**Scientific Report**

# Prevalence and ultrastructural study of *Aegyptianella* spp. in domestic birds from southwestern area, Iran

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## Summary

Aegyptianellosis is a disease caused by small intraerythrocytic inclusions which is restricted to the area of Africa, Asia and extreme southern Europe. In this study the prevalence of *Aegyptianella* spp. was evaluated in four genera of domestic poultry in the northern, southern and central regions of Lorestan province from April to September 2008. A total of 275 native adult birds including chickens, ducks, geese, and turkeys which were bred in free range pasture condition were used for blood sampling in the rural areas of the regions. Twenty one (7.6%) out of 275 birds used in this study were infected with the organisms. The detected *Aegyptianella* were found in 7 chickens (33.3%), 2 ducks (9.5%), 7 geese (33.3%), and 5 turkeys (23.9%), respectively. The majority of the records were from the northeastern regions. Therefore, more than one-half of the infected birds with the *Aegyptianella* species were located in these regions. The structure morphology of *Aegyptianella* spp. was studied using light and electron microscopy. The results of the study by electron microscopy demonstrated the developmental stage as well as implemented similar and different extra/intra genus.

**Key words:** *Aegyptianella* spp., Blood parasite, Electron microscopy, Native poultry, Iran

## Introduction

The intraerythrocytic inclusion named after Egypt where the organism was first described by Carpano (1929). *Aegyptianella* is a genus of the family of rickettsia-like organisms called *Anaplasmataceae* (Castle and Christensen, 1985). Some of the genera in this family include *Anaplasma*, *Ehrlichia*, *Neorickettsia* and *Wolbachia* (Kawahara *et al*., 2004).

The organism is an obligate intracellular parasite that can be transmitted by soft tick *Argas persicus* (*Persicargus*) and is capable of infecting an extensive gamut of amphibians, reptiles and bird species (Ahmed and Soliman, 1966; Hadani and Dinur, 1968; Pierce and Bevan, 1977; Castle and Christensen, 1985; Desser, 1987; Barta *et al*., 1989; Desser and Barta, 1989; Mutinga and Dipeolu, 1989; Huchzermeyer *et al*., 1992; Werner, 1993).

Up to now, there have been different reports on the ultrastructural studies concerning Aegyptianellosis in birds in the world, but no data have been recorded in Iran (Gothe, 1967; Bird and Garnham, 1969; Castle and Christensen, 1985). Here, we determine the prevalence of *Aegyptianella* spp. in some bird populations of the different areas of Lorestan province. In addition, the study elicited more details about the ultrastructure and comparison of the morphological characteristics with the organisms-like (*Rickettsiae*) with an affinity to blood cells in other species.

# Materials and Methods

## Survey area

This study was conducted between April and September 2008 in Lorestan province, Iran. Selection of collecting areas was confined to three divisions with three different geographical districts including cold in the north, moderate in the center and warm in the south.

## Sample collection

A total of 275 native adult birds including 99 chickens; 39 ducks; 87 geese; and 50 turkeys were bred in free range pasture condition and were selected for blood sampling in the rural areas of the three regions. Ethical approval of the study was obtained from the ethics committee of Lorestan University.

## Light microscopy

One ml of blood was taken from the brachial wing of each bird subjected to study. The blood smears were air-dried, fixed in methanol and stained with Giemsa's stain in phosphate buffer saline (pH = 7.2). The blood films were examined with an oil immersion lens at a 1000 × power field, Olympus CX31 microscope.

## Electron microscopy

Infected blood was provided by fresh peripheral blood smears and their infections were reconfirmed. Some collected blood drops were clotted at room temperature and prefixed with 2.5% glutaraldehyde solution (TAAB Laboratories-3 Minerwa, Calleva park, Aldermaston, Berks, RG78NA, England - EM grade, Batch No.58030) in 0.1 M PBS (pH = 7.2) for 2 h at 4°C for preparation of transmission electron microscopy (TEM). Then, the samples were washed three times in the PBS (10 min, each time), post-fixed in 1% osmium tetroxide solution (Batch No. 48290) in the same buffer at room temperature for 2 h. Then washed in the PBS (10 min), dehydrated in ascending alcohol series and finally embedded in TAAB resin (Berks, RG74QW, England, Data Sheet No. 3), polymerized in 60°C for 48 h. 50 nm ultra thin sections were then prepared by LKB ultratome 4801A (LKB-producer AB-Stockholm 12-Sweden) and double stained with 20% uranyl acetate solution (B.D.H. Laboratory Chemical Division, England, No. 0148860) in pure methanol (E. Merck, D-6100 Darmstadt, F-R. Germany) for 45 min and Reynold solution (Lead nitrate and sodium citrate) around 1 h. Finally, the samples were examined (observed) with a Philips 208 S transmission EM at X resolution. The photographs were captured by Olympus digital camera (model. E-20 p, 5M.P).

## Statistical analysis

Data analysis of the relationship between prevalence rates and different areas were evaluated by the Chi-square test. Statistical significance was defined as $P<0.05$.

# Results

Of the 275 birds examined, 21 (7.6%) harbored *Aegyptianella* spp. including 7 chickens (33.3%), 2 ducks (9.5%), 7 geese (33.3%), and 5 turkeys (23.9%), respectively. The highest population of infected birds (57.1%) was located in the rural area of Alashtar city in the northeast of Lorestan province. There was a significant increase in the frequency of infection in the birds located in the north area as compared to the birds in the other studied areas ($P<0.05$). The results are summarized in Table 1.

The rickettsial bodies were densely stained and located either alone or as multiples in the cytoplasm of infected erythrocytes. In the malignant infections severe leukocytosis was prominent (Fig. 1).

Most of the erythrocytes were infected with one and/or more than one membrane bound vacuoles (as single or morula), in each from 1 to 11 round shaped individual bodies, so called elementary, initial or Anaplasmal bodies were observed, in which the whole surface of the cytoplasmic vacuole was virtually filled. Vesicles were found within some vacuoles (Fig. 2). In some instances, the vacuoles protruded from the host cell membranes and it seemed that membrane of the cell and inclusion body integrated with each other and, therefore, there was no distinction between the host

**Table 1: Prevalence of *Aegyptianella* spp. in infected domestic birds in the three regions of Lorestan province**

| Areas | Prevalence (%) | No. of infected birds | | | | Statistical analysis ($x^2$)* |
|---|---|---|---|---|---|---|
| | | Chickens (%) | Ducks (%) | Geese (%) | Turkeys (%) | |
| Northern | 57.1[a] | 3 (14.3) | 1 (4.7) | 5 (23.8) | 3 (14.3) | a:b, $P<0.05$ |
| Central | 28.6[b] | 2 (9.5) | 1 (4.8) | 2 (9.5) | 1 (4.8) | |
| Southern | 14.3[c] | 2 (9.5) | 0 (0.0) | 0 (0.0) | 1 (4.8) | a:c, $P<0.05$ |
| Total[§] | 100 | 7 (33.3) | 2 (9.5) | 7 (33.3) | 5 (23.9) | |

*Chi-square test. [§]A total of 275 domestic birds were examined

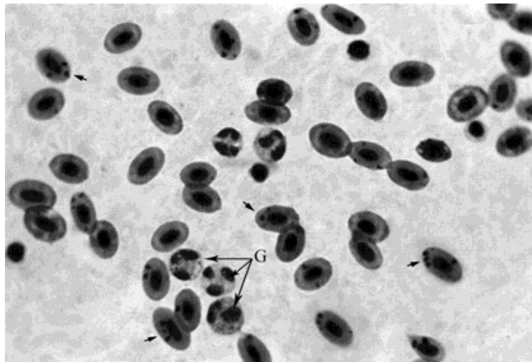


**Fig. 1: Photomicrograph of Giemsa stained *Aegyptianella* spp. within erythrocytes of geese (short arrows). The granulocytes (G) are visible, (Giemsa stain, ×1000)**

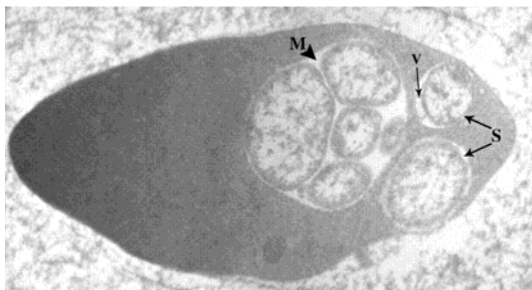


**Fig. 2: Electronmicrograph of a goose erythrocyte containing both singles (S) and multiple organisms or morula (M) in their inclusion bodies. The vesicle (V) is present within the vacuole, (×14000)**

cell layer and the membrane of the vacuole (Fig. 3).

The Anaplasmal bodies were covered by 2 well-defined electron-dense membranes which were separated from each other by an electron-lucent zone as periplasmic space. The outer membrane as cell coat, which is a component of the external surface, made direct contact with the vacuole matrix and the inner layer or plasma membrane was adherent to the internal constituents of the organism. The internal structure of the organism was seen as several forms. The numbers had a central electron-dense core substance in protoplasm which is a pale zone area located around it. Some individual bodies exhibited dispersed electron-dense material and the rest had a clear central electron-lucent area, containing fine strands of DNA filaments, surrounded by a narrow rim of granular dense peripheral zone, suggestive of ribosomes.

In several erythrocytes, individual inclusions were demonstrated, either penetrating or thoroughly engulfed by these

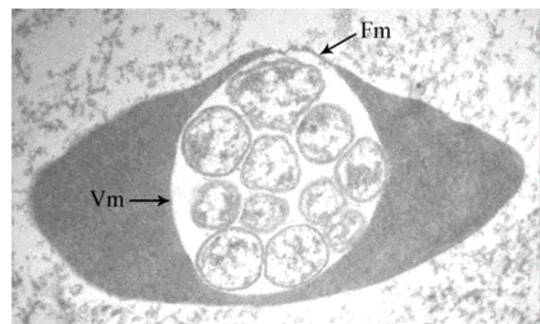


**Fig. 3: Electronmicrograph of erythrocyte from a goose with single inclusion. The membrane of vacuole (Vm) and plasma membrane of the erythrocyte appear as a thin fused membrane (Fm) also the developmental stage is completed, and the organisms are ready to release from the host cell, (×18000)**

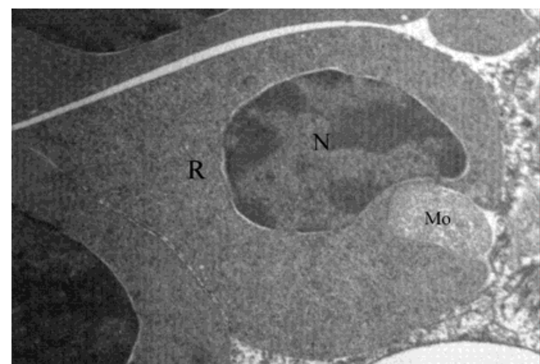


**Fig. 4: Electron micrograph of an infected goose erythrocyte. The single microorganism (Mo) is penetrating to the cytoplasm of red blood cell (R), (×18000)**

cells. The structure of the organism of these types consisted of a thickened electron-dense mono layer of plasma membrane, with a reticular network in their protoplasm (Fig. 4).

## Discussion

The present study demonstrated that *Aegyptianella* spp. (known as *A. pollurum*) infections are distributed in the Lorestan province bird population. The results of our investigation showed that 57.1% of infected birds were located in the north of the province. A low infection was found in different birds in other regions (south and southwest) of Lorestan.

Our study revealed that *Aegyptianella* in birds somewhat differed structurally from their counterparts in amphibians, like *A. bacterifera* and *A. ranarum*. The ultrastructure of these organisms, described as an elongated structure arranged in a membrane bound vacuole and enveloped by a typical gram negative-like cell wall membrane, contained either double trilaminar or three distinct membranes in *A. ranarum* and *A. bacterifera*, respectively (Desser, 1987; Desser and Barta, 1989), but this matter is a little different for *Aegyptianella*, *A. pollorum*, *Ehrlichia*, and *Anaplasma* in this survey (Sells *et al*., 1976; Castle and Christensen, 1985; Ribeiro *et al*., 1997; Arraga-Alvarado *et al*., 2003). All of them were surrounded by only two distinct membranes, which is the determinant of the *Rickettsia*, without any extra laminar membrane. Nevertheless, the intra erythrocytic organisms in frogs are more and/or larger than in birds. Desser (1987) concluded that ultrastructural similarity between *Aegyptianella* in birds and those pointed out in amphibians suggest that they must be species of the same genus.

On the other hand, Gothe and Kreier (1977) showed up to 26 rod-like bodies of *A. pullorum* in a single inclusion, whereas in our survey as well as other studies the organisms in the individual vacuole fluctuate between 10 and 12.

This considerable discrepancy may be interpreted as variations exist in the same species, therefore, we preferred to use *Aegyptianella* spp. rather than *A. pullorum* in the present study. The single inclusion which was described earlier indicated the primary stage of the life cycle of the organism, after it has been phagocytosed by erythrocytes. Next to the ripeness of the individual body, through asexual reproduction, the vacuolar membrane is ruptured and the organisms (*Rickettsia*) are released, thereafter each parasite penetrates the uninfected red blood cells as the primary or immature inclusion body, developed until divided into multiple parasites as secondary or mature inclusion bodies when the maturation is completed (Sells *et al*., 1976; Castle and Christensen, 1985; Desser, 1987). The penetration of these organisms to the host cell by phagocytosis has not been reported yet, so our finding confirms the presumptive theory suggested by other investigators (Sells *et al*., 1976; Desser, 1987). The double membranes that were observable in mature inclusions were undefined when the bodies were in an initial developmental phase. This finding is consistent with the primary stage of infection with *Ehrlichia platys* (*Anaplasma platys*) in platelets (15), and also the difference of the interior of the organism in this study, indicated by the various developmental stages, as: Initial, intermediate and mature phase. These are similar to those reported as the initial body of *Anaplama* inclusions in erythrocytes and *Ehrlichia platys* in platelets (Kocan *et al*., 1978; Ribeiro *et al*., 1997; Arraga-Alvarado *et al*., 2003).

Conclusively, we believe that the taxonomy of an organism, based on the ultrastructure may be efficient in its classification, but it does not seem to be constantly reliable. Molecular phylogenetic revealed that Texas strain of *A. pullorum* (morphologically similar with our strain) is most closely related to *Anaplasma* species and the family of *Anaplasmataceae* (Rikihisa *et al*., 2003), but as mentioned before, for the sake of diversity of the ultrastructural results in this and other studies (Bird and Garnham, 1969; Gothe and Kreier, 1977), we were not convinced that our strain is *A. pullorum*, so we suggest that the genotyping in all isolates of *Aegyptianella*, whether in birds or amphibians, is the best means in the

phylogeny of this genus.

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