BARTONELLACEAE

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Bartonellaceae (1) form a group of blood parasites characterized by their smallness and their position close to red blood cells. Bartonella bacilliformis (Strong, Tyzzer, Sellards), causing human bartonellosis (Oroya fever and verruga peruana) in Peru, Colombia and Ecuador, grows on culture media and was early recognized by Noguchi (2) as a bacterium. Other bartonellae found all over the world as animal parasites that cause a latent infection activated by splenectomy cannot be cultured with certainty on artificial media and still withstand definite classification. Weinman (3) in his comprehensive monograph, although listing all bartonellae as bacteria, contrasts the biologic traits of B. bacilliformis with those of animal bartonellae. In his article with Bengtson in Bergey's Manual of Determinative Bacteriology (1) he lists three families within the order of Rickettsiales: Rickettsiaceae, Bartonellaceae, and Chlamydozoonaceae; the family of Bartonellaceae comprises four genera: Bartonella, with the only species B. bacilliformis; Haemobartonella, or animal bartonellae; Eperythrozoon; and Grahamella.

Since light microscopy is not adequate for the minute structural details of these bodies, ranging between the size of rickettsiae and that of large viruses, electron microscopy was used for B. bacilliformis of human blood (4) and cultures (5), Haemobartonella muris (Mayer) of rat blood (6, 7, 8), and Eperythrozoon coccoides (Schilling, Dinger) of mouse blood (9, 10). The results of this work and those of other authors on the systematics are summarized in this paper. The main features of the three parasites are shown in table 1.

Light microscopy shows that all bartonellae readily take the Giemsa stain. Rod shapes prevail in B. bacilliformis (figure 1), whereas H. muris has more coccoid forms (figure 2) and E. coccoides has more ring-shaped bodies (figure 3). Such rings probably arise from the process of air-drying; wet preparations seen in phase contrast microscopy suggest that E. coccoides in the circulating blood has a coccoid or vesicular form. Annular forms rarely occur in H. muris either (8).

1 We use the term for brevity with the reservations as to classification made in the text.

In electron microscopy, the contrast between B. bacilliformis and the other two species is striking. Cultured B. bacilliformis shows retracted cytoplasm and cell walls typical of bacteria (figure 4). Also like bacteria, they are rod-shaped in young cultures and mostly coccoid in older ones (5). For comparison with animal bartonellae, however, B. bacilliformis had to be studied as a blood parasite. Also, as such it is mostly rod-shaped with cell walls often visible (figure 5, (4)). Annular and coccoid particles which are seen also in light microscopy (3) must be considered degenerate forms, in contrast with the ring forms of E. coccoides. Such structures preferably occur during the final stages of infection with B. bacilliformis under antibiotic treatment and in old cultures.

H. muris shows coccoid particles in electron microscopy. The rod forms of H. muris, as seen in light microscopy, proved to be chains of coccoid particles in electron optics (figure 7). Similar findings are valid for Haemobartonella muris musculi (Schilling) (11), whose light-optical aspect is marked by rod forms. No structural details or cell walls like those of bacteria could be discerned in H. muris [(figures 7, 8 (6)]². After hemolysis, the identification of H. muris is often rendered difficult by the residues of reticulocytes present in the anemic blood. Such reticulofilamentous particles, however, are translucent and less uniform in size (13), resembling the vesicular structures recently described as “endoplasmic reticulum” (14).

Also in E. coccoides, cell walls and inner structure could not be proved (9). Although ring shapes predominated (figure 9), signet-ring, racket and comma shapes were also seen. This polymorphism is probably due to injuries of the fragile bodies during the act of mounting and does not suggest developmental stages (10).

The action of crystallized enzymes on the previously fixed microorganisms has been studied in light and electron microscopy. After trypsin

² Eyer and Ruska (12), in a paper on the morphology of typhus rickettsiae, pointed out that Haemobartonella muris does not show a cell wall like that of Rickettsia prowazekii.

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treatment, B. bacilliformis shows distinct cell walls [figure 6, (4)]; note that nearly the whole plasmatic substance appears to have oozed out, as happens with other gram-negative bacteria (15, 16). With H. muris, trypsin leaves only an ill-defined rest, not to be interpreted as a cell wall, while E. coccoides is entirely dissolved by trypsin (17).

H. muris and E. coccoides contain ribo- and deoxyribonucleoproteins which are decomposed by the combined action of nucleases and pepsin (17). An arrangement of nucleoproteins in separate areas could not be observed.

The motility of B. bacilliformis seen in the blood (18) and in cultures (5, 19) was found to be due to unipolar flagella. They regularly showed up in culture material under both types of microscopes (figure 4), whereas in blood material, so far, the presence of flagella was not demonstrated. Diameter and arrangement of flagella resemble those found in bacteria. H. muris and E. coccoides lacked motility in dark-field examinations and phase-contrast microscopy. Reports of other authors (20, 21) agree that active movements are not to be seen in these parasites; flagella are absent.

The favorite position of all three microorganisms is on the surface of red blood cells (eperythrocytic). Disputes in this respect could be definitely settled by the pseudo-replica method (6), although the possibility of some parasites entering the red cell cannot be wholly excluded.

B. bacilliformis is found in human bartonellosis not only as an eperythrocytic parasite, but also within the endothelial cells. H. muris and E. coccoides, in contrast, are strictly limited to the blood. For this reason, Tyzzer and Weinman (22) have separated animal bartonellae from B. bacilliformis and given them the generic name of Haemobartonella.

B. bacilliformis and H. muris do not pass filters retaining bacteria; the opposite is reported
for *E. coccoides* (23) as well as for *E. parvum* and *E. suis* (24). The filtrability of erythrozoa may be explained by variation in size and by structural plasticity. *H. muris*, however, although of similar size, is always attached to erythrocytes or their residues (8). *E. coccoides*, however, is also found free in the plasma (figure 3). This difference in the behavior is a further explanation for the filtrability of this organism.

*B. bacilliformis* readily grows on culture media as well as on chick embryos (25) and tissue cultures of the Maitland type (26); *H. muris* and *E. coccoides* never multiply with certainty outside the host blood (7). All authors agree that *B. bacilliformis* multiplies by binary fission. Nothing definite is to be said about *H. muris* and *E. coccoides* in this respect.

Both *B. bacilliformis* and *H. muris* cause anemia of high degrees, though the pathogenic action and accompanying symptoms differ (3). Anemia due to *E. coccoides* is an exception (27). Skin eruptions, such as verruga peruana caused by *B. bacilliformis*, are never seen in animal bartonelloses. The spleen seems to exert little, if any, influence on the course of *B. bacilliformis* infection in men and monkeys. It plays an obvious part in all animal bartonelloses and erythrozoonas, for only after splenectomy does the animal blood become infected with parasites in fair number.

*B. bacilliformis* infection in men and monkeys leads to a true immunity subject to differences in degree and duration. *H. muris* and *E. coccoides* infections, as known so far, bring about a state of premunition only. An affinity of *B. bacilliformis* and *H. muris* to rickettsiae could not be proved by serological tests. Complement-fixation reactions of bartonella and haemobartonella carriers were always negative with rickettsial antigen, and the Weil-Felix reaction showed only occasional positive titers of doubtful specificity (7, 28).

*B. bacilliformis* is transmitted by sandflies, *H. muris* and *E. coccoides* by lice. Since lice become infected experimentally with *B. bacilliformis* only by the intracelomic injection (29), the human louse most probably will not act as a vector of *B. bacilliformis*. The strictly regional occurrence of verruga peruana and Oroya fever may be due to limited habitats of the transmitting sandfly. No such restrictions exist for *H. muris* and *E. coccoides* infections, which are found everywhere.

*B. bacilliformis* can be transmitted with certainty to monkeys only; cultured *B. bacilliformis* and tissue taken from verruga peruana are more virulent for monkeys than blood bartonelloses which merely produce a latent infection. Laboratory rodents such as mice, rats, and Syrian hamsters (8, 10) are readily infected by blood containing *H. muris* or *E. coccoides*.

As to chemotherapy, it is known that organic arsenical substances, such as nearsphenamine, and also arseneic-antimony compounds do not act in infections with *B. bacilliformis* (3), but have a marked influence on *H. muris* as well as on *E. coccoides*, and may effect complete disappearance of parasites. All three parasites are refractory to sulfa compounds (30, 31, 32, 33). The antibiotics cited in table 1 act bacteriostatically on *B. bacilliformis in vitro* (31). Penicillin (34), streptomycin (35), and chloramphenicol (36) show a curative effect in *B. bacilliformis* infections. To date, no reports have been made about the clinical action of aureomycin and terramycin. *H. muris* and *E. coccoides* are resistant to penicillin and streptomycin (33, 37), but not to aureomycin (7, 33, 38) and terramycin (33, 39). Chloramphenicol has little, if any, effect. The action of chemotherapeutic agents is remarkably uniform against *H. muris* and *E. coccoides*, in contrast with their action against *B. bacilliformis*.

**DISCUSSION**

As has been outlined, the morphology and biology of *Bartonella bacilliformis* differs notably from that of *Haemobartonella muris* and of *Eperythrozoon coccoides*. *B. bacilliformis* has characteristics typical of bacteria, including: size and form, growth on culture media, propagation by binary fission, flagella, cell walls, and behavior in serological tests. Contrary to the opinion of Lwoff (40) no just criteria exist to classify *H. muris* and *E. coccoides* among bacteria. Haemobartonella and eperythrozoa can be set apart from protozoa on account of their small size and lack of cellular structure. The pleuropneumonia-like organisms (PPLO) resemble haemobartonella and eperythrozoa in their coccoid and annular shapes visible with both types of microscopes (41, 42, 43). They differ, however, in that they can be grown on culture media.

A relationship to rickettsiae often supposed because of analogies in size and transmission by insects may be excluded. In fact, rickettsiae definitely differ from *B. bacilliformis*, which is
flagellated and can be cultivated on artificial media, and also, in structural details, from H. muris and E. coccoides. Serological relations are lacking and, moreover, multiplication of bartonellae in the insect vector is questionable. Transmission by insects is too common in protozoa, bacteria and viruses to form a criterion for taxonomy.

The electron-optical findings of de Robertis and Epstein (44) in Anaplasma marginale suggesting a likeness to H. muris are not sufficient to link haemobartonellae to the anaplasma group as proposed in older publications (45). Relations to grahamellae, which can be cultivated on artificial media, are likewise questionable.

It is tempting to compare H. muris and E. coccoides with viruses on account of their small size, which allows E. coccoides to pass filters that retain bacteria, and because of their inability to grow on culture media. Yet viruses of the psittacosis (46) and pox group (47, 48), approaching haemobartonellae in size, are structurally different. They possess cell walls that are missing in H. muris and E. coccoides, and, moreover, nothing is known about an intracellular multiplication, which is essential for viruses.

At present it seems advisable to reserve a special place within the system of microorganisms for haemobartonellae and eperythrozoa and to set animal bartonellae apart from B. bacilliformis than has been customary up to now. Although B. bacilliformis must keep its place among bacteria, H. muris and E. coccoides should be excluded. It is to be presumed also that other species of haemobartonellae and eperythrozoa resemble H. muris and E. coccoides as described in this review.

Since the characteristics of H. muris and E. coccoides reported in table 1 correspond in nearly every respect, it must be questioned whether two generic names are justified by trifling differences in morphology.

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REFERENCES


Nucleasen und Proteasen. Z. Naturforsch., 9b, 585-596.


PLATES I–IV
Figure 1. Bartonella bacilliformis, human blood, light microscopy, Giemsa stain. [Original published in Z. Tropenmed. u. Parasitol., 4, 539–548, 1953 (4)]

Figure 2. Haemobartonella muris, Syrian hamster blood, Giemsa stain. [Original published in Z. Tropenmed. u. Parasitol., 3, 437–452, 1952 (8)]

Figure 3. Eperythrozoon coccoides, mouse blood, Giemsa stain. [Original published in Z. Tropenmed. u. Parasitol., 3, 461–472, 1952 (10)]
Figure 4. Bartonella bacilliformis from 7 days' culture, electron microscope, Pd-shadowed. Rod-like parasites, retracted cytoplasm, cell walls and flagella. [Original published in Z. Tropenmed. u. Parasitol., 3, 313–326, 1952 (5)]

Figure 5. Bartonella bacilliformis, pseudo-replica from human blood. Thin blood smears are covered by collodion films which are taken off by diluted hydrofluoric acid and transferred to electronoptical grids. The erythrocytes have left their impression on the film as less opaque "negative" areas, while the parasites stick to the film and appear "positive." Rod with cell wall and "annular" form on erythrocyte replica. [Original published in Z. Tropenmed. u. Parasitol., 4, 539–548, 1953 (4)]

Figure 6. Bartonella bacilliformis, pseudo-replica, Chabaud fixation, trypsin-treated. Empty cell walls, marginal folds. [Original published in Z. Tropenmed. u. Parasitol., 4, 539–548, 1953 (4)]
Figure 7. *Haemobartonella muris*, attached to red cell ghost after osmotic hemolysis, Pd-shadowed. Coccosid particles without structure, partly in chains. [Original published in Z. Tropenmed. u. Parasitol., 3, 437-452, 1952 (8)]
Figure 8. *Haemobartonella muris*, pseudo-replica. Parasites on erythrocyte replica. [Original published in Z. Tropenmed. u. Parasitol., 3, 437-452, 1952 (8)]

Figure 9. *Eperythrozoon coccoides*, pseudo-replica. Annular parasites on erythrocyte replica. [Original published in Z. Naturforsch., 6b, 326-333, 1951 (9)]