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In vitro efficacy of drugs against *Trichomonas gallinae* in pigeon in Assam

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Abstract
A study was carried out in order to establish the *in vitro* efficacy of five different drugs against the flagellate protozoa, *Trichomonas gallinae*. The five drugs namely Flagyl 400 (Metronidazole), Ornida (Ornidazole), Tiniba 300 (Tinidazole), Sulcoprim (sulphadiazine and trimethoprim) and Vetfur-TL (Metronidazole, Furazolidone and Loperamide) were taken at concentration of 10, 20 and 30 mg/ml for the *in vitro* study. The results showed highest efficacy against the drugs Metronidazole followed by Ornidazole and combination of Metronidazole, Furazolidone and Loperamide.

Keywords: *Trichomonas gallinae*, pigeon, Assam, antiprotozoal drug, *in vitro* test

Introduction
Among all the domestic birds duck, chicken, geese, pigeon, quail, turkey etc. are more popular throughout the world. Poultry meat and eggs are among the most common animal protein source. Pigeon meat contains high protein and minerals that are beneficial for our body. 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 (Dey *et al*., 2010; Jahan *et al.*, 2011; Eljadar *et al*., 2012) [1, 2, 3]. Avian trichomoniasis is caused by *Trichomonas gallinae*, a flagellate protozoan parasite belonging to the class Zoomastigophorea and order Trichomonadida. The disease also known as canker, has significant health and economic impact on poultry industry, especially in pigeon and game birds rearing and breeding (Stockdale *et al*., 2015) [4] and is considered as a major factor for regulation and even decline of avian populations (Robinson *et al*., 2010) [5]. *T. gallinae* primarily infects the columbiformes birds (pigeon and mourning dove) although several other birds including the wild carnivorous ones may be infected (Girard *et al*., 2014) [6]. 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. Typical lesions include development of caseous masses (canker) in the upper digestive tract which in severe cases become completely blocked and cause death by starvation (Mesa *et al*., 1961) [7]. Surviving birds remain as carrier and become a source of infection for others. Variation in the pathogenic process depends on the virulence of the strains of the parasite (McDougald, 1992) [8]. The disease has been recognized as an emerging and potentially fatal disease of birds (Girard *et al*., 2014) [6]. Columbiformes could be treated with Metronidazole in drinking water (Rupiper and Ehrenberg, 1994) [9]. Several workers (Munoz *et al.*, 1998 [10]; Biswas *et al.* (2010) [11] performed *in vitro* susceptibility test on *T. gallinae* isolates using antiprotozoal drugs (carnidazole, tinidazole, dimetridazole, metronidazole, Secnidazole, ornidazole and ronidazole) at different concentrations with varying results. Youssefi *et al.* (2017) [12] evaluated the *in vitro* activity of *Trichomonas gallinae* using antitrichomonal drug metronidazole and essential concluded that metronidazole can eliminate the full parasite after 5 days of treatment at the dose rate of 50mg/kg. There is no such report of *in vitro* efficacy of commercially available drugs on pigeon trichomoniasis in India and limited knowledge on this subject prompted us to perform this study.
As regards to trichomoniasis in pigeons of Assam, no work had been carried out on *in vitro* effect of antiprotozoal drugs for treatment of canker. Hence, owing to the gaining importance of trichomoniasis in pigeons of Assam, the current study was designed to provide information on the use of effective drugs against *T. gallinae* infection.

Materials and Methods

The aim of the study was to assess the efficacy of different groups of drugs under *in vitro* condition against *T. gallinae* isolates obtained from domestic pigeon. The method used for the *in vitro* drug assay was that described by Munoz *et al*. (1998) [10] with slight modifications. For this, five different drugs namely Flagyl 400 (Metronidazole, Sanofi aventis), Ornida (Ornidazole, Aristo), Tiniba 300 (Tinidazole, Zydus Alidac), Sulcoprim (Sulphadiazine and Trimethoprim, Concept Pharmaceuticals Ltd.) and Vetfur-TL (Metronidazole, Furazolidone and Loperamide, Boehringer Ingelheim) at final concentration of 10, 20 and 30 mg/ml of culture medium was used. A volume of 100 µl of culture medium (Medium 199) containing 1×10^4 trophozoites was pipetted into each tube with prediluted drugs to give final concentrations of 10, 20 and 30mg/ml respectively and no drugs were used in the control tubes. All tubes were incubated at 37 ºC. After cultivation for 24 hours, the number of survived parasites were counted by haemocytometer and compared with control tubes and relative survival rate were used to evaluate the efficacy.

Viability assessment

Culture tubes kept at 37 °C were observed at 24, 48 and 72 hours post treatment (PT) using a light microscope to check live trophozoites by assessing their number and motility. Assessment was carried out in triplicate and the average value was taken for each concentration as well as the control and the percentage of mortality was recorded.

$$\text{Cell viability} = \frac{\text{Live cell count}}{\text{Total cell count}} \times 100\%$$

Results and Discussion

The results for the *in vitro* anti-trichomonad activity/efficacy of the drugs are shown in Table 1. Under *in vitro* condition, different commercial drugs Metronidazole, Ornidazole and combination of Metronidazole, Furazolidone and Loperamide showed the highest efficacy (100%) at different time intervals. Before treatment the concentration of trophozoite inoculum was 100 cell/ml for all five drugs.

The efficacy of Metronidazole @10 mg/ml after 24, 48 and 72 hours of incubation were 65%, 74% and 85% respectively; @20 mg/ml the respective efficacy percentage was 72%. 87% and 91% while @ 30mg/ml concentration, the drug efficacy after 24 hours was 95% and it was 100% both after 48 and 72 hours. Examination of culture resulted in no viable trophozoite after 48 hours and mortality of trophozoites was confirmed by the lack of resumption of growth in the subsequent 48 hours cultures. Drug Ornidazole @ 10 mg/ml showed efficacy of 59% at 24 hours, 71% at 48 hours and 83% at 72 hours while the corresponding percentage @ 20mg/ml was 68%, 79% and 90% after 24, 48 and 72 hours respectively of counting of live trophozoites in the culture. Ornidazole was 100% effective on *T. gallinae* culture @ 30 mg/ml after 72 hours of incubation followed by 97% at 48 hours and 92% after 24 hours. In case of Tinidazole, the drug @ 10mg/ml showed least efficacy with 56%, 67% and 79% death of trophozoites after 24, 48 and 72 hours of incubation while @ 20mg/ml, 64% efficacy was observed after 24 hours, 76% after 48 hours and 81% after 72 hours. However, Tinidazole @ 30mg/ml concentration showed efficacy and trophozoite reduction percentage of 82%, 90% and 94% at the end of 24, 48 and 72 hours respectively. Combination of Sulphadiazine and Trimethoprim was least effective in *in vitro* @ 10mg/ml with only 68%, 55% and 44% reduction at 24, 48 and 72 hours respectively. With 20mg/ml drug concentration, 81% efficacy was recorded in *in vitro* culture after 72 hours incubation followed by 70% at 48 hours and only 59% at 24 hours interval while @ 30mg/ml, highest drug efficacy was observed after 72 hours (86%) followed by 81% at 48 hours interval and 73% after 24 hours incubation of the treated culture. Metronidazole, Furazolidone and Loperamide showed efficacy of 61% at 24 hrs, 70% at 48 hrs and 78% at 72 hours @10mg/ml. At concentration of 20 mg/ml, the trophozoite reduction percentage at the end of 24, 48 and 72 hours was 72%, 80% and 89% while @ 30 mg/ml, the drug was 100% effective in killing the trophozoites *in vitro* after 72 hours followed by 95% efficacy at 48 hours observation and 87% at the end of 24 hours. From the *in vitro* drug experiment, it can be concluded that Metronidazole was the most effective among the five drugs followed by Ornidazole and combination of Metronidazole, Furazolidone and Loperamide @ 30 mg/ml while Tinidazole and combination of Sulphadiazine and Trimethoprim was the least effective drugs. Among the three doses, 10 mg/ml and 20 mg/ml was unable to show 100% efficacy in any drug and time interval. Hence, Metronidazole @ 30 mg/ml can be considered as highly effective in causing 100% mortality of *T. gallinae* trophozoites at *in vitro* condition. In the control tubes, there was tremendous growth of *T. gallinae* trophozoite even after 72 hours of incubation of the culture.

The present study suggests that metronidazole is the most effective drugs tested against pigeon trichomoniasis and also cost effective. Our findings are supported by the reports of some other scientists in different parts of the world. Munoz *et al.* (1998) [10] performed *in vitro* susceptibility of five nitroimidazolic drugs (carnidazole, dimetridazole, metronidazole, ornidazole and ronidazole) and tested on four *T. gallinae* isolates in Barcelona, Spain and showed greater potency of ornidazole than the other drugs. Franssen and Lumeij (1992) [13] also proved several nitroimidazoles including metronidazole, dimetridazole, ronidazole and carnidazole as effective drugs against *T. gallinae* under *in vitro* condition. Biswas *et al.* (2010) [11] evaluated the efficacy of five commercial antiprotozoal drugs in Bangladesh and reported 100% efficacy of Metronidazole, Ornidazole and Secnidazole @ 30 mg/kg body wt. whereas Nitozoxanide @30mg/kg body wt. had the lowest efficacy (84.61%) in *in vitro* condition. In China, Luo *et al.* (2006) [14] tested the efficacy of common antitrichomonal drugs against *T. gallinae* from pigeon and observed that dimetridazole and tinidazole were able to inhibit and control *T. gallinae* under *in vitro* condition.

In avian veterinary medicine, several nitroimidazoles including metronidazole, dimetridazole, ronidazole and carnidazole, have been considered effective drugs against *T. gallinae* (Franssen and Lumeij, 1992) [13]. The only works known on nitroimidazoles are that of Lumeij and Zwijnenberg (1990) [15] and Inghelbrecht *et al.* (1996) [16] carried out in pigeons, and of Franssen and Lumeij (1992) [13] dealing with the *in vitro* susceptibility of this parasite. Contrary to our findings, Meingassner and Thurner (1979) [17] documented that resistance in trichomonads was always higher to metronidazole than to other nitroimidazoles. Youssefi *et al.* (2017) [12] evaluated the *in vitro* activity of metronidazole and essential oil on *Trichomonas gallinae* and concluded that EO

can eliminate the full parasite after 4 days of treatment @ 50mg/kg and for metronidazole at the same dose rate, it was 5 days.

Table 1: *In vitro* Effects of Anti Trichomonal Activity of different drugs against *Trichomonas gallinae*

Name of drugs	Doses (mg/ml)	Pre-treated live *T. gallinae* counted	Post-treated live *T. gallinae* counted			% Efficacy of drug		
			24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
Metronidazole	10	100	35	26	15	65	74	85
	20	100	28	13	9	72	87	91
	30	100	5	0	0	95	100	100
Ornidazole	10	100	41	29	17	59	71	83
	20	100	32	21	10	68	79	90
	30	100	8	3	0	92	97	100
Tinidazole	10	100	44	33	21	56	67	79
	20	100	36	24	19	64	76	81
	30	100	18	10	6	82	90	94
Sulphadiazine & Trimethoprim	10	100	56	45	32	44	55	68
	20	100	41	30	19	59	70	81
	30	100	27	19	14	73	81	86
Metronidazole, Furazolidone & Loperamide	10	100	39	30	22	61	70	78
	20	100	28	20	11	72	80	89
	30	100	13	5	0	87	95	100
Control	-	100	132	169	143	0	0	0

Conclusion

The present work on *in vitro* efficacy of five drugs on *T. gallinae* of pigeons in Assam is the first report from this part of the country. Metronidazole was the most effective among the five drugs tested against pigeon trichomoniasis and also cost effective.

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References

1. Dey AR, Begum N, Paul SC, Noor M, Islam KM. Prevalence and pathology of blood protozoa in pigeons reared at Mymensingh district, Bangladesh. International Journal of Biological Research 2010;2(12):25-29.
2. Jahan N, Chandra R, Shoeb M. Parasitimic load of haematozoan parasites in Rock pigeons (*Columba livia*). Recent Research in Science and Technology 2011;3(6):9-11.
3. Eljadar M, Saad W, Elfadel G. A study on the prevalence of endoparasites of domestic pigeons (*Columba livia domestica*) inhabiting in the Green Mountain Region of Libya. Journal of American Science 2012;8(12):191-193.
4. Stockdale JE, Dunn JC, Goodman SJ, Morris AJ, Sheehan DK, Grice PV *et al*. The protozoan parasite *Trichomonas gallinae* causes adult and nestling mortality in a declining population of European Turtle Doves, *Streptopelia turtur*. Parasitology 2015;142:490-498.
5. Robinson RA, Lawson B, Toms MP, Peck KM, Kirkwood JK, Chantrey J. Emerging infectious disease leads to rapid population declines of common British birds. PLoS ONE 2010;5:122-125.
6. Girard YA, Rogers KH, Woods LW, Chouicha N, Miller WA, Johnson CK. Dualpathogen etiology of avian trichomonosis in a declining band-tailed pigeon population. Infection, Genetics and Evolution 2014;24:146-56
7. Mesa CP, Stabler RM, Berthrong M. Histopathological changes in the domestic pigeon infected with *Trichomonas gallinae* Jones' barn strain. Avian Diseases 1961;5:48-60.
8. McDougald LR. Other protozoan diseases in the intestinal tract, In: *Diseases of Poultry*. Hofstad, M.S. (Ed.), 8th Edition, Panima Educational Book Agency, New Delhi, India, 1992, 723-725.
9. Rupiper DJ, Ehrenberg M. Introduction to pigeon practice. Proceedings of Annual Conference Association of Avian Veterinarians, 1994, 203-211.
10. Munoz E, Castella J, Gutierrez JF. *In vivo* and *in vitro* sensitivity of *Trichomonas gallinae* to some nitroimidazole drugs. Veterinary Parasitology 1998;78:239-246.
11. Biswas PG, Begum N, Mamun MAA, Bari MA, Mondal MMH. Efficacy of herbal and commercially available antiprotozoal drugs on pigeon trichomoniasis. International Journal of Biological Research 2010;2(11):29-33.