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At the end of the last century, Smith and Kilborne (1893) made clear the role of ticks in the spread of piroplasmosis among domestic animals. Later investigators discovered that the agent was transmitted thru the egg, and that the larva, nymph, and adult ticks could infect the vertebrate host thru blood sucking. However, in spite of all the studies made on the development of the piroplasmid in ticks, little is known and the data are conflicting.

The first work was done by Koch (1906) on the development of *Babesia bigemina* in *Margaropus australis*, *Rhipicephalus evertsi*, and *Hyalomma aegyptium*. He described stellate and club-shaped forms of parasites in the tick intestine. Dennis (1932), Regendanz (1936), and others reported the development of *B. bigemina* in other species of ticks, *Margaropus annulatus* and *Boophilus microplus*. They observed various stages of the parasite in the intestine and developing eggs of female ticks and in the larvae which subsequently hatched from these eggs.

Christophers (1907), Regendanz and Reichenow (1932, 1933), Shortt (1936), Brumpt (1937), and others studied the development of *Babesia canis* in *Rhipicephalus sanguineus* and *Dermacentor reticulatus*.

Martsinovskiĭ and Belitser (1908), Tsaprun (1940, 1941, 1952, 1954), Shepelev (1942), and Abramov (1955) described in different degrees of detail the development of *Piroplasma caballi* in *Dermacentor reticulatus*, *D. pictus*, *D. marginatus*, and *Hyalomma plumbeum*.

Abramov (1952) and Tsaprun (1954) studied the development of *Nuttalia* in *Dermacentor marginatus* and *Hyalomma scupense*. Markov and Kurchatov (1940), Abramov and Stepanova (1952), and Tsaprun (1952, 1954) studied the growth of *Babesiella bovis* in *Rhipicephalus bursa*. Petrov (1938, 1939, 1949) described various stages of development of *B. bovis* in *Ixodes ricinus*. He found oval, spherical, amoeboid, pear- and club-shaped forms of *Babesiella* in the intestine of female ticks which had sucked blood from infected animals. In the eggs which were laid by these ticks he found pear-shaped bodies with flagella (?), large forms of different dimensions (sporoblasts), and club-shaped forms. He also found oval and pear-shaped forms of the parasite in the salivary glands of the larvae and nymphs.

Serial sections, 5 to 7 microns thick, were cut and stained with Giemsa. The smears and sections were stained according to the method of Nocht-Maximow.

The cows for the experiment were obtained from the Kemskiĭ region of KASSR where Babesiella has never been observed because this region lies considerably north of the area where I. ricinus and I. persulcatus are found. The cows were injected intravenously or subcutaneously with blood taken from acutely ill animals. Six cows in all were infected; and in three of them, a large number of parasites was found in the blood on the third and fourth days but dropped off sharply by the 6th to 7th day.

Part of the ticks were kept for egg laying. Dry and wet smears were made from the eggs on the 5th, 12th, 17th, and 31st days. Ten to 15 eggs were crushed in a drop of physiological salt solution, and smears were prepared from this emulsion. They were fixed and stained in the same way as the smears from the intestine of the tick.

RESULTS

A small number of parasites was found in the intestine 2 to 3 hours after the ticks had been removed from the cow. They were oval, spherical, or pear-shaped bodies measuring 1 to 2 by 0.5 to 0.8 microns; the cytoplasm stained blue and the nucleus a violet-red (Fig. 1). In size and shape they resembled the forms of Babesiella found in the peripheral blood of horned cattle. In the pear-shaped bodies the nucleus is always located in the broad end, and in the oval and spherical ones the nucleus may lie in the center or at the edge of the cell (Fig. 1). Sometimes we observed dividing forms (pear-shaped bodies in pairs) similar to those found in the peripheral blood. We believe these bodies are Babesiella bovis. The number of parasites found in the intestine at this time is far smaller than the number found in the blood of the vertebrate host while the ticks are engorging. Probably the majority of the parasites were digested in the intestine and only a few continued to grow. The pear-shaped and oval stages of Babesiella remained in the lumen of the intestine up to 72 hours. During this time we did not find any forms which could be compared with the endoglobular stages of Babesiella.

Oval, spherical, or pear-shaped extra-cellular bodies (Fig. 2) were found in the hemolymph on the 2nd and 3rd days. These are similar to the ones we found in the intestine; however, multi-nucleated bodies were also present. In the pear-shaped forms two nuclei were located on each side of the broad end (Fig. 2). These can be identified as dividing forms. Because of the lateral position of the nuclei in some and in view of the fact that two uni-nucleated, pear-shaped bodies are found together, it can be assumed that these forms divide longitudinally. Besides these forms, we observed a stage which could be considered schizogony (Fig. 2). The schizonts were spherical or oval, 2 to 3 microns in diameter, and contained from one to 6 nuclei located near the periphery. Several times we saw 5 to 6 uni-nucleated, pear-shaped bodies (nearly 1 micron long) lying close together (Fig. 2). The position and shape of these forms indicate that they may be dissociated schizonts. These stages were found in the hemolymph 96 to 168 hours after the ticks had been removed from the infected cow. We could not find any stages that resembled the sexual stages found in the Sporozoa, either in the smears or in the microscopic sections.

The eggs were incubated at room temperature for 5 to 17 days. In them we found spherical and egg-shaped bodies with the nucleus located in the broad end of the latter (Fig. 3). The latter can be called bean-shaped or pear-shaped. They were 2 to 3 microns long and a small vacuole was present in some. All these stages in the egg are similar in size and shape to the stages found in the hemolymph of the tick. The greater number of these bodies, which we consider developing stages of B. bovis, were found from the 6th to the 12th day. We did not find these forms on the 30th day (temperature 16 to 18°). The conclusion can be drawn that multiplication of the babesiellas continues in the same manner in the egg as in the tissues of the adult tick.

We did not find forms which could be considered developing stages of the parasite in eggs from control ticks. However, we feel that it is important to describe those forms found in the tick which could be confused with the stages of the parasite. Buchner (1930) and Steinhaus (1949) in various insects and Mudrow (1932) in ticks described some bacteria and yeast-like forms as symbionts. These organisms multiplied in the internal organs of their hosts and the new generations were transmitted thru the egg. We sometimes found in significant numbers crescent-shaped forms of various sizes in the hemolymph, ovocytes, and eggs. These bodies are oidia of some fungus, and mycelia could be seen in some cases. The oidia measured 5 to 7 microns, contained a large, central dark-violet nucleus and two vacuoles at the periphery, had light blue cytoplasm, and were surrounded by a distinct wall (Fig. 4). It is not difficult to distinguish these from Babesiella. In the eggs of three ticks (one a control) we found spindle-shaped bodies, measuring 2 to 8 by 1 to 2 microns and containing a centrally located nucleus (Fig. 5). Frequently we observed elongate bodies, blunt at both ends, having blue-violet cytoplasm and a red nucleus. The above forms greatly resemble the spindle-, club-, and lanceolate-shaped bodies described by many authors as piroplasmids. Petrov (1949) described them as developing stages of B. bovis. We found these forms in especially large numbers in smears that contained mycelia (Fig. 5). Mycelia, broken up into spindle- or lanceolate-shaped bodies and possessing one or two nuclei were seen. Thus, in spite of the extremely close resemblance of some of these bodies to Babsiella, their origin from mycelia indicates that they are not protozoa.

Very small forms (up to one micron in size) were found in some of the ticks. They were pear-shaped, without nuclei, sometimes situated in pairs and closely resembling Babesiella. They more closely resemble those forms described from insects by Buchner (1930) and are most likely bacteria or yeast-like symbionts. Dennis (1932) probably thought that analogous yeast-like organisms were developing stages of B. bigemina (see his Fig. 51).

Besides symbionts, cells resembling schizonts are found in the hemolymph. They are spherical, nearly 5 microns in diameter and contain several small nuclei. They can easily be mistaken for schizonts of Babesiella, but differ from the latter in having strongly basophilic protoplasm, in having a wide size range, and in being present in the control as well as the infected ticks.

DISCUSSION

We observed the first part of the life cycle of B. bovis in adult female ticks and eggs. The description of the developing stages follows.

Pear-shaped, oval, spherical and ameboid forms of Babesiella liberated from the vertebrate erythrocyte in the intestine penetrate the epithelium and enter the hemolymph. Some migrate to the ovocytes, while others probably go to other tissues. Here in the internal organs of the tick, the Babesiellas undergo reproduction by binary fission or schizogony (forming not more than 6 schizonts). Those dividing by binary fission retain their original shape, and in general are similar to the endoglobular stages; whereas, those multiplying by schizogony become spherical and grow to 3 to 4 microns in diameter. The latter stages are not found in the blood of the vertebrate host. In the hemolymph, reproduction occurs extracellularly, and in the ovocytes and eggs both processes of asexual reproduction continue.

Comparing our findings with those of Petrov (1939, 1949), we are convinced that reproduction by longitudinal division and schizogony are characteristic of Babesiella. We disagree with Petrov on the nature of the large vermicular forms (6 to 8 microns) which contain a dark-violet nucleus with vacuoles on either side. Petrov described them as zygotes, but we believe that they are oidia of yeasts.

Also, Petrov considered the pear-shaped forms gametocytes and thought that they merged to form a zygote and that the pear-shaped bodies lying close together and the spherical ones containing two nuclei were the result of this process; whereas, we consider that the former undergo longitudinal division and the latter, schizogony. We did not find any forms which corresponded to the ookinetes, non-motile zyogtes, or oocysts of various Sporozoa. Judging from Petrov's illustrations, he did not find sexual stages of Babesiella either. Petrov stated that copulation of isogametes took place in the tick intestine and that a vermicular (club- or crescent-shaped) zygote was formed which penetrated the egg of the tick and eventually rounded up and underwent multiple division of the nucleus (Petrov, 1949, p. 101). If Petrov's description is correct, then why does he call the pear-shaped, lanceolate, paired lanceolate, and spherical forms in the eggs parasites? Undoubtedly these forms are asexual stages of Babesiella, and the "zygotes" have no connection with the parasite at all. Just where the pear-shaped forms with flagella (?), described by Petrov, fit into the life cycle is not known. Thus, we believe that agamous and not sexual reproduction takes place in the tick. Regendanz (1936), Regendanz and Reichenow (1932, 1933), and Shortt (1936) believed that only agamous reproduction occurs in B. canis; and Reichenow (1935) doubted that Dennis (1933) observed the sexual process in B. bigemina.

Abramov (1955) studied Piroplasma caballi in the tick and said that reproduction occurred only by "transverse and longitudinal division of the individuals, by budding, by multiple division of a single schizont into [either] a large number of spherical, angular forms or 2 to 4 (rarely more) individuals of cigar-shaped form." This author did not discuss a sexual process. On the other hand, Tsaprun (1952, 1954) described a sexual process for P. caballi. He stated that micromerozoites were present with the blood in the tick intestine, and that the majority died. The remainder penetrated the epithelial cells, rounded up, and developed into large schizonts, which formed macromerozoites. He stated that this process of schizogony could repeat itself. "Some of the macromerozoites before the sexual process temporarily unite in pairs." From them "apparently are formed macro- and microgametocytes". He stated that microgametes form on the surface of the microgametocytes, and are "apparently" supplied with two flagella. The macrogametocytes are spherical, with "globules of reserve material" in the cytoplasm, and two sporozoites are formed in the oocyst after fertilization. The sporozoites were not observed beyond this stage, but Tsaprun thought that they entered the intestinal epithelial cells, became schizonts, and formed macromerozoites which penetrated the egg cells. The latter again form schizonts which produce merozoites. These enlarge and apparently encyst. Subsequent "final development occurs in the salivary glands of the adult ticks...." When the tick begins to suck blood, the macromerozoites leave the cyst, and again develop into large- and small-nucleated schizonts. The former give rise to new schizonts, while the latter represent the infective stage.

This cycle of development does not in any way resemble any of the well-known life cycles of the Sporozoa or other protozoa. It seems to us that this cycle is not only doubtful, but more than likely does not occur. The author failed to prove its existence, and since he had no illustrations to refer to, it is impossible to know exactly what he mistook for stages of the piroplasmid. All of Tsaprun's data must be checked thoroughly, for we doubt that this cycle of development for P. caballi could realistically take place.

Thus, the existence of a sexual process has not been proved either in Babesiella bovis, or in other species of piroplasmids. In any case, none of the authors has proved the presence of the sporozoite-type of sexual reproduction. It is therefore premature to consider them Hemosporidia, which they resemble only in the fact that they are found in (or on) the erythrocytes. The true Hemosporidia (their agamonts) multiply by schizogony in the blood of the vertebrate host, while the piroplasmids multiply by longitudinal division; and, since no alternate cycle with a change in form was found, the fact that the piroplasmids are not Sporozoa is confirmed. Longitudinal division and schizogony, characteristic of Babesiella in the tick, are not stages peculiar to the Sporozoa, but are stages more typical of the flagellates or sarcodines. A similar process of reproduction is found in some trypanosomes (Trypanosoma cruzi and others).

Tsaprun's (1954) opinion has no factual or logical basis. He assumes the presence "of a phylogenetic connection of the gregarines" with Piroplasma caballi, on the basis of a temporary union "of macromerozoites" in pairs before the sexual process. This has nothing in common with the union of gamonts and the formation of gamontocysts of the gregarines.

The narrow host specificity of the piroplasmids hampers placing them with the flagellates. This is a characteristic usually not peculiar to the flagellates but is typical for many Sporozoa. This characteristic of the piroplasmids, however, could have occurred as a secondary adaptation. In addition, there is no proof that the piroplasmids have kinetids, which would indicate that they do not belong with the flagellates. Dennis (1922) described a rhizoplast in them, but this has not been definitely established.

Longitudinal division in the vertebrate and invertebrate hosts, schizogony in the tick, absence of a sexual process similar to the Sporozoa, and the absence of agamous reproduction in the blood of the vertebrate place the piroplasmids closest to the class of flagellates or sarcodines. Their position is still not definitely decided.

SUMMARY

1. Reproduction of Babesiella bovis by binary division or schizogony occurs in the tissues of the tick.

2. Stages of sexual reproduction and sporogony were not observed, and it can be assumed that only asexual reproduction takes place in the tick.

3. Forms of B. bovis were found in the developing and laid eggs of infected ticks that were similar to the forms found in the tissues of the adult tick.

4. Characteristics (except for parasitism of the erythrocytes and narrow host specificity) which would place B. bovis with the Haemosporidia or other Sporozoa are absent.

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