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REVIEW ARTICLE

The quest for piroplasms: from Babeş and Smith to molecules

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Abstract. Throughout history, several deadly plagues of cattle were attacking animals around various parts of the world. Among all, perhaps the most intriguing was the "*blood urination*", currently known as babesiosis. Two distinct geographical areas (Eastern Europe and North America) are particularly important from historical point of view. At the end of the 19th century (1888), Victor Babeş, a Romanian microbiologist, discovered in the agent of the disease in cattle. One year later, Theobald Smith, a medical doctor from New York, described the agent of Texas fever in USA and was the first to elucidate the tick-borne nature. It was the first time ever when arthropods were shown to transmit a disease.

Keywords: Piroplasms; Victor Babeş; Theobald Smith; *Babesia*; Babesiosis.

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Throughout history, several deadly plagues of cattle were attacking animals around various parts of the world. Among all, perhaps the most intriguing was the "*blood urination*". Seemingly, the first written report of a disease in cattle with clinical signs consistent with „bovine blood urination" is found in the Bible. In Exodus 9:3, cattle of Pharaoh Rhamses II, together with other livestock, were to be attacked by *"a very grievous murrain*" (Gelfand, 2000). It is debatable if this plague was indeed what we know today as babesiosis, but even nowadays, the word "*murrain*" is still used in some parts of Ireland to describe the red-water fever of cattle (Dammin, 1978). Moreover, in USA, blood urination of cows was also known as "*bloody murrain*" (Archibald, 1898). However, according to several medical dictionaries, "*murrain*" of cattle could also refer to rinderpest.

Even if "blood urination of cattle" was probably spread worldwide, two distinct geographical areas (Eastern Europe and North America) are particularly important from historical point of view. It was not until the end of the 19th century that a Romanian microbiologist, Victor

Babeş, discovered in Romania the agent responsible for what we know today as cattle babesiosis (Babeş, 1888). One year later, Theobald Smith, a medical doctor from Albany, New York described the agent of Texas fever in USA (Smith, 1889) and elucidated the tick-borne nature (Smith and Kilborne, 1893).

In Europe, the disease was known from France and Spain, through Germany, up to Finland and Norway. The main symptom was haemoglobinuria, and it was believed that the disease was caused by different poisonous plants (Köhler and Köhler, 2003).
In Romania, the disease was known for ancient times as "*blood urination*", and since 1874 it officially drew the attention of the State Veterinary Services. In the same year, Măgureanu published the first epidemiological overview, reporting the disease in 20 villages and stating that the condition was different than rinderpest. At Măgureanu's suggestion, the Romanian Government appointed a special commission to investigate this disease. The commission, formed by three vets, named the new disease "*gastroenteronephritis*", based on the main pathological findings. An official Romanian report stated that in 1879, more than 50,000 cattle died in the country because of this disease of unknown etiology. Following this report, in 1883, a permanent commission including three vets, one chemist and one botanist was founded by the time's Government. First observation of the commission was that "*gastroenteronephritis*" was more common in wet environments, leading to the conclusion that the condition was similar to malaria (Cernăianu, 1957). In 1880, another researcher, Constantinescu, strongly suggested that the cause of this disease might be an infective microorganism and Vasilescu noted the higher predisposition of animals newly introduced to certain areas in contrast to an apparent resistance of local ones (Cernăianu, 1957).

In 1884, during the Veterinary Congress in Romania, due to the high economical impact of the disease, the third commission was created, this time with Victor Babeş as the head investigator. After accurately describing the symptoms and lesions, Babeş and his team successfully transmitted the disease by injecting blood from sick to healthy cows (**Cernăianu, 1957**).

After several years of research within the Institute of Pathology and Bacteriology, in 1888, Babeş noted intracellular, round, often paired microorganisms in the red blood cells of the sick cows. But Babeş did one crucial mistake: he considered them to be bacteria and named the new species *Hematococcus bovis* (currently *Babesia bovis*). Monsieur Bouchard presented Babeş's results on 29 October 1888 in the Academy of Sciences from Paris (Babeş, 1888) bringing some blood smears to show to the audience. The original publication was without figures but subsequent reprints were accompanied by two black and white drawings (figure 1) (Köhler and Köhler, 2003), later published in color in the famous Virchow's Archiv (Babeş, 1889) (figure 2). In these two publications, Babeş accurately describes the morphology of the erythrocytic stages and differential diagnosis.

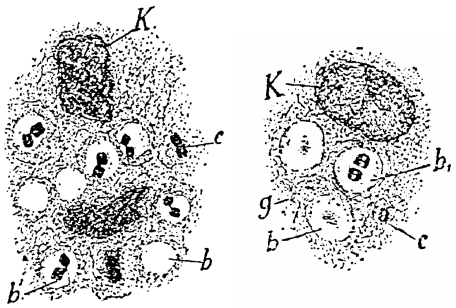

Figure 1. First line drawings of *Babesia* published by Babeş

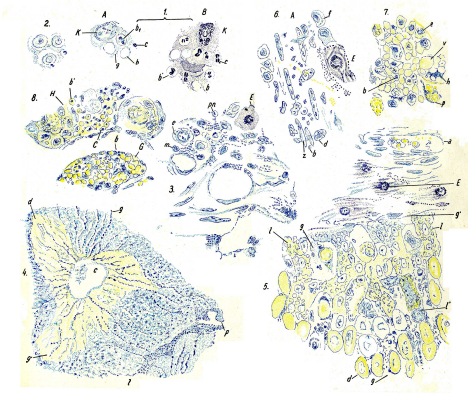

Figure 2. First color drawings of *Babesia* published by Babeş.

Overseas, in USA, another plague of cattle was puzzling the scientists. Known through time under various names (Southern cattle fever, splenetic fever, Spanish cattle fever, Texas cattle fever, Carolina cattle distemper, bloody murrain, Mexican cattle disease, Indian cattle disease, red water, haematuria, splenic fever, tick fever, acclimatization fever etc.), it seems the disease was brought to North America by Spanish colonists as early as 16th century (Archibald, 1898). If this was true or not, one might argue, but a certain fact is they spread it. At the beginning of the 18th century, when Spanish colonists moved with their animals from South to North, they brought to the cattle of English colonists a deadly gift, as Archibald (1898) shows: "*The Spanish invader on the continent was responsible for many things, but could he planned, as diligently as he did inquisition methods, to have left behind him a cattle plague, which would harass his foemen, he could scarcely have found a better means than by distributing this disease which time has proved to have been the greatest known curse to the stock industry of our southern States*".

There is relatively high amount of data on Texas fever published prior to the description of the agent of the disease, by Smith and Kilborne in 1893. As early as 1796, Plase showed how the Texas fever spread from south into Pennsylvania (Cernăianu, 1958). A report from 1868 shows in great extent the lesions of the Texas cattle disease with probably the first graphical output of lesions (figure 3) (Baumgarten, 1868). In 1882, the US Department of Agriculture publishes an extensive report with detailed clinical and pathological findings as well as about the spreading pattern of the disease in certain states (***, 1882).

The epidemic character of the disease suggested to the scientists the involvement of an infectious agent. A report on the pathology of Texas cattle fever was probably the first documentation of microscopical alterations of red blood cells (Stiles, 1868), but no intraerythrocytic infective agents were found. However, in the bile ducts of infected cattle Stiles found some "*micrococci*", described later as a new species of fungus, *Coniothecium stilesiarum* (Hallier, 1868). Billings and Curtis (1871) criticized these observations, considering that fungi can easily contaminate any sample. Another researcher named Billings (note that the two authors named Billings were different: one was John S., the other Frank S. - see references for details) claimed to have discovered the "true germs" of Texas cattle fever, and considered them to be similar to those causing hog-cholera: "*Hence the germ of Southern cattle plague has been discovered, and I think that I may be pardoned to egotism of claiming this to be the first occasion in American medicine that not only one but two germ diseases of animals have been traced out and their origin placed upon an impregnable basis*" (Billings, 1888). Smith (1889) and Smith and Kilborne (1893) strongly criticized all these findings: "*it is impossible to discover from the descriptions anything concerning the nature of the germ, except perhaps that it does not exist*" (Smith and Kilborne, 1893). And they were right to do so…

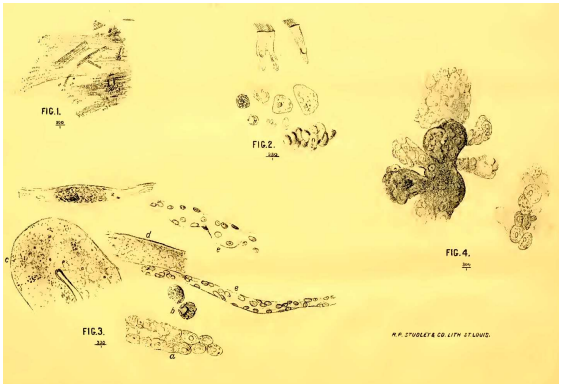

Figure 3. Lesions in the muscles, trachea, kidneys and lungs of cattle suffering of Texas fever (after Baumgarten, 1868)

Theobald Smith was the first to find a round or oval organism inside the red blood cells of cattle affected by Texas cattle fever (Smith, 1889). After four more years, Smith together with Kilborne described the new species responsible for Texas fever and named it *Pyrosoma bigeminum* (currently *Babesia bigemina*). They correctly included it among protozoans and brilliantly elucidated its tick-borne nature (Smith and Kilborne, 1893). It was the first time ever when arthropods were shown to transmit a disease. In the same year, Starcovici erects genus *Babesia* to include both

species known at that particular time (Starcovici, 1893). As the genus name *Pyrosoma* was preoccupied when it was proposed by Smith and Kilborne in 1893 (genus *Pyrosoma* Péron, 1804 is a genus of free-floating colonial tunicates), *Babesia* is the valid name for the genus (Uilenberg, 2006). Other generic names were used since then, including *Piroplasma* and *Babesiella*, but subsequently all were synonimized to *Babesia*. The name ''piroplasm'' derives from the pear-shaped aspect of the intraerythrocytic stages (Uilenberg, 2006).

However, all reviews which refer historically to the discovery of piroplasms, omit one very interesting fact. In 1889, an Italian microbiologist (Count Vittore Trevisan) used the genus name *Babesia* for two species of bacteria (Trevisan, 1889). The first, indicated as the type species was *B. xanthopyrethica*, referring to the bacteria found by Victor Babeş in 1893 from cases of human yellow fever (Babeş, 1883). The second, *B. erysipeloides* was used by Trevisan to name the agent of human erysipeloid as described by Rosenbach (1887). In 1954, genus *Babesia* Trevisan, 1889 was rejected by the International Committee on Systematics of Prokaryotes (***, 1954).

A scientific debate on the priority of the discovery started soon between Babeş on one side and Smith and Kilborne on the other side, as epically shown by Köhler and Köhler (2003). In 1903, Babeş published a review on the piroplasms of domestic animals where he claims priority on his discovery: "*I decided to take again the word [...] to defend myself against some researchers who [...] criticize my research*". Indeed, Babeş was the first to actually see and describe the agent of cattle blood urination, but he erroneously considered it to be a bacterium, as himself shows: "*The objections to my results partly rest on insufficient information though my studies were published manifold in Romanian, French and German, and on the other hand because my papers were often too short ones, although my paper [...] published in January 1889, that is before Th. Smith's first announcement, is rather long and contains many figures. But even this publication seems to be read seldom...*" (Babeş, 1903).

The discoveries of Babeş and Smith have opened new insights in the medical research. A disease, similar to blood urination, known as "*cârceag*" or "*cârcean*" caused massive losses in sheep along Danube plains. The first description of this condition in ovine hosts dates back to 1880, when Focşa noted the similarities with the cattle blood urination in a sheep flock, near Constanţa, Romania. The disease was mentioned again in 1884 by Măgureanu at the Romanian Veterinary Congress (Cernăianu, 1958). Babeş was the first to describe this parasite as an endoglobular bacterium, *Hematococcus ovis* which he also succeeds to transmit experimentally (Babeş, 1892). Motaş, another Romanian researcher, was the first to extensively study "cârceag" (Motaş, 1902; 1904) and to prove the tick-borne character of it (Motaş, 1903). Wenyon (1926) suggested that this organism should be called *Babesia motasi*. In 1925, Lestoquard showed that in sheep, there are two different species (Cernăianu, 1958), and named the second one *Piroplasma ovis*.

Since these early discoveries, many different genera and species were described from various wild and domestic animals. It was in the second half of the 20th century when Skrabalo and Deanovic (1957) described the first human case.

To date, more than 100 species are known from mammals and birds, and their identification and taxonomy are based mainly on morphology and aggregation pattern of blood stages, location of initial merogony, host specificity (both vertebrate and vector) and susceptibility to drugs (Hunfeld et al., 2008; Chauvin et al., 2009).

Morphologically, piroplasms are traditionally divided into small species (length of intraerythrocytic stages between 1 and 2.5 μm) and large species (2.5-5.0 μm). Interestingly, during the molecular era of parasitology, phylogenetic characterization based on 18S rDNA sequences, showed that the two morphological groups are part of two distinct phylogenetic clusters (Hunfeld et al., 2008).

Genome was sequenced for several species of *Babesia*, mostly for the ones with zoonotic importance. Babesiosis is considered an emerging disease by several authors, with high clinical importance in humans and domestic animals. The new current perspective of global warming and the geographical spreading of tick vectors, makes the disease highly attractive to scientists, as it was during Babeş and Smith. It seems everything and nothing changed during the 122 years of *Babesia*'s history.

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