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In vivo Efficacy of Drugs against *Trichomonas gallinae* in Pigeons of Assam

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A B S T R A C T

A study was carried out in order to establish the *in vivo* efficacy of five different drugs against the flagellate *Trichomonas gallinae*.The drugs included Flagyl 400 (Metronidazole), Ornida (Ornidazole), Tiniba 300 (Tinidazole), Sulcoprim (sulphadiazine and trimethoprim) and Vetfur-TL (Metronidazole, Furazolidone and Loperamide) at the concentration of 20 and 30 mg/kg which revealed highest efficacy of Metronidazole, Ornidazole and combination of Metronidazole, Furazolidone and Loperamide against *T. gallinae* in naturally infected and experimentally induced pigeons.

Introduction

Birds have been domesticated by human from the ancient time to provide supplementary income to most of the landless families. The world has over 23 billion poultry population which counts at about three per person on the planet (FAOSTAT, 2016) and about 5 times more than that of 50 years ago. They are raised in a wide range of production systems to provide mainly meat and egg (protein source) and also manure for crop fertilization. Among the various types of parasites, digestive tract protozoan parasites mainly coccidia and *Trichomonas* are known to be harmful to domestic birds in different countries including India (Dey *et al.*, 2010; Eljadar *et al.*, 2012). 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). *T. gallinae* primarily infects the columbiformes birds (pigeon and mourning dove) although several other birds and wild carnivores may be infected (Girard *et al.*, 2014). Typical lesions include development of caseous masses (canker) in the upper digestive tract which in severe cases become completely blocked to cause death by starvation (Mesa *et al.*, 1961). Surviving birds remain as carrier and become a source of infection for others. The disease has been recognized as an emerging and potentially fatal disease of birds (Robinson *et al.*, 2010; Girard *et al.*,

2014). Different antiprotozoal drugs (Metronidazole, Carnidazole, Ronidazole, Tinidazole, Ornidazole, Secnidazole, Nitazoxanide etc.) have been used for treatment of canker in columbiformes, mainlypigeons by several workers abroad (Rupiper and Ehrenberg, 1994; Munoz *et al.*,1998; Biswas *et al.*, 2010). Nagel and Aronoff (2015) considered Nitroimidazole drugs as the first choice for the treatment of avian trichomonosis. Under *in vivo* conditions, the drugs were in general administered to the birds at different concentrations either in drinking water for 5 to 7 days or orally as single dose tablet. Hawkins *et al.,* (2013) reported that for Columbiformes, Dimetridazole, Carnidazole or Ronidazole has to be employed in drinking water, food or by direct administration to the bird for treatment of trichomonosis. In most of the cases, the *T. gallinae* organisms were completely eliminated by 7^{th} day of treatment. Metronidazole was considered to be the most effective drug by many workers under *in vivo* conditions in terms of improvement of lesions and complete elimination of the organisms (Munoz *et al.*, 1998; Biswas *et al.*, 2010). Trichomoniasis is a common problem in pigeons in Assam including India and since no detailed study on treatment aspect was done earlier, the present work of evaluating the *in vivo* efficacy of drugs against *T. gallinae* was carried out.

Materials and Methods

Five 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) were included for *in vivo*efficacy study in pigeons which were either naturally or experimentally infected with *T. gallinae*. A total of 44 pigeons were made available for this experiment. All birds were housed in wire netted iron cages and supplied with feed and water *ad libitum*. Out of the 44 birds, 22 were infected by oral inoculation of *T. gallinae* (4×10^4 parasites). The remaining birds were found naturally infected and included to the experimentation. Birds were divided into 6 groups namely group I, II, III, IV, V and VI with the former 5 groups consisting of 8 infected birds and the group VI consisted of 4 birds. Birds of 5 groups (group I-V) were treated with Metronidazole, Ornidazole, Tinidazole, Sulphadiazine and trimethoprim and Metronidazole, Furazolidone and Loperamide respectively at dose rate of 20mg/kg body wt. orally for 5 consecutive days to 4 birds of each group and @ 30 mg/kg for the same duration in other 4 birds of each group.

Aqueous suspension of the drugs was prepared at first as stock suspension. Birds of group VI remained as infected but untreated control. Birds were observed for 6 days after administration of last dose of drugs.

Efficacy assessment was done by monitoring the birds for their appetite to feed and water, persistence or disappearance of oropharyngeal lesions and grading of oral lesion was done as severe/more (+++), moderate (++), mild/less (+) and absent (-).

Estimation of parasite concentration in the throat swabs at 24 hours interval was done for confirmation on the growth of parasites, if any. Throat swab for parasite was counted as high (+++), medium (++), less (+) and absent (-).

Results and Discussion

The *in vivo* assay demonstrated the effectiveness of two doses (20mg/kg and 30mg/kg) of metronidazole, ornidazole, tinidazole, combination of sulphadiazine and trimethoprim and combination of Metronidazole, Furazolidone and Loperamide administered orally to the pigeons (22 naturally infected with *Trichomonas gallinae* and another 22 experimentally induced).

Five days post inoculation of *T. gallinae* in experimental birds (day 0) and before initiation of treatment, number of trophozoites recovered from crop of birds was counted. *In vivo* efficacy was counted on the basis of grading of oral lesion and

throat swab examination. The results for the *in vivo* efficacy of the drugs are shown in Table 1 and Fig.1. With Metronidazole @ 20mg/kg, 3 out of 4 infected pigeons recovered fully with drug efficacy of 75% while @ 30mg/kg, the drug was successful in eliminating 100% infection with decrease of concentration of *T. gallinae* trophozoites.

There was total disappearance of organisms in throat swab from 3rd day of treatment and improvement of gross oral lesions and general health from 4th day onwards. Metronidazole @ 30 mg/kg on the 1st and 2nd day after the treatment resulted in significant reduction in number of *T. gallinae* by throat swab examination in comparison to all other groups of drugs. In the 3rd, 4th and 5th day, no motile trophozoite were recovered. This drug led to full recovery of infected pigeons.

Similar results were obtained with Ornidazole, with 100% drug efficacy @ 30mg/kg and disappearance of gross lesions and trophozoites of *T. gallinae* from 4th day of treatment.

However, @ 20 mg/kg, there was 75% recovery of infected squabs with persistence of both oral lesions on gross and trichomonads on throat swab examination. Pigeons treated with Ornidazole @ 30 mg/kg showed full recovery on the 5th day reach. No mortality was recorded for treatment groups and no clinical side effects were observed in treated birds.

Combination of Metronidazole, Furazolidone and Loperamide gave similar results as obtained for Metronidazole. At dose of 30mg/kg, 100% drug efficacy was recorded along with complete disappearance of organisms from 3rd day of treatment of *T. gallinae* infection whereas @ 20mg/kg, 75% efficacy of the drug was seen.

This combination drug @ 30 mg/kg was able to cause full recovery of infected pigeons and in throat swab examination no parasites were detected from 3rd day onwards. Tinidazole @20 mg/kg revealed 50% drug efficacy with persistence of infection up to 5th day of treatment whereas @30mg/kg the efficacy percentage was 75%. In both the doses, trophozoites of *T. gallinae* and associated oral lesions could be observed till 5th day of treatment indicating that this drug was less effective in comparison to Metronidazole, Ornidazole and combination of Metronidazole, Furazolidone and Loperamide. In case of tinidazole, only 3 nos. of birds recovered after treatment and parasite was observed till 5th day of throat swab examination.

Sulphadiazine and Trimethoprim was the least effective among the five drugs used in the *in vivo* efficacy test as it failed to eliminate the infection of *T. gallinae* in pigeons with persistence of organisms and lesions up to 5th day after treatment. The efficacy of this drug @30mg/kg was 50% while it was only 25% @ 20mg/kg.

Moreover, the number of trophozoites along with associated gross lesions increased/ remained same throughout the observation period @ 20mg/kg. Combination of Sulphadiazine and Trimethoprim was able to eliminate the infection in only one bird out of four nos. of bird.

In order of efficacy it was observed that drug Metronidazole, Ornidazole and combination of Metronidazole, Furazolidone and Loperamide were equally effective (100%) followed by Tinidazole (75%) and Sulphadiazine and Trimethoprim (50%) @ 30mg/kg, whereas @ 20mg/kg; Metronidazole, Ornidazole and combination of Metronidazole, Furazolidone and Loperamide showed 75% efficacy in eliminating the *T. gallinae* infection followed by Tinidazole (50%) and Sulphadiazine and Trimethoprim (25%). There was clinical improvement of the recovered birds along with body condition, feed intake, activity etc.

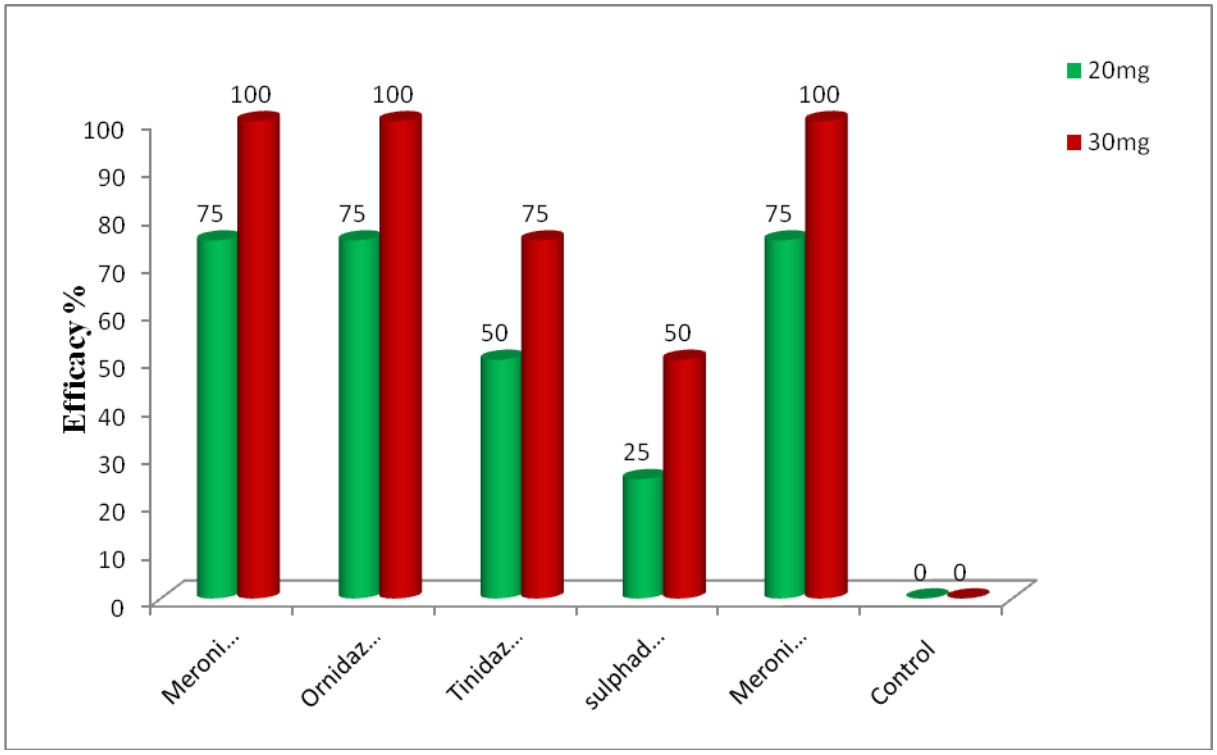
There are several reports to show the efficacy of Metronidazole in eliminating/reducing *T. gallinae* infection as has been observed in our study. There is no literature available on *in vivo* drug efficacy against *T. gallinae* in India and ours was an attempt to carry out the work in small group of birds using five different drugs.

Table.1 *In vivo* Efficacy of Drugs against *T. gallinae* in Naturally Infected and Experimentally Induced Birds

Name of drugs	Doses (mg/kg)	No. of pigeons treated	Throat swab result after treatment					Grading of oral lesion					No. of *T. gallinae* Infected pigeon	Drug efficacy %
			Day1	Day2	Day3	Day4	Day5	Day1	Day2	Day3	Day4	Day 5		
Metronidazole	20	4	+++	+++	+	+	-	+++	+++	+++	++	+	1	75%
	30	4	++	+	-	-	-	+++	++	+	-	-	0	100%
Ornidazole	20	4	+++	++	++	++	-	+++	+++	++	++	++	1	75%
	30	4	+++	++	+	-	-	+++	++	++	-	-	0	100%
Tinidazole	20	4	+++	+++	++	++	+	+++	+++	+++	+++	++	2	50%
	30	4	+++	++	+	+	+	+++	+++	++	+	+	1	75%
Sulphadiazine and Trimethoprim	20	4	+++	+++	++	++	++	+++	+++	+++	+++	+++	3	25%
	30	4	+++	+++	++	++	+	+++	+++	+++	++	++	2	50%
Metronidazole, Furazolidone and Loperamide	20	4	+++	+++	++	+	-	+++	+++	+++	++	++	1	75%
	30	4	++	+	-	-	-	+++	++	+	-	-	0	100%
Control (4 nos.)	-												4	0%

Throat swab for parasite: +++High no., ++Medium no., + Less, - Absent
Grading of oral lesion: +++Severe/more ++ Moderate +Mild/Less, - Absent

Fig.1 *In vivo* Efficacy of Drugs against *T. gallinae* in Naturally Infected And Experimentally Induced Birds



Mohamed *et al.,* (2009) experimentally produced *T. gallinae* infection in 20 apparently healthy squabs in Sharkia governorate orally by inoculation of 1 ml Glucose serum broth media containing 800–1000 parasite and treated with Flagyl (Metronidazole) @ 1 gm/liter drinking water for five successive days. The treated squabs against trichomoniasis with Flagyl led to improvement in the clinical signs and general condition of the birds with decreased mortality rate within few days after the beginning of the treatment similar to our present findings.

Bussieras *et al.,* (1961) reported that metronidazole, which has been used successfully against *T. vaginalis*, is also effective against *T. gallinae*. According to Rupiper and Ehrenberg (1994), Columbiformes could be treated with Metronidazole @ 50 mg/kg PO every 12 hours for 5 days or with 10.57 mg/ml in drinking water. Abd El-Rahman *et al.,* (2008) also carried out a treatment trial in 15 naturally infected pigeons with *T. gallinae* and treated with metronidazole (Flagyl®) @ 100 mg/pigeon in drinking water for 7 successive days. The population density of *T. gallinae* gradually decreased reaching the lowest degree (+) starting from the 4th day of treatment till the 6th day and the parasite completely disappeared on the 7th day of treatment which agrees to our present observation. McLoughlin (1965) used white Carneau pigeons and common pigeons having both naturally acquired asymptomatic infections of *T. gallinae* and also experimentally infected with a virulent local strain of *T. gallinae* in U.K. Metronidazole @ 50mg/kg body wt. was administered by pipetting 1 ml of an aqueous suspension into the crop of one group of birds and for the remaining birds, a 0.05% aqueous solution of dimetridazole was the sole source of drinking water during the treatment period. In the experiments, trichomonads were not recovered from any bird given metronidazole or dimetridazole while none of the control birds eliminated the infection during the experimental period. The results confirmed the effectiveness of metronidazole and closely related compound dimetridazole against *T. gallinae*. Contrary to this finding, Munoz *et al.,* (1998) in their *in vivo* sensitivity test of carnidazole

(10mg tab) orally as single dose and dimetridazole (0.18g/l) in drinking water for 5 consecutive days against *Trichomonas gallinae* infected racing pigeon flock in Barcelona, Spain failed to eliminate infection in 13 of 17 and 20 of 21 pigeons respectively.

In vivo drug efficacy test against *T. gallinae* carried out for the first time in pigeon in Assam and perhaps in India using five drugs showed highest efficacy of Metronidazole, Ornidazole and combination of Metronidazole, Furazolidone and Loperamide followed by Tinidazole and Sulphadiazine and Trimethoprim at both the concentrations taken along with clinical improvement, body condition, feed intake, general activity etc. of the recovered birds. Hence the drugs under the present study can be suggested for treatment of canker in pigeon.

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