

## SOME STUDIES ON TRICHOMONAS GALLINAE INFECTION IN PIGEONS

(with 2 Fig. & 4 Tables)

By

T.Y. ABD-EL-MOTELIB and BAHY EL-GAMAL GALAL\*

(Received at 4/10/1993)

### بعض الدراسات عن الاصابة بطفيل التريكوموناس جاليني في الحمام

طلبه غبط المطلب ، باهى الجمال جلال

تم تجميع ٢٨٨ عينة من حمام ذات أعمار مختلفة من مصادر مختلفة من محافظة أسيوط شاملة أنظمه مختلفة من التربية في دراسة لطفيل التريكوموناس جاليني وبالفحص المباشر لعينات الحوصلة بواسطة الميكروسكوب وكذلك الانماء على المستنبت الصناعي أتضح أن الاصابة ٢٣ ٪ في زغاليل الحمام ٢٠ ٪ ، في الحمام حديث العمر ، ٥٧ ٪ في الحمام البالغ .  
تمت دراسة الاعراض الاكلينيكية والافات التشريحية من حمام ذات أعمار مختلفة والمصاب طبيعياً بطفيل التريكوموناس جاليني .

بأجراء العدوى الصناعية لزغاليل حديثة الفقس بطفيل التريكوموناس جاليني المعزول من حالات ذات آفات تشريحية شديدة كانت نسبة الوفاء ٣٠ ٪ وكانت الأعراض والافات التشريحية تشبه إلى حد كبير تلك التي شوهدت في العدوى الطبيعية لزغاليل شديدة الاصابة .

بأجراء اختبار الحساسية في المعمل أتضح أن الفلاجيل ، والفاسيجين ذو فاعلية ممتازة في القضاء على طفيل التريكوموناس جاليني بنسبة ١٠٠ ٪ بينما أظهر الاكريفلافين كفاءة ضئيلة بلغت ٢٥ ٪ .

بأجراء اختبار الحساسية على الطيور الحية المصابة صناعياً بطفيل التريكوموناس جاليني أثبت الفلاجيل والفاسيجين كفاءتهما العلاجية الممتازة أما الاكريفلافين فكان ذو كفاءة علاجية ضئيلة وقد كانت النسب متطابقة تماماً مع النسب المعملية .

\*: Animal Health Research Institute Assiut.

## SUMMARY

Random samples of 95 pigeon squabs, 87 youngsters and 106 adult living pigeons were collected from different sources in Assiut province including domestic and racing pigeons of both individually housed and loft pigeons. Examination of crop samples directly under microscope and cultural trial on artificial media revealed that the infestation with *trichomonas gallinae* was 24.2% in pigeon squabs, 40.2% in youngsters and 57.5% in adult pigeons. The clinical signs and post mortem lesions of naturally infested pigeon squabs, youngsters and adult pigeons were studied. Experimental transmission of the disease to pigeon squabs by feeding of the cultured trichomonads showed that all of the squabs became infested. The result of in vitro sensitivity test revealed that the parasite was very sensitive (100%) to both Flagyl and Fasigyn but less sensitive to acriflavin (25%). The in vivo sensitivity test showed that both Flagyl and Fasigyn were of high efficacy in treatment of all squabs experimentally infested with *Trichomonas gallinae*, while acriflavin was of low therapeutic effect.

## INTRODUCTION

Very little attention was directed toward diseases of pigeons and this may be due to the majority of these birds were unconfined as they usually wandered over a long distance scratching for food and also the population of pigeons is few if compared with chickens that reared intensively. Many species of birds have been found to harbour *trichomonas gallinae* (*T. gallinae*) as chickens, doves, budgerigars, turkeys, quails, canaries, sparrows and birds of prey. Trichomoniasis is a serious cause of mortality in squabs and representing one of the most popular problems facing pigeons (NIAK, 1968; ISMAIL *et al.*, 1979; VOGEL, 1983; Narcisi *et al.*, 1991). Most deaths from this parasite are attributable to oesophageal obstruction by caseous lesions (STABLER, 1954; JESSUP, 1980; BAKER, 1986, and COOPER and PETTY, 1988) Different strains of the organism vary greatly in pathogenicity (WHITEMAN and BICKFORD, 1989). In adult pigeons, low mortality rate have been noticed and the disease is observed only sporadically and with a variable



degree of involvement. The debilitating effect of this disease has a drastic effect on body weight and reduces the value of the birds for flying and display. In Egypt, individual attempts concerning sporadic affection with pigeon trichomoniasis were tried by SOLIMAN (1960); AHMED and EL-SISI (1966) and SHIHATA (1978). A significant percentage of mortalities was occurred in young pigeons especially during first few weeks after hatching that were sent for poultry Diseases Department, Fac. of Vet. Med., Assiut University. Examination of wet preparations from crops of live or freshly dead squabs revealed the presence of elongated, pear shaped, flagellated organisms, therefore the attention was directed to study the followings:

- Incidence of *T.gallinae* in pigeons of different ages.
- Trials for propagation of *T.gallinae* on artificial media.
- Studying the pathogenicity of *T.gallinae* in newly hatched pigeon squabs.
- In vitro and in vivo sensitivity of many drugs against *T.gallinae*.

#### MATERIAL and METHODS

##### Specimens:

Crop samples were obtained from 95 squabs, 87 youngsters and 106 adult pigeons collected randomly from individually housed pigeons and several lofts distributed at different localities in Assiut province including domestic and racing pigeons. Five ml of physiological saline solution were introduced into the crop by a bulbed dose pipette, mixed with the contents and resyphoned. Wet film preparations of the washings were examined directly under the microscope, 1 ml of the washings was also inoculated into two tubes of Clausen's glucose broth serum medium (MAFF, 1971) to which 3000 i.u penicillin and 5000 ug streptomycin/ml were added in order to control bacterial growth. Inoculated tubes were incubated at 37°C and one drop from the bottom of each tube was microscopically examined after 4 days and again on 7<sup>th</sup> day for the presence of trichomonads. Positive tubes were subcultured into new tubes of media, incubated for 3 days and checked for growth of the parasite.

##### Blood film:

Blood film was prepared from each bird and examined for the presence of blood parasites.



### Faecal examination:

Faecal materials were examined directly under ordinary microscope for intestinal coccidiosis.

### Pathogenicity test:

The number of pigeon squabs used in this experiment was 20, 10 of which were selected for studying the pathogenicity of *T.gallinae*. Each squab was fed 1 ml of trichomonas cultures ( $2 \times 10^6$  viable trichomonads) by pipette. The pipette was introduced into the oesophagus far enough so that the material would enter the crop but not go directly into the gizzard. The other 10 birds were left as uninfected control. All birds proved free from pathogenic organisms before experiment. During experiment the clinical signs and mortalities were noticed and the dead birds were necropsied for recording the lesions. One month after feeding with *Trichomonas* cultures, all survivors were necropsied for evidence of infection.

### In vitro sensitivity tests:

Four sets, of 8 tubes each, of the cultured tubes with *T.gallinae* were used in this experiments. The first three sets were used for in vitro sensitivity testing of the parasite to Flagyl (Alexandria Pharm Co.) Fasigyn (Pfizer) and a prepared solution of 5% Acriflavin, respectively. The fourth set was left as untreated control. All tubes, after the addition of the antiparasitic drugs, were incubated at  $37^\circ\text{C}$  and they were examined after 24, 48 and 72 hours for the presence of alive motile parasites. The sensitivity to the drugs was evaluated on the basis of the number of the motile parasites in 10 microscopical field before and after the addition of the drug.

### In vivo sensitivity tests:

Pigeon squabs used in the present study were infested with *T.gallinae* cultures ( $2 \times 10^6$  viable trichomonads/squab) after they proved free from natural infection. Four sets of 4 pigeons each were used for testing the efficacy of Flagyl, Fasigyn and Acriflavin for treatment of the parasitic infestation. The first set of pigeons was given 1 ml/bird of Flagyl suspension (50 mg/ml) directly into the crop once daily for 3 successive days. The second and third sets of pigeons were given 1 ml/bird of Fasigyn suspension (50mg/ml) and 1 ml/ bird of 5% Acriflavin solution, respectively once daily for 3 successive days. The fourth set was left as untreated control. Samples were collected directly from the crop by aspiration and microscopically examined 3, 6 and 10 days after the third dose of treatment.



## RESULTS

A total of 23 out of 95, 35 out of 87 and 61 out of 106 examined squabs, youngsters and adult pigeons respectively were positive for *T. gallinae* as illustrated in Table (1). *T. gallinae* was successfully cultured and maintained on Clausen's glucose broth serum medium. The trichomonad have a pear shape body, moved actively with characteristic spiral, jerky movement. Examination of fixed and stained (Giemsa-stain) preparation, revealed the presence of four anterior free flagella and marginal flagellum with undulating membrane Fig. (1). Squabs severely infested with *T. gallinae* showed difficulty in closing their mouth because of lesion in the oral cavity, depression, inappetance, drooling of watery foetid discharge from the mouth, faecal material was liquid with the feathers around the anus in some cases being soiled. Watery discharge from conjunctival sacs was observed in five birds. The squabs were also underdeveloped and poorly feathered. yellow colour could be noticed in the skin of the throat region in two squabs. The necropsy revealed, in majority of infested birds, a caseous materials varying in size in the mouth, oesophagus, crop and only in four cases in the proventriculus. These lesions occluded the entrance of the oesophagus in some cases. Superficial erosions or even ulcer formation was observed in crop of two cases. The clinical signs and necropsy findings of youngsters were more or less similar to that reported in squabs but of milder type. Most infested adult pigeons showed no signs or lesions of the disease but were severely emaciated and in few cases, drips of foetid odour fluid from the buccal cavity was observed. Examination of Giemsa's stained blood films and faecal materials obtained from infested pigeons with trichomoniasis revealed the presence of *Haemoproteus columbae* in 17 cases and *Eimeria* species in 18 cases.

Pathogenicity test of pigeon squabs resulted in death of 3 out of 10 squabs, 8 to 14 days post infection as shown in Table (2). The clinical signs of infested pigeon squabs included depression, inappetance, drooling of watery fluid from buccal cavity. Severe emaciation was observed after 2 weeks of infestation. In 7 out of 10 infested pigeon squabs pin point caseous materials were present in the buccal cavity and in 3 cases in the crop. Extensive caseous clumps occluded the entrance of the oesophagus was observed in 3 of them (Fig.2). Yellowish fluid exudate was abundant in the crop of all infested squabs in which heavy trichomonads were present.



Neither lesions nor trichomonads were present in squabs of the control group. This experiment indicated that newly hatched pigeon squabs are very sensitive to infection with cultures of trichomonas previously isolated from lesions in the upper digestive tract of pigeons.

The in vitro sensitivity test revealed that *T. gallinae* was highly sensitive (100%) to Flagyl and Fasigyn and this has evidenced by no trichomonads were found on microscopic examinations of the inoculated tubes with the protozoal parasite. On the other hand, *T. gallinae* was proved to be less sensitive (25%) to acriflavin (Table 3). Control tubes remained positive throughout the experiment.

The in vivo sensitivity test revealed that all pigeons treated with Flagyl in the first experiment cured by examination of crop samples at 3, 6 and 10 days after third dose of treatment. The same result was observed in the second experiment of Fasigyn treated pigeons while only 25% were cured in the third experiment of pigeons treated with Acriflavin (table 4). Control squabs remained positive throughout the experiment.

#### DISCUSSION

Trichomoniasis is frequently encountered in pigeons predominantly causing caseous accumulation in the crop, oesophagus and oral mucosa. The severe morbidity and mortality in young pigeons may be attributed either to a highly pathogenic strains of *T. gallinae* or to a highly susceptible population of young squabs. The lethal effect of severe trichomoniasis was only observed in squabs (STABLER, 1954; JESSUP, 1980; BAKER, 1986; COOPER and Petty, 1988; KUCERA *et al.*, 1988, LUMEIJ and Zwiijnenberg, 1990). The natural infection reported in the present study has the clinical signs and upper digestive tract lesions similar to those reported by TREES, 1990; CHARLTON *et al.*, 1991 and NARCISI *et al.*, 1991.

In the present paper, the positive percentage in adult pigeons (57.5%) was higher than that reported in youngsters (40.2%) or pigeon squabs (24.2%). This may be due to the severely infested pigeon squabs are oftenly die during the first few weeks after hatching. This finding coincided with the previous report of ISMAIL *et al.*, (1979). The successful trial in cultivation and maintaining of the protozoal parasite on artificial media is considered an encouraged step for preparation of vaccine.

Experimental infestation of healthy pigeon squabs proved the pathogenic nature of the isolated protozoal parasite and



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mortality rate of 30% was observed after oral infestation. The death of squabs may be due to oesophageal obstruction by caseous materials. The observed clinical signs and gross lesions in diseased and dead infested squabs resembled to some extent those mentioned by *NARICISI et al.* (1991). No yellow discolouration of throat region skin that observed in naturally diseased squabs, was occurred among experimentally infested birds. Detection of the protozoal parasite from diseased and freshly dead infested squabs was successfully obtained from examined crops. The birds of the control group remained negative throughout the experiment. It could be concluded that pigeon squabs are highly susceptible to *T.gallinae* infestation. The parasite was very sensitive to Flagyl and Fasigyn drugs. Moreover, on the other hand, a low proportion (25%) of the parasite was sensitive to Acriflavin.

Oral administration of Flagyl was successful in treatment of pigeons experimentally infested with *T.gallinae*. A nearly similar results were reported by *BUSSIERAS et al.* (1961); *LUTHGEN and BERNAU* (1967) who controlled pigeon mortality due to trichomoniasis by oral administration of Flagyl for 5 successive days. Conversely it was reported by *LUMEIJ and ZWIJNENBERG* (1990) that treatment with Flagyl failed to control pigeon trichomoniasis. Successful treatment was also achieved in trichomonas-infested pigeons given Fasigyn by the oral route. Low therapeutic efficacy for pigeon trichomoniasis was observed by the application of Acriflavin. unfortunately, there is no available data on the use of Fasigyn or Acriflavin in treatment of pigeon trichomoniasis. It is worthy to mention that the experimentally-infested pigeons became in good condition after a short time of treatment with either Flagyl or Fasigyn.

It is concluded that pigeon trichomoniasis is widely spread causing great problem to all ages of pigeons, therefore, Flagyl or Fasigyn should be administered in a protective dose as routine program for all pigeons either reared individually or intensively but must be changed each period to avoid drug resistance.

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Fig.1. *Trichomonas gallinae* from the crop of pigeon showing anterior flagellae, undulating membrane and marginal flagellum.



Fig2. Photograph of experimentally infected opened squab display caseous materials in the upper digestive tract.



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Table (1): Incidence of *Trichomonas gallinae* in pigeons.

Age of birds	No. of pigeons examined	(No. of positive)	10% of positive	No. of birds with lesions	Mixed infection	
					Haemoproteus	Eimeria
Squabs	95	23	24.2	18	0(0%)	0(0%)
Youngster	37	35	40.2	16	7(20%)	6(17.1%)
Adult	106	61	57.5	0	10(16.4%)	12(19.7%)

Table (2): Results of pathogenicity test of *Trichomonas gallinae* in pigeon squabs.

Group No.	No. of tested pigeon squabs	Route of infection	Dose	Daily death post infection					Mortality No. %
				1	8	9	14	30	
1	10	orally	1 cc of abundant <i>Trichomonas</i> culture	-	1	1	1	-	3 30
2	10	non infected control	---	-	-	-	-	-	-



Table (3): Results of sensitivity (In vitro) of isolated Trichomonads to antiparasitic drugs.

Drugs	No. of samples	No. of sensitive	No. of non-sensitive	Sensitivity
Flagyl 50mg/ml	8	8	-	100%
Fasigyn 50mg/ml	8	8	-	100%
Acriflavin 5%	8	2	6	25%
Control	8	-	-	-

Table (4): Results of treatment (in vivo) of infested pigeons with antiparasitic drugs.

Drugs	No. of pigeon	No. of cured	No. of non-cured	Efficiency
Flagyl 50mg/ml	4	4	-	100%
Fasigyn 50mg/ml	4	4	-	100%
Acriflavin 5%	4	1	3	25%
Control	4	-	-	-