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DESCRIPTION OF *AEGYPTIANELLA BOTULIFORMIS* N. SP. (RICKETTSIALES: ANAPLASMATACEAE) FROM THE HELMETED GUINEAFOWL, *NUMIDA MELEAGRIS*

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ABSTRACT

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Aegyptianella botuliformis n. sp. (Rickettsiales: Anaplasmataceae) isolated from helmeted guineafowls *Numida meleagris* from the Kruger National Park is described. The rickettsia occurs within a membrane-bound vacuole in the cytoplasm of erythrocytes with up to 8 organisms in a mature inclusion. The initial body resembles that of *Aegyptianella pullorum*. The tightly packed, sausage-shaped intermediate forms are a distinctive morphological feature, seen as irregular, pleomorphic forms under light microscopy.

While more larvae and nymphs of *Amblyomma hebraeum* and *Amblyomma marmoreaum* were found on the birds than larvae of an *Argas* sp., it is believed that the latter are the vectors of *A. botuliformis* n. sp.

In addition to the Kruger National Park, positive blood smears were obtained from guineafowls at other localities in the Transvaal.

INTRODUCTION

Carpano (1929) erected the genus *Aegyptianella* to contain a blood parasite, *Aegyptianella pullorum*, which he had found in domestic fowls in Egypt, believing it to be a piroplasm. After an ultrastructural study of the developmental stages of *A. pullorum*, Bird & Garnham (1969) showed it to be a rickettsia, while Gothe (1971) found that it had close morphological and biological affinities with the Anaplasmataceae. Ristic & Kreier (1974) placed the genus *Aegyptianella* in the family Anaplasmataceae, along with the genera *Anaplasma, Eperythrozoon, Haemobartonella* and *Paranaplasma*.

The isolation of an *Aegyptianella* species from helmeted guineafowls, *Numida meleagris*, in the Kruger National Park (KNP) was reported by Huchzermeyer, Horak & Braack (1991) and their prevalence described by Earlé, Horak, Huchzermeyer, Bennett, Braak & Penzhorn (1991). The present paper describes the ultrastructure of this organism and records its prevalence and geographic distribution in guineafowls from two other areas of the Transvaal. It also speculates on the role played by argasid and ixodid ticks, found on guineafowls in the southern region of the KNP, as vectors of the organism.

MATERIALS AND METHODS

Four pure isolates of the *Aegyptianella* sp. (isolates S and T collected on 1989-02-28 and A and B collected on 1990-03-08) were obtained from blood samples taken from four guineafowls shot at Skukuza, KNP, as previously reported (Huchzermeyer *et al.*, 1991; Earlé *et al.*, 1991). Parasite-free guineafowls were obtained from eggs produced by the Veterinary Research Institute's own flock. All birds were reared in an isolation unit under tick free conditions on a commercial poultry ration and were 12–20 weeks of age when used. Passaging of the *Aegyptianella* sp. was done by intravenous injection of fresh or heparinized infected blood into the brachial vein of susceptible guineafowls. Blood smears were taken 5 times a week, fixed with May Grünwald Giemsa and stained with Giemsa's stain for 1 h at pH 6,5. The stained smears were examined at a magnification of 1000 × and parasitaemias were expressed as parasites per 100 fields of view at approximately 100 erythrocytes per field of view.

Electron microscopy

Blood for transmission electron microscopy was taken from the second passage of isolate T, the first passage of isolate A and the seventh passage of isolate B, when high parasitaemias were found to be present (Table 1). A modified version of the method described by Desser & Barta (1989) was used for the preparation of the guineafowl blood for transmission electron microscopy (TEM). Infected blood was collected in heparinized haematocrit tubes. Without centrifugation, drops of this blood were directly fixed in 2,5 % glutaraldehyde in 0,1 M Na-cacodylate buffer containing 0,02 % (w/v) $CaCl_2$ and 4 % (w/v) sucrose (pH 7,3). After 1 h the hardened clots were sliced into 0,5 mm^3 cubes, the fixative solution replaced and the samples left overnight at 4 °C. After 2 rinses in 0,1 M Na-cacodylate containing 4 % sucrose (w/v) pH 7,3, the cubes were post-fixed in 1 % OsO_4 in 0,1 M Na-cacodylate buffer containing 0,5 % (w/v) $K_3Fe(CN)_6$ and 4 % (w/v) sucrose for 1 h. After 3 rinses in double distilled water, the samples were *en bloc* stained for 1 h in 0,5 % (w/v) aqueous uranyl acetate. An ascending ethanol series (50, 70, 90, 95 and 3 × 100 %) was used for dehydration after which the cubes were embedded in Taab 812 resin (Taab Laboratories Equipment Ltd, Berkshire, England) and sectioned using a Reichert-Jung Ultracut microtome (C. Reichert, A. G., Wien, Austria) and a Diatome diamond knife

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(Diatome Ltd, Bienne, Switzerland). The ultrathin sections were contrast stained using 5 % (w/v) aqueous uranyl acetate followed by lead citrate (Reynolds, 1963). The sections were viewed at 60 kV using a Jeol JEM-1200-EX transmission electron microscope. The various developmental stages of the organism were measured from electron micrographs.

Survey of guineafowl blood smears

Between November, 1984, and August, 1990, 46 blood smears from guineafowls were submitted to the Veterinary Research Institute. The sources of these smears varied but included those taken from birds shot during the hunting season as well as from birds (both living and freshly dead) submitted to the institute for diagnostic purposes. In addition four undated smears from guineafowls in the collection of the institute were also examined. The smears were stained as described above and subsequently examined at a magnification of 1000 ×.

Ticks collected from the guineafowl

The species and numbers of ticks collected from 118 birds in the KNP, including the 4 from which the isolates were made, have been recorded by Horak, Spickett, Braack & Williams (1991).

RESULTS

Passaging

The peak parasitaemias during the passages of the 4 isolates are shown in Table 1 and occurred in passage 1 of isolates A and B, passage 4 of isolate S and passage 6 of isolate T. There was no particular pattern to the levels of parasitaemia, with occasional high levels occurring sporadically in various passages. Unfortunately, it was not possible to take samples for the TEM study each time a high parasitaemia was observed. Consequently, the highest parasitaemia available for study was 520 parasites/100 fields (Table 1: isolate A, passage 1).

TABLE 1 Peaks of parasitaemias during the passages of 4 isolates of *Aegyptianella botuliformis n. sp. in laboratory reared guineafowls (Number of organisms per 100 fields at 1 000* × magnification).

Passage	Isolate			
	A	B	S	T
1	520*	1 440	280	240
2	220	340	100	260*
3	84	360	180	130
4	200	420	450	200
5	200	120	150	240
6	200	360	340	400
7	185	420*	285	175
8	85	350	16	56
9	130	150	130	8
10	200	28	140	—
11	—	20	75	—
12	—	28	360	—
13	—	—	260	—

* Passages used for TEM study

Light microscopy

The ring-shaped (Fig. 1a, b) and the irregular (Fig. 1c, d) intraerythrocytic forms and their measurements have been described in a previous paper (Huchzermeyer *et al.*, 1991).

Electron microscopy

The intracellular stages were contained in a membranebound vacuole within the cytoplasm of the erythrocyte (Fig. 2, 7, 8). This vacuole appeared to be formed when an initial body penetrates into the cell (Fig. 2). The cell wall of the organism consisted of a trilaminar membrane (Fig. 3). The organisms contained floccular and filamentous material of high electron density distributed throughout areas of low electron density. In the early initial stages and in growing intermediate stages (Fig. 3) the electron dense material appeared to be evenly distributed throughout the organism, whereas in the more

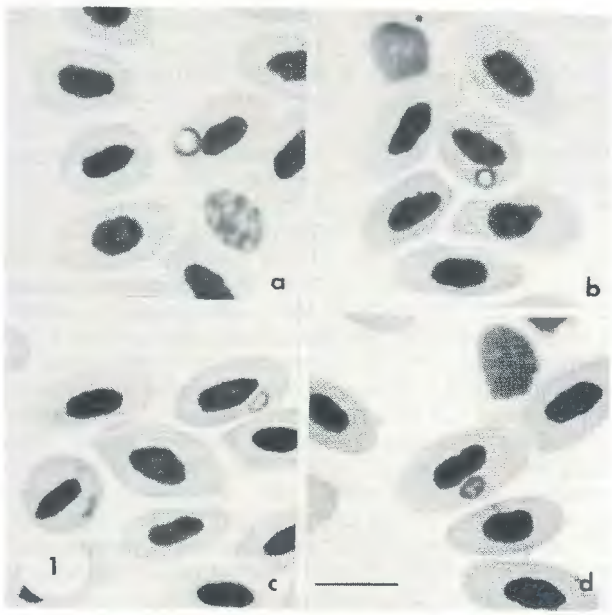

FIG. 1 *Aegyptianella botuliformis* n. sp.: Ring-shaped forms (a, b) and irregularly shaped forms (c, d). Bar = 10 µm

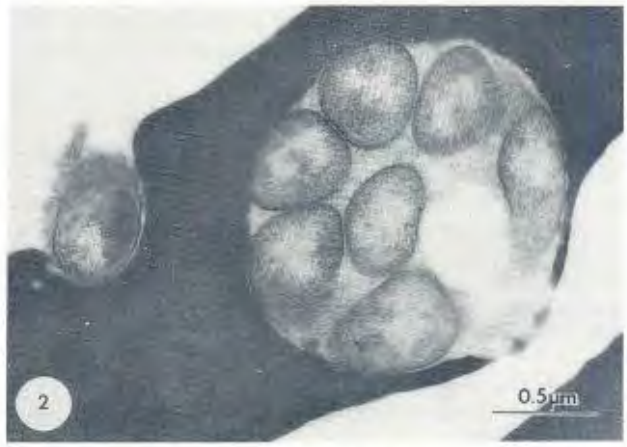

FIG. 2 *A. botuliformis* n. sp.: Initial body apparently penetrating into erythrocyte cytoplasm and forming membranebound valuole and mature daughter organisms in near spherical vacuole

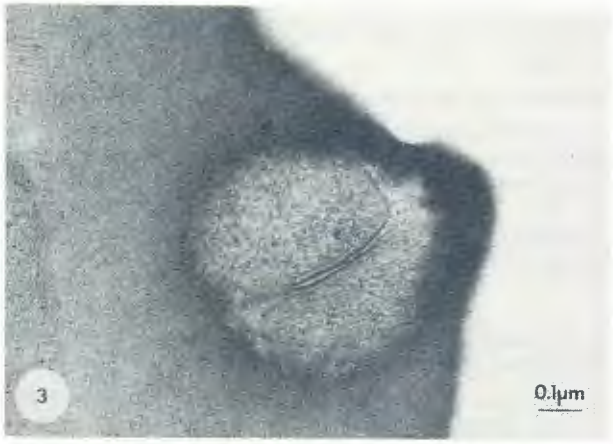

FIG. 3 *A. botuliformis* n. sp.: Initial body undergoing binary fission

FIG. 6 *A. botuliformis* n. sp.: Intermediate body packed into tight knot

FIG. 4 *A. botuliformis* n. sp.: Sausage-shaped intermediate body folding over

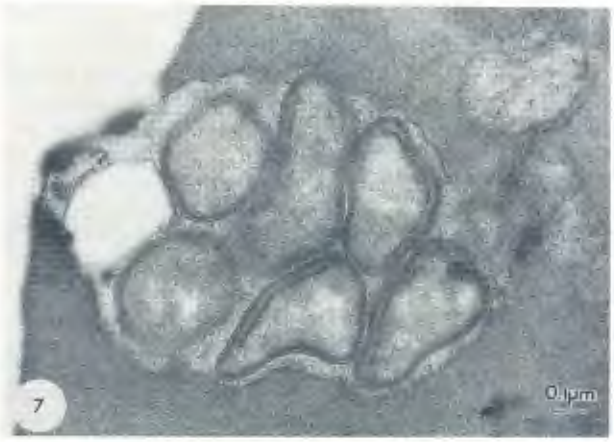

FIG. 7 *A. botuliformis* n. sp.: Intermediate bodies undergoing multiple binary fission with apparent doubling of the trilaminar membrane in places

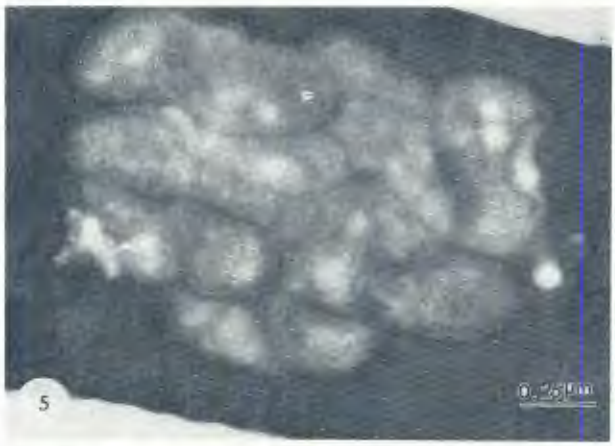

FIG. 5 *A. botuliformis* n. sp.: Intermediate body stretching vacuole into irregular shape

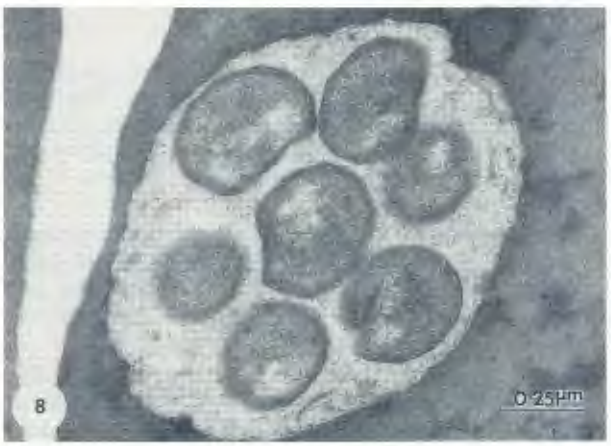

FIG. 8 *A. botuliformis* n. sp.: Mature vacuole with daughter organisms

mature forms it appeared to be congregating closer to the cell wall (Fig. 2).

Soon after penetration the initial body appeared to undergo binary fission (Fig. 3). The daughter organisms then appeared to grow in length without further division, folding on themselves and stretching the vacuole into irregular, pleomorphic shapes, with the vacuole membrane following the outline of

the folded, sausage-shaped organisms (Fig. 4–7), while the vacuole containing the early and mature stages assumed a spherical or ovoid shape, leaving clear space around the organisms. When the organism reached its maximum growth it appeared to be packed in a tight knot (Fig. 6). It then appeared to begin a series of divisions accompanied by an apparent doubling of the trilaminar membrane in places (Fig. 7). In the final stage a varying number (4 to 8) of subspherical to ovoid daughter organisms occupied a near spherical vacuole (Fig. 8).

Survey of guineafowl blood smears

In all, 180 blood smears from 3 areas in the Transvaal were examined. Of the 130 smears collected in the Kruger National Park, 59 (45,4 %) were positive for *A. botuliformis* n. sp., while 24 (48 %) of the 50 birds from the other 2 areas (1 farm in the Grobblersdal area and 11 out of 17 farms in the Warmbaths district) were infected.

Ticks collected from guineafowls

Only 26 of the 118 guineafowls sampled in the Kruger National Park were infested with *Argas* sp. and harboured a total of 1 274 larvae (Horak *et al.,* 1991). These larvae appeared not to be those of *Argas walkerae* (Heloise Heyne, personal communication, 1991). The latter tick is commonly found on domestic fowls in South Africa (Howell, Walker & Nevill, 1978), and transmits *Aegyptianella pullorum* (Gothe, 1978).

With one exception all the birds were infested with the immature stages of the bont tick, *Amblyomma hebraeum,* of which 21 813 larvae and 1 965 nymphs were recovered. Also found on 89 of the birds were 2 284 larvae and 103 nymphs of the tortoise tick, *Amblyomma marmoreum* (Horak *et al.,* 1991).

Taxonomic Summary

Aegyptianella botuliformis *n. sp.*

Host and locality: Helmeted guineafowl *(Numida meleagris),* the Skukuza area of the Kruger National Park and 2 other localities in the Transvaal. Unable to infect domestic fowl *(Gallus gallus)* and domestic turkeys *(Meleagris gallopavo)* (Huchzermeyer *et al.,* 1991).

Description: In Giemsa-stained blood smears spherical, ring-shaped, as well as densely staining spherical inclusions, up to 2,2 μm in diameter and pleomorphic, irregularly shaped inclusions ranging from 1,5–3 μm in length and 0,4–1,8 μm in width.

At transmission electron microscope level the organisms are contained in a membrane-bound vacuole within the cytoplasm of the erythrocyte and are bound by a trilaminar membrane. The interior consists of flocculent as well as filamentous material. The organisms initially appear to divide by binary fission and this appears to be followed by a growth stage during which they assume a "sausage" shape, folding over and packing tightly. This appears to be followed by another series of divisions leading to the final stage, in which the mature organisms assume a near spherical shape and are arranged in varying numbers in a near spherical vacuole.

Ethymology: *botuliformis* = sausage-shaped.

Specimens deposited: Giemsa stained slides of guineafowl blood infected with *A. botuliformis* n. sp. have been deposited in the blood parasite collection of the Protozoology Section of the Veterinary Research Insitute, Onderstepoort, Republic of South Africa (No. 6145–6154).

Discussion

A. botuliformis n. sp. differs from *A. pullorum* in the following aspects:

- Its host spectrum, by being unable to infect the domestic fowl and by producing a higher and longer lasting parasitaemia in guineafowl than the experimental infection with *A. pullorum* (Huchzermeyer *et al.,* 1991).
- The morphology of the intermediate stages, which under light microscopy are seen as large irregular, pleomorphic forms (Fig. 1c, d). Under TEM these appear as tightly packed, even knotted, sausage shapes, from which the species was named.
- The vacuole containing the final stage, which seems to contain fewer organisms than that of *A. pullorum: A. botuliformis* n. sp. up to 8; *A. pullorum* up to 20 (Bird & Garnham, 1969; Gothe, 1967).

In *Aegyptianella ranarum* and *A. bacterifera* of frogs the organisms are elongate and rod-shaped in the final stage (Desser, 1987; Desser & Barta, 1989). In *A. botuliformis* n. sp., it is the intermediate stage which is elongate, before the final series of divisions.

The relatively low number of daughter organisms in mature vacuoles of *A. botuliformis* n. sp. could be the cause of a lower reproductive potential and hence of the relatively low parasitaemias achieved during passaging (Table 1), when compared with those achieved during passaging of *A. pullorum* (Huchzermeyer, 1967). A lower parasitaemia could possibly be responsible for stimulating a lesser immune response, which in turn whould be insufficient to suppress the organisms completely. This could explain, why microscopically positive birds were found at most times of the year (Earlé *et al.,* 1991).

Quick passaging, which increases pathogenicity and the peak levels of parasitaemia in *A. pullorum* (Huchzermeyer, unpublished data), did not have the same effect on *A. botuliformis* n. sp. The decrease in peak parasitaemias in passages 9–12 of isolate B, however, might have resulted from increasing age of the guineafowls used, as there may be an age resistance similar to that found in the common fowl to *A. pullorum* (Gothe, 1971).

While only 45,4 % of the blood smears taken in the Kruger National Park contained *A. botuliformis* n. sp., six out of six blood samples yielded isolates when injected into susceptible birds (Huchzermeyer *et al.,* 1991), demonstrating the superiority of subinoculation as a technique for discovering the presence of low levels of infection. This technique

should, where-ever possible, be employed in further studies of the prevalence of this organism.

Larvae and nymphs of *Amblyomma hebraeum* and *A. marmoreum* were found more frequently and in larger numbers on the guineafowls than larvae of *Argas* sp. However, the only previously known avian species of *Aegyptianella, A. pullorum,* is transmitted exclusively by the bite of argasid ticks (Gothe, 1978).

Norval, Short & Chisholm (1985) reported a seasonal variation in the presence of nymphs and adults of *Argas walkerae* in an experimental fowl run. The percentage infestation rate of the KNP guineafowls with *Argas* sp. larvae in the present study closely fits the curve of their seasonal abundance as reported by Horak *et al.,* (1991). In addition to the presence of the larvae nightly feeding by nymphs and adults is presumed to take place. In this way the birds would be more regularly exposed to infection than is apparent from the small number of larvae found. We therefore assume this *Argas* sp. to be the vector of *Aegyptianella botuliformis* n. sp.

Examination of blood smears and/or samples from a population of guineafowls with an abundant infestation with both species of *Amblyomma* in the absence of the *Argas* sp. (e.g. from the Andries Vosloo Kudu Reserve, Horak *et al.,* 1991) could help to elucidate this question. Attempts to collect live nymphs or adults of the *Argas* sp. in the KNP for transmission studies and identification have so far been without success.

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