An Experimental Study of *Borrelia anserina* in Four Species of *Argas* Ticks

2. Transstadial Survival and Transovarial Transmission*

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Summary. The role of 4 species of bird-parasitizing *Argas* ticks common to Egypt [*Argas (Persicargas) persicus*, *A. (P.) arboreus*, *A. (P.) streptopelia*, and *A. (Argas) hermanni*] in transmitting *Borrelia anserina* to chickens was investigated experimentally.

Transstadial survival: Nymphs and adults developing from experimentally infected larval *A. (P.) persicus* and *arboreus* successfully transmitted the spirochetes to clean (previously uninfected) chickens during feeding; those of *A. (P.) streptopelia* failed to do so. Only the first and second nymphal instars (N₁, N₂) developing from experimentally infected larval *A. (A.) hermanni* transmitted spirochetes to clean chickens; the N₃ and the adult stage failed to do so.

Transovarial transmission: In *A. (P.) persicus* and *arboreus* experimentally infected as N₁ and N₃, respectively, spirochetes were transovarially transmitted to the F₁ generation; the percentage of females depositing infected eggs was 84% in *A. (P.) persicus* and 24% in *arboreus*.

Filial infection rates [determined by 2 methods: (a) individual microscopic examination of 60 eggs from 3 positive egg hatches produced by adults originally infected as nymphs and by F₁ adults of each species, and (b) individual feeding of 60 N₁ of the F₁ and F₂ generations on clean chickens and afterward examining the host blood for spirochetes] were: 80.0 and 83.3% for eggs deposited by originally-infected *A. (P.) persicus* and *arboreus*, respectively (method a), and 83.3% for N₂ of the F₁ generation of both species (method b); 100% for eggs deposited by F₁ females of both species (method a) and 100% for N₂ of the F₂ generation of both species (method b).

Spirochetes were not transovarially transmitted to the F₁ generation of *A. (P.) streptopelia* and *A. (A.) hermanni*.

Introduction

Data from numerical counts and observations of localization of *Borrelia anserina* in 4 species of bird-infesting *Argas* ticks, which were infected in the adult stage by feeding on infected chickens, showed heavy spirochete development and survival in

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internal organs, including salivary glands and reproductive systems, of A. (Persicargas) persicus (Oken) and A. (P.) arboreus Kaiser, Hoogstraal and Kohls. In A. (P.) streptopelia Kaiser, Hoogstraal and Horner, spirochetes did not invade the salivary glands and reproductive systems; in A. (A.) hermanni Audouin, salivary glands were irregularly invaded but reproductive systems were not invaded (Diab and Soliman, 1977).

In the present investigation, each of these 4 tick species was used to determine the capacity of B. anserina spirochetes to survive from originally infected larvae through the nymphal instars to the adult stage and to be transovarially transmitted from originally infected nymphs to eggs and F$_1$ and F$_2$ nymphs.

**Materials and Methods**

**Tick Species and Basic Techniques.** The 4 tick species (from NAMRU-3 Medical Zoology laboratory colonies), feeding methods, sources of Borrelia anserina and chicken hosts, techniques for studying spirochete localization and densities, and for microscopic examination, and methods for establishing clean (uninfected) tick colonies were the same as recorded by Diab and Soliman (1977).

For this investigation, clean larvae were infected by feeding on the wing of clean chickens beginning on the same day the host was inoculated with infected blood (the chickens became highly infected 3 days post-inoculation, remained so for 4–5 days, and then died; this period coincided with the optimum time of larval feeding and thus ensured larval infection). Clean nymphs and adults were also infected by feeding on heavily spirochetic chickens.

**Transstadial Survival.** To investigate transstadial survival of spirochetes from initially infected larvae to successive nymphal instars and to the adult stage, and to evaluate the capability of transstaddially infected nymphs and adults to transmit spirochetes to clean chickens during feeding processes, 100–150 clean larvae of each of the 4 Argas species were fed on infected chickens. Each test with each species was repeated 4 times.

Engorged larvae dropping from the chickens were collected, counted, and observed for molting. First instar nymphs (N$_1$) were fed on clean chickens; the host chicken blood was examined for the presence of spirochetes daily for 15 days after tick feeding. Subsequent nymphal instars (N$_2$, N$_3$, N$_4$) and adults ($\varphi$ and $\delta$) were similarly investigated.

**Transovarial Transmission.** To investigate the occurrence of transovarially transmitted spirochetes to successive tick generations, 90–120 clean N$_2$ A. (P.) persicus, A. (P.) streptopelia, and A. (A.) hermanni and 140 uninfected N$_3$ A. (P.) arboreus were fed on heavily infected chickens. Adults molting from these nymphs were paired separately in vials after feeding on clean chickens and were observed for oviposition. When oviposition was completed, 25 females of each species were selected. On day 1 or 2 after each female oviposited, a sample of 10 eggs from each batch was crushed in 0.2 cc phosphate buffer (pH 7.2) and examined microscopically by dark field illumination for the presence of spirochetes, and the percentage of infected batches was determined. The other eggs from each batch were left to hatch. Ten F$_1$ larvae from each batch were crushed in 0.5 cc phosphate buffer and examined microscopically for the presence of spirochetes. The remaining F$_1$ larvae were fed on clean chickens and the chicken blood was examined daily for 20 days for the presence of spirochetes.

F$_1$ nymphs and adults from negative larval batches were similarly tested to determine whether these stages were infected and, if so, could transmit the spirochetes. The host chicken blood was examined microscopically for the presence of spirochetes each day for 15 days after each feeding.

To determine the percentages of spirochete infection in eggs and in the F$_1$ generation, 2 methods were used:

a. 30 eggs from 3 positive batches (10/batch) were selected; each egg was crushed separately in a drop of phosphate buffer and examined by dark field illumination on day 1 or 2 after oviposition.
b. 30 N$_2$ from the same 3 batches (10/batch) were fed individually on 30 clean chickens and the host blood was examined microscopically daily for 15 days for the presence of spirochetes.

The same methods were used to determine the percentage of transovarial transmission to eggs of the F$_1$ generation and to nymphs of the F$_2$ generation.