dead parasites took blue colour and it was easy to count the parasites. Firstly, the cultured samples were diluted in Trypan Blue dye by preparing a 1:1 dilution of the cell suspension using 0.3% solution of Trypan Blue. After adding the stain solution, the trophozoites of *T. gallinae* could be easily differentiated within a minute.

The cultured tubes were incubated at 37°C in BOD incubator for seven days in aerobic condition for giving the protozoan parasites sufficient time to multiply. Culture tubes were monitored every 24 hours post inoculation for 7 days for assessment of growth and motility of the parasites and were kept tightly capped after sampling. Positive culture materials were transferred into new fresh medium with antibiotics and passages continued every 48-72 hours till bacterial contamination was controlled.



RESULTS AND DISCUSSION

The result showing comparison of direct smear and culture methods for detection of *Trichomonas gallinae* infection is presented in Table-1. Out of 464 nos. of samples examined from pigeon, 330 nos. were positive (either method) with positivity of 71.12%. Only 87 samples were found positive by direct smear (Giemsa stained) and also confirmed by culture, with percentage of 18.75% while remaining 377 were negative by microscopy. *In vitro* culture done on these 377 microscopic negative samples showed positive result for *T. gallinae* in 243 no. while 134 samples were true negative by both microscopy and culture (28.87%). Culture method proved to be sensitive and superior in comparison to microscopic examination for detection of *T. gallinae* infection in pigeon.

In chicken, 304 samples were collected out of which 5 were microscopically positive (1.64%), remaining 299 samples were microscopic negative (98.35%) while 14(4.60%) were positive only by culture method. Overall, 19 samples were positive by either method with percentage of 6.25% while 285 (93.75%) were true parasite negative samples. Culture method detected more positive cases than microscopy.

Diagnosis of trichomonads is performed conventionally by microscopic observation of motile protozoa in wet mount preparation or stained smear. The sensitivity of detection in wet mount preparations is low, especially when the number of parasites in the host is marginal. Smears prepared from cultures and treated with different staining methods such as Giemsa, Gram, silver, iron-hematoxylin, Malachite green or methylene blue, Papanicolaou, acridine orange or other stains have proved to be the better method for diagnosis ([20,21]). However, this method is not used in routine examination because of laborious and expensive dilution of the original sample. Furthermore, they might result in false negative result if direct smears are made from birds harboring only low numbers of parasites ([21]). Inoculation of swabs into a suitable growth medium and their incubation at optimal temperature was shown to be helpful to enrich the number of trichomonads. Cultivation of trichomonads in growth medium for the detection of trichomonads has been the gold standard, easy to interpret and gives valid results, even in poorly infected birds and shown to be more sensitive than wet mount preparations as has been carried out by several investigators ([22, 21, 23]) thus agreeing to our reports.

Several other workers also used Giemsa stained method for identification of parasite ([24, 10, 25, 6]). Prevalence of *T. gallinae* from domestic pigeon was recorded in Kirkuk City, Iraq and recorded 49.26% prevalence based on wet mount preparation, different staining techniques (Giemsa, Gram stain and Fields stain) and four culture methods with Giemsa staining and In-pouch media being more efficient than wet mount preparation ([15]) similar to our reports.

Trichomonas gallinae can be cultured and maintained in a variety of media including liquid and semi liquid types as has been carried out by several workers viz. cysteine-peptone-liver infusion-maltose (CPLM) medium ([26]), simplified trypticase-serum (STS) medium ([27]), trypticase-yeast extract-maltose (TYM) medium ([18]), Diamond's medium ([28, 29]), in Pouch TF kits ([12, 23]), Hollander fluid ([29]), Medium 199 ([14]), *Trichomonas* CM0161 media ([15]) and modified thioglycolate medium ([16]). In our present study, five different media viz. Modified Diamond's medium, Medium 199, Minimum Essential Medium (MEM), Nutrient Broth, and RPMI-1640 were used for culture of *T. gallinae* and all the media supported the growth of the organisms.

CONCLUSION

From the present study it can be concluded that culture of *T. gallinae* has been proved to be superior to wet mount preparation or staining methods in detection of the flagellate.

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Table 1. Comparative Evaluation of Direct Smear and Culture Methods towards Detection of *T. gallinae* Infection

Method of examination	Pigeon Sample examined	Chicken Sample examined
Direct smear +ve	87/464	5/304
Direct smear -ve	377/464	299/304
Direct smear +ve, Culture +ve	87/464	5/304
Direct smear -ve, Culture +ve	243/377	14/299
Direct smear -ve, Culture -ve	134/464	285/304
Overall positive	330/464	19/304

REFERENCES

- Dey, A.R., Begum, N., Paul, S.C., Noor, M., and Islam, K.M. 2010. Prevalence and pathology of blood protozoa in pigeons reared at Mymensingh district, Bangladesh. *Int. J. Bio. Res.* 2 (12), 25-29.
- Jahan, N., Chandra, R., and Shoeb, M. 2011. Parasitomic load of haematozoan parasites in Rock pigeons (*Columba livia*). *Recent. Res. Sci. Tech.* 3(6), 9-11.
- Eljadar, M., Saad, W., and Elfadel, G. 2012. A study on the prevalence of endoparasites of domestic pigeons (*Columba livia domestica*) inhabiting in the Green Mountain Region of Libya. *J. American Sci.* 8 (12), 191-193
- Girard, Y.A., Rogers, K.H., Woods, L.W., Chouicha, N., Miller, W.A., and Johnson, C.K. 2014. Dualpathogen etiology of avian trichomonosis in a declining band-tailed pigeon population. *Infect. Genet. Evol.* 24:146-56
- Saleem, M., Khan, Ms. A.S., Chaudry, A., and Samad, H. 2008. Prevalence of trichomoniasis in domestic and wild pigeons and its effects on hematological parameters. *Pak. Vet. J.* 28(2), 89-91.
- Borji, H., Razmi, G. H., and Movassaghi, A. H. 2011. Prevalence and pathological lesion of *Trichomonas gallinae* in pigeons of Iran. *J. Parasit. Dis.* 35(2),186-189.
- Nematollahi, A., Ebrahimi, M., Ahmadi, A., and Himan, M. 2012. Prevalence of *Haemoproteus columbae* and *Trichomonas gallinae* in pigeons (*Columba livia domestica*) in Isfahan, Iran. *J. Parasit. Dis.* 36, 141-142.
- Saikia, M. 2016. Studies on protozoa infection of domestic pigeon (*Columba livia domestica*) in Assam. M.V.Sc. Thesis, Assam Agricultural University, Khanapara, Guwahati-22.
- Cauthen, G. E., and M. M. Harris. 1935. The cultivation of *Trichomonas columbae* free from bacteria. *Am. J. Hyg.* 22, 364-365.
- Stabler, R. M. 1954. *Trichomonas gallinae*: a review. *Exp. Parasitol.* 3, 368-402.
- Honigberg, B.M., Becker, R.D., Livingston, M.C., and McLure, M.T. 1964. The behaviour and pathogenicity of two strains of *Trichomonas gallinae* in cell cultures. *J. Protozool.* 447-465.
- Honigberg, B.M. 1978. Trichomonads of importance in human medicine. In: *Parasitic Protozoa*. Kreiger, J.P. (Ed), Academic Press, Inc., New York.
- DeCarli, G., and Tasca, T. 2002. *Trichomonas gallinae*: a possible contact-dependent mechanism in the haemolytic activity. *Vet. Parasitol.* 106, 277-283.
- Grabensteiner, E., Bilic, I., Kolbe, T., and Hess, M. 2010. Molecular analysis of clonal trichomonad isolates indicate the existence of heterogenic species present in different birds and within the same host. *Vet. Parasitol.* 172, 53-64.
- Hamad, S.S., Hazha, H., and Hassan, H. 2017. Isolation, Diagnosis and Cultivation of *Trichomonas gallinae* from Domestic Pigeon in Kirkuk City, Iraq. *Int. J. Curr. Res. Aca. Rev.* 5 (2), 10-18
- Raza, A., Qamar, M.F., Rubtsova, N., and Saneela, S. 2018. Pathogenicity and Diagnostic Sensitivity of Culture Media for Identification of *Trichomonas gallinae* in Domestic Pigeons of Lahore, Pakistan. *J. Protozool. Res.* 28, 11-21.
- Tasca, T., and De Carli, G.A. 2003. Scanning electron microscopy study of *Trichomonas gallinae*. *Vet Parasitol.* 118, 37-42.
- Diamond, L.S. 1957. The establishment of various *Trichomonas* of animals and man in axenic cultures. *J. Parasitol.* 43, 488-490.



19. Kharofa, W.A. 1999. An epidemiological study and cultivation of *Trichomonas vaginalis* in Mosul City. M.Sc. Thesis, College of Science, University of Mosul.
20. Borchardt, K.A., and Smith, R.F. 1991. An evaluation of an in-pouch TV culture method for diagnosis *Trichomonas vaginalis* infection, *Gentourin. Med.* **67**, 49-52.
21. Kaufman, R. H., Faro, S., and Brown, D. 2004. *Benign Diseases of the Vulva and Vagina*, 5th Edn., Mosby, Maryland Heights, MO, USA.
22. Cooper, J. E., and Petty, S. J. 1988. Trichomoniasis in free-living goshawks (*Accipiter gentilis gentilis*) from Great Britain. *J. Wildl. Dis.* **24**, 80-87.
23. Bunbury, N., Bell, D., Jones, C., Greenwood, A., and Hunter, P. 2005. Comparison of the InPouch TF culture system and wet-mount microscopy for diagnosis of *Trichomonas gallinae* infections in the pink pigeon *Columba mayeri*. *J. Clin. Microbiol.* **43**,1005–1006. doi: 10.1128/JCM.43.2.1005-1006.
24. Kietzmann, G.E. 1993. Relationship of *Trichomonas gallinae* to the palatal–esophageal junction of ring doves (*Streptopelia risoria*) as revealed by scanning electron microscopy. *J. Parasitol.* **79**, 408-415.
25. Coles, E.H. 1980. *Vet. Clinical Pathology*. Sounder’s Company, Philadelphia.
26. Johnson, G., and Trusseil, R. E. 1943. Experimental basis for the chemotherapy of *Trichomonas vaginalis* infestations. *Proc. Soc. Exp. Biol. Med.* **54**,245-249.
27. Kupferberg, A. B., Johnson, G., and Sprince. H. 1948. Nutritional requirements for *Trichomonas vaginalis*. *Proc. Soc. Exp. Biol. Med.* **67**, 304-308.
28. Mckeon, T., Dunsmore, J., and Raidal, S.R. 1997. *Trichomonas gallinae* in budgerigars and columbid birds in Perth, Western Australia. *Aust. Vet. J.* **75**, 652-655.
29. Amin, A., Neubauer, C., Liebhart, D., Grabensteiner, E., and Hess, M. 2010. Axenization and optimization of *in vitro* growth of clonal cultures of *Tetratrichomonas gallinarum* and *Trichomonas gallinae*. *Exp. Parasitol.* **124**, 202–208

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