# Pathological Studies on Naturally Argas reflexus-Infected Squabs

#### Mouchira Mohamed Mohi El-Din

Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, South Valley University, Qeuna, Egypt

## Abstract

This work is the first to describe Argas reflexus larval induced lesions in squabs (Columba livia; Gmelin). A huge number of adult oval soft ticks (2 by 3 mm with narrow anterior end) were found in wooden crevices of pigeon lofts in Dakahlia Provience, Egypt, Such huge numbers of Argas reflexus persuaded the investigator to look for attached larvae to the squab-skin. One hundred squabs (20-30 day-old), were examined. Three squabs, revealed clusters of bluish-pink larvae tightly embedded in the underwing-skin, were highly emaciated. Lactophenol preparation revealed larvae (Argas reflexus) with 6 relatively long legs and roughly circular bodies. Giemsa-stained blood smears revealed small glistening spots of equal size around the erythrocytic nuclei. The most predominant microscopic lesions were ulcerated skin and subcutaneous hematomas without any inflammatory reaction at the sites of parasitic bites. Moreover, lesions were encountered in the kidneys, brain, esophagus, crop, proventriculus and intestine. It could be concluded that Argas reflexus induces hemorrhagic anemia, anorexia, emaciation and skin ulcerations, besides severe lesions in kidneys, brain and most of the internal organs.

# Introduction

The pigeon is one of the oldest domesticated birds on record. The ancient Egyptians used it as postal messengers. Nowadays, it contributes to the animal-protein required for human consumption in Egypt. Argas reflexus infests domestic pigeons and a variety of wild birds in Western Europe. The subspecies hermanni Audouin, is common in Egypt and certain areas of French West Africa, besides Kenya, Sudan and Ethiopia (Hoogstraal and Kohls, 1960). The available literature didn't contain much about the pathology of Argas reflexus in squabs. Kral and Schwartzman (1964), Wall and shearer (2001) and Taylor et al. (2007) mentioned that Argas reflexus infestation may cause irritation, sleeplessness, loss of egg productivity and anemia. The heavy infestation kills birds within 1-2 weeks.

Small granulomatous reaction may form at the sites of tick bites consisting of mixed inflammatory cells and fibrosis. Previous study recorded the importance of Argus reflexus hermani, in Egypt, in transmitting West Nile and Chenuda viruses, besides the Quaranfil virus group among pigeons and is implicated in transmission of Q fever rickettsia in humans (Saif 2003). Kral and Schwartzman (1964) and Songer and Post (2005) reported that Aegyptianella pullorum causes Aegyptianellosis in chickens, turkeys, ducks and geese transmitted by soft ticks of the Sp. Argas. The infected birds show anemia, diarrhea, anorexia, fever and high mortality. Kral and Schwartzman (1964) reported that Argasidae larvae, nymphs and adults (imago) show a predilection for young (4-8 days old), pigeons, fowl, ducks and geese. Argasidae life cycle includes an egg, a larval,

two nymphal and an adult stage. The larvae hatch after 3 weeks or more, attach themselves to the unfeathered parts of host-skin to suck blood for 5 c and moult into nymphs. Both nymphs and adults are nocturnal bloodsuck. The fact that the larvae attach for short period to the squabs made it difficuting them attached to squabs to be examined. Consequently, the aim of investigation was to follow up such larval attachment to squab-skin and describe the pathological changes, induced in infested squabs.

### Material and Method

#### Examined birds

One hundred (20-30 day-old) squabs, from pigeon lofts in Dakahlia provie Egypt were examined. Three emaciated squabs displayed clusters of bluish-larvae embedded in the underwing-skin during the summer season. The la were removed for parasitological examination.

#### **Blood smear**

Blood smears, from the ulnar vein, were prepared and stained with Giemsafor blood examination.

## Parasitological examination

Larvae were collected from the 3 infested squabs and immersed in lactoph drop (Gurr 1961) on a glass slide and covered by a glass-slip for microsc examination.

# Pathological examination

The clinical signs and gross lesions, observed in the infested squabs vecorded. Specimens were collected from the affected skin, kidneys, b esophagus, crop, proventriculus and intestine, then immediately fixed in neutral buffered formalin. Five micron-thick paraffin sections were prepared stained with hematoxylin and eosin, then examined microscopically.

#### Results

#### Signs and gross appearance

The affected squabs were emaciated with slightly raised their wings above sides of the body (Fig.1). The unfeathered skin of the inner side of the w showed clusters of tightly embedded bluish-pink larvae (Fig. 2). After hiremoving the larvae, bleeding ulcers were noticed.

#### Parasitological results

Microscopically, the lactophenol whole-mount revealed Argas reflexus-la with 6 relatively long legs and roughly circular bodies (Fig. 3).

#### Blood smear results

The blood-smears from the infested squabs showed glistening small glot surrounding the erythrocytic nuclei (Fig.4). Few erythrocytes prese basophilic hemoglobin and partially fragmented nuclei.

## Histopathological findings

The affected skin was ulcerated and large subcutaneous hematoma was without any inflammatory reaction (Figs. 5& 6). The ulcers were plugged eosinophilic mass containing scattered bluish bacterial colonies (Fig. 7). larvae were represented by irregular circles on ulcerated skin (Fig. 8). The convoluted tubules suffered albuminous degeneration (Fig. 9) and the colle tubules were cystic (Fig. 10). Exaggerated normal extramedullary hematopo foci focally replaced the renal parenchyma (Fig. 11). Congestion encountered in the cerebral subarachnoid space (Fig. 12) and

hemorrhages in the white matter of the cerebellar folia (Fig. 13). Cerebral edema was evidenced by clear circular rings around nuclei of cerebral neurons (Fig. 14) besides dilated Virchow Robin spaces. Aggregation of round cells was seen in lumen of lateral ventricle which developed hyperplastic ependymal lining with vacuole (Fig. 15). Some Purkinje cells were lost and replaced with clear vacuoles, and the neurons of the cerebellar granular layer were widely separated by eosinophilic material (Fig. 16). The esophageal wall was relatively thin with atrophic mucous glands, near the junction with proventriculus, when compared with the preceding segment (Figs. 17& 18). The crop-mucosa was papillary folded (Fig. 19) due to anorexia. The lumina of the proventricular lobular-tubules were dilated. The mucous columnar epithelial-lining of the glandular lobules was partially lost (Fig. 20). Intestinal villous atrophy and sloughing were prevalent (Fig. 21).

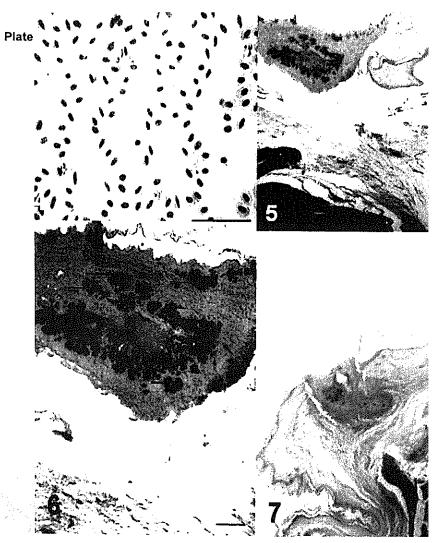
#### Plate 1



Figure 1 Emaciated squabs with slightly raised wings

Figure 2 Clusters of tightly embedded bluish-pink larvae showing in the unfeathered skin of the inner side of the wings.

Figure 3 Argas reflexus-larvae with 6 relatively long legs and roughly circular bodies HE, Bar =  $50 \, \mu$ 



**Figure 4** The blood-smears from the infested squabs showed glistening sm globules surrounding the erythrocytic nuclei HE, Bar =  $50~\mu$ 

Figures 5& 6 Ulceration and large subcutaneous hematoma was seen with any inflammatory reaction HE, Bars =  $100 \mu$ ,

Figure 7 The ulcers plugged with eosinophilic mass containing scattered blui bacterial colonies HE, Bar = 100  $\mu$ 



Figure 8 Larvae were represented by irregular circles on ulcerated skin HE, Bar =  $100 \mu$ 

Figure 9 The renal convoluted tubules suffered albuminous degeneration HE, Bar =100  $\mu$ 

Figure 10 Cystic collecting tubules HE, Bar =100  $\mu$ 

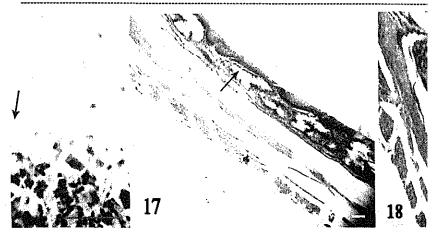


Figure 11 Exaggerated normal extramedullary hematopoietic foci focally replaced the renal parenchyma HE, Bar =

Figure 12 Congestion was encountered in the cerebral subarachnoid space HE, Bar = 100  $\mu$  Figure 13 Focal hemorrhages in the white matter of the cerebellar folia HE, Bar = 50  $\mu$ 

Figure 13 Flocal Helmonthages in the William Hallow of the School of the Figure 14 Cerebral edema was evidenced by clear circular rings around nuclei of cerebral neurons and dilated Virchow Robin spaces HE, Bars =  $50 \,\mu$ 

Figure 15 Aggregation of round cells showing in lumen of lateral ventricle which developed hyperplastic ependymal lining with vacuole HE, Bars =  $50~\mu$ 



**Figure 16** Some Purkinje cells were lost and replaced with clear vacuoles, the neurons of the cerebellar granular layer were widely separated eosinophilic material HE, Bars =  $100~\mu$ 

**Figure 17, 18** The esophageal wall was relatively thin with atrophic muglands, near the junction with proventriculus, when compared with the prece segment HE, Bar =  $100~\mu$ 

# Plate 6

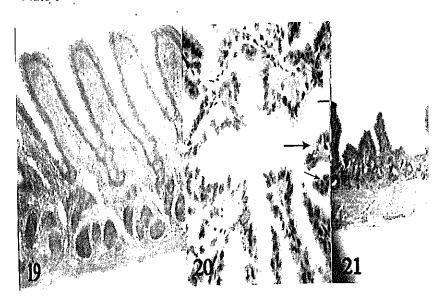


Figure 19 The crop-mucosa was papillary folded HE, Bar =  $100 \, \mu$  Figure 20 The mucous columnar epithelial-lining of the glandular lobules was partially lost HE,  $50 \, \mu$ 

Figure 21 Intestinal villous atrophy and sloughing were prevalent HE, Bar =100  $\mu$ 

# Discussion

The larvae of Argas reflexus remain attached to host for several days to feed before dropping off to molt into first stage nymphs (Roberts and Janovy, 2005). This short period of attachment to the host makes it difficult to find larvae attached to squabs. Consequently, the available literature about the larval induced skin lesions is almost nonexistent. This work is the first to describe the larval induced lesions in squabs by its mouthparts. The sites of larval attachment to skin were ulcerated due to the damaging effect of the parasites on the skin. Large hematomas were encountered in the subcutaneous tissue, underlying the ulcers due to anticoagulant factors produced by the larval salivary glands (Markward, 1994). Spiewak etal. (2006) found that Argas reflexus bitten humans developed generalized urticaria with asthma, and local allergic reaction. Dal Monte, Pajello (1994) reported a case of anaphylactic shock caused by an Argas reflexus-sting in a young woman with a history of recurrent undefined urticaria-angioedema syndrome.

The small glistening dots, surrounding the erythrocytic nuclei, could be rickettsial infection. Tarello (2006) reported round, oval and clover-like Aegyptianella-like large inclusion transmitted by Argasedae. The enlarged size of the extramedullary hemopoietic renal centers could be due to excessive loss of blood by the blood-sucking larvae. It is known that larvae keep attached to the host for several days to feed on its blood (Roberts and Janovy, 2005). Sipos et al. (2005) reported simultaneous porcine dermatitis and nephropathy. The current study revealed disturbed renal metabolism, which was probably induced by toxin absorption, from the ulcerated skin. Skin affections is usually associated with renal lesions (Segales & Domingo 2003), probably due to the excretion of the absorbed toxic metabolites via the kidneys. Drolet et al. (1999) found systemic vasculitis with marked tropism to porcine skin and kidneys. It could be concluded that Argas reflexus infestation induced anemia, hemorrhage, skin ulceration and various visceral lesions, besides emaciation and death.

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# ص العربي

ات باثولوجيه على العدوى بيرقة الارجس رفلكسس في الزغاليل

ا محمد محيى الدين

باثولوجيا والباثولوجيا الإكلينيكية- كلية الطب البيطرى- جامعة جنوب الوادى (بقنا)

بدنا عددا هاتلا من القردة وكان حجمها من ٢ الى ٤ ملى داخل الشقوق الخشبيه الموجودة بابراج الحمام لم الدقهلية جمهورية مصر العربية. مما اوحى الينا بالبحث على وجود يرقات القردة على جلد صغير الحمام اليل) لتسجيل وشرح الاصابات التى تحدثها يرقة الارجس رفلكسس فى الزغاليل حيث انه من الصعب ها على جلد الطائر لقصر مدة التصاقها بالجلد. وقد قمنا بفحص عدد منه من الزغاليل داخل الابراج ووجدنا يعانون من هزال شديد وتحت اجنحتها مجموعات من اليرقات ذات اللون الازرق الوردى منغمدة بشدة بازالتها. وقد ازلنا بعض منها بصعوبه مما احدث نزيف وقرح بالجلد. واخذنا اليرقات ووضعناها فى فينول لفحصها باراسيتولوجيكل. فوجدنا يرقة الارجس رفلكسس لها ارجل طويله وجسم دانرى خشن. واخذنا من دم الزغاليل وفحصناها تحت الميكروسكوب فوجدنا بقع لامعه بداخل كرات الدم الحمراء تحيط بالنواه. من الانسجه تحت الميكروسكوب وجدنا قرحات على سطح الجلد وكدمات تحت الجاد مع اختفاء الخلايا ابية مكان قرصة اليرقة ونزول لعابها وكذلك اصابات بالكلى والمخ والمرئ والحويصلة ومقدمة القناصة ماء وقد استنتجنا من هذة الدراسة ان يرقة الارجس رفلكسس تحدث انزفة تسبب انيميا وفقدان الشهية وهزال الم وقرحات جلديه بجانب اصابتها بمشاكل فى الكاية والمخ ومعظم اجهزة الداخليه بالجسم.