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Laurence Malandrin

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Equine piroplamosis: epidemiology of the disease in Europe.

Laurence Malandrin, UMR BIOEPAR, INRAE, Oniris, Nantes F-44300, France.

1- Introduction

Equine piroplasmosis is a tick-borne disease of equids (horses, donkeys, mules, and zebras), and is caused by two protozoan parasites, *Babesia caballi* and *Theileria equi* (formerly *Babesia equi*). Equine piroplasmosis is the most prevalent tick-borne disease in equids and has a wide geographical distribution being endemic in most equine-habited parts of the world, with cases reported from Central and South America, Cuba, Europe, Asia and Africa. The few non-endemic countries (Australia, Canada, Great Britain, Ireland, Japan, New Zealand and USA) sometimes impose strict regulation on horses' importation to prevent the introduction of carrier animals (Wise et al. 2013, Tirosh-Levy et al. 2020). Equine piroplasmosis is responsible for important economic losses to the equine industry due to the treatments and their side effects, the decrease in performance of the animals or the negative impact on the horse international racing industry (Knowles 1996).

Clinical signs of equine piroplasmosis are not pathognomonic, and vary from sub-clinical (apathy, loss of appetite, poor exercise tolerance) to severe (fever, anemia, jaundice, haemoglobinuria, petechial hemorrhages of the mucous membranes). These symptoms are variable, non-specific, and infections with either *B. caballi* or *T. equi* cannot be distinguished clinically. The infection can also be asymptomatic. Equids may be infected without development of clinical signs. Infected animals recovering from acute or primary infection with *T. equi* remain life-long carriers, whereas horses infected with *B. caballi* may remain carriers for up to 4 years. Even with an appropriate treatment, only *B. caballi* can be cleared (de Waal 1992). This biological difference greatly influences the maintenance and spread of the disease as carriers represent a permanent source of infection for ticks.

2- Equine piroplasmosis: a vector transmitted disease

The two parasites responsible for equine piroplasmosis, *B. caballi* and *T. equi*, are transmitted by a very diverse range of *Ixodidae* tick species, depending on the geographical area. Equine piroplasmosis incidence therefore depends on the distribution of the vectors of each of the two parasites. About 33 tick species belonging to six genera have been implicated as vectors. In Europe, *Dermacentor reticulatus* / *D. marginatus* in the northern Europe and *Hyalomma marginatum / Rhipicephalus bursa* in the southern Europe are the main vectors. However, *Ixodes ricinus* (Italy), *Hyalomma detritum* (= *scupense*?) and *H. dromedarii* (Western Europe), *Rhipicephalus sanguineus* (Southern Europe), and *Haemaphysalis punctata* are also suspected to be vectors (Scoles and Ueti 2015, Onyiche et al., 2019). However, their vectorial competence needs to be experimentally proven, as the vectorial suspicion relies solely on parasite DNA detection in ticks.

3- Prevalence of equine piroplasmosis in Europe

3.1- Serological versus molecular detection to address *T. equi* and *B. caballi* prevalences

Serological tests are indirect tests, they detect antibodies produced by the infected animals. As antibodies persist after infection clearance, they do not distinguish infected from cured animals, leading to an overestimation of prevalence. Molecular tests are direct tests, generally detecting parasite DNA. As the quantities of circulating parasites can be low in the case of asymptomatic carriage, these methods may underestimate prevalence.

Serological tests are very diverse in terms of technics and antigens used (CFT and IFAT with the use of non-standardized antigens, ELISA with specific but possibly variable antigens), that therefore influence differently their specificities and sensitivities. Molecular analysis, even if diverse technics are used (PCR, nested PCR, quantitative PCR, multiplex PCR, LAMP PCR, reverse line blot…), is based most of the time on the molecular amplification of the same gene target, the 18S ribosomal DNA. The presence of highly conserved and variable regions in this gene allows at the same time its amplification despite sequence heterogeneity, and further evaluation of this diversity through amplicon sequencing. The importance of this feature will become evident in the next section on the genetic diversity of both parasites.

3.2- A great diversity of equine piroplasmosis prevalences in Europe

In this section, the author will focus only on the molecular assessment of equine piroplasmosis prevalence.

Data on equine piroplasmosis prevalence are available from 15 countries in Europe (Nadal et al. 2021, Coultous et al., 2020). The first trend is a higher prevalence of *T. equi* (0 - 68%) compared to *B. caballi* (0 - 8.3%). This difference might be explained by the ability of horse immunity and treatment to clear *B. caballi* compared to the life-long persistence of *T. equi*. The second trend is the higher prevalence of both parasites in southern countries (Portugal, Spain, Italy, Romania, Hungary, Serbia with equine piroplasmosis prevalence higher than 30%) compared to the northern countries (Ireland, Netherlands, Poland, Slovakia with prevalence lower than 5%). The third trend is the great heterogeneity of prevalences within a country according to the study sites. For example, in Italy, equine piroplasmosis prevalence varies between 11.7 to 70.3 and in Spain between 3.9 to 66%. These differences may be related to horse management or geographical location, that influence tick contact or abundance respectively.

4- Genetic diversity of both parasites

With the first studies using molecular detection of equine piroplasmosis and subsequent sequencing of the 18S rDNA (Nagore et al. 2004; Criado-Fornelio et al., 2004), the unsuspected genetic diversity of both *B. caballi* and *T. equi* was revealed. After around 20 years of studies from different countries around the world, an ever-increasing quantity of 18S rDNA sequences available as well as a few complete sequenced genomes, a more accurate picture of this diversity is emerging (Tirosh-Levy et al., 2020).

B. caballi can be divided into 3 genotypes, named A, B1 and B2. Genotype A predominates worldwide.

T. equi is divided into 5 genotypes, A to E, distributed worldwide. A geographical repartition of these genotypes is not clear. Genotypes A and C are present worldwide, while genotype E seems to have an eurasian preference, and genotypes B and D an African preference (Tirosh-Levy et al., 2020). However, this distribution needs to be refined, and is blurred by the genetic analysis of parasites from infected horses that remain carriers for life and are moved geographically from one country to another.

Recently, a novel equine *Theileria* species, *T. haneyi*, has been proposed on the basis of genome sequencing (Knowles et al, 2018). This named species seems in fact to correspond to the genotype C described worldwide. The five *T. equi* genotypes may indeed correspond to different species, but at present, we have no life traits that we could be linked to these cryptic species: neither pathogenicity, nor geographical distribution, nor vectorial transmission, nor any other aspect of the disease.

Conclusively, even if we have no evidence of it yet, genetic diversity may play an important role in influencing disease transmission and pathogenicity. However, it can greatly impact the degree of sensitivity of the diagnostic tools, especially the serological diagnostic based on highly variable antigens.

5- Are equids the only hosts of *T. equi*? Discussion about their epidemiological relevance.

T. equi is described as a parasite infecting equids (horse, zebra, mule and donkey). However, the use of molecular piroplasmosis diagnostic and subsequent sequencing have highlighted the presence of *T. equi* on non-equid hosts. The most frequently described host is the dog, symptomatic or asymptomatic, in very diverse countries in Europe as well as outside Europe (Croatia, France, Paraguay, Romania, Spain) (Oniyche et al, 2019). As a widespread domestic animal prone to canine piroplasmosis, the dog has been the subject of numerous studies, which may explain why *T. equi* has been described so frequently in this animal. However, studies have also shown *T. equi* to infect camel as well as waterbuck (*Kobus ellipsiprymnus defassa*) (Githaka et al., 2014). We may only be at the beginning of the discovery of the host range of *T. equi*, and for the moment we may have only uncovered the tip of the iceberg.

The epidemiological relevance of these hosts needs to be studied, as they often share the same vectors. For example, *Dermacentor reticulatus* and *Rhipicephalus* spp. are frequent tick species on dogs as well as on horses in Europe. The accidental infection of dogs by *T. equi* infected ticks may therefore occur in areas where both animals are present. However, it has yet to be demonstrated that dogs play an epidemiological role in the *T. equi* biological cycle. One of the most critical steps in the transmission of Piroplasmas is the production of presexual stages (gametocytes) in the vertebrate host. These stages can then be transmitted to the tick during the blood meal and are the only ones that can evolve within the vector to ensure vectorial transmission (transstadial as well as trans-ovarial) (Jalovecka et al., 2018). Some parasites may be able to multiply in erythrocytes of a vertebrate host, but not to differentiate into gametocytes, and therefore not to be transmitted by ticks. Such hosts constitute epidemiological dead ends.

6- Equine piroplasmosis and the PiroGoTick project in France

The PiroGoTick project has started in 2020 for at least 5 years (www.pirogotick.fr in French, and Pirogotick Facebook page). It is supported financially by three French founders: IFCE, Fonds Eperon and France Futur Elevage. With a central activity at Nantes in the UMR BIOEPAR, the project brings together staff from the 4 french national veterinary schools, veterinary students and uses citizen science to cover the whole of mainland France.

The aim of the PiroGoTick project is to gain knowledge at the same time on parasites and vectors involved in equine piroplasmosis. The project encompasses the evaluation of prevalence using molecular detection tools, the characterization of the genetic diversity of the French *T. equi* and *B. caballi* isolates on the basis of multiple molecular markers, and the inventory and spatio-temporal analysis of ticks from outdoor horses in different regions of France. It has been divided into several programs.

The PiroQuest program aims to assess and compare the prevalence of carriage of *T. equi* and *B. caballi* in 4 French regions and determine the 18S rDNA genotypes present in France. This study involved 566 asymptomatic equids living at least part of the time outdoors and received at the 4 national veterinary schools for various reasons (lameness, reproduction, etc). The overall prevalence of equine piroplasmosis carriers was 38%, dominated by *T. equi* carriers (37%) and presence of a few carriers of *B. caballi* (3.4%). We highlighted a prevalence difference between regions with less impacted horses in the northern part of France (Paris 18.6%) compared to the Southeastern part (Lyon 56.1%). Most of the positive samples were sequenced generating 199 *T. equi* 18S rDNA partial sequences and 19 *B. caballi* partial sequences. The E genotype of *T. equi* is predominant in France (98% of isolates) with some A genotypes. For *B. caballi*, only genotype A has been detected (Jouglin et al., 2023).

The PiroTick and Pirosentinel programs focus on ticks from horses, to perform a combined inventory, spatio-temporal and abundances analyses of ticks linked to the horse carrier status (Malandrin et al., 2022). For these programs, we are using citizen science to collect ticks from all over the country and volunteer veterinary local practitioners to perform blood sampling. Two very different levels of involvement are proposed. The PiroTick program involves sending ticks on a one-off basis, while the PiroSentinel program involves monitoring over time. The combined two programs have enabled an inventory to be made of the ticks mainly found on horses in France and their period of activity to be pinpointed.

7- Conclusion and take-home messages

Even if equine piroplasmosis has been described for decades, we just begin to uncover the complexity of these parasites. There is still so much to discover on the two parasites, their genetic diversity, their interactions with the host target cells, their host range and their vectorial transmission in order to implement reliable and efficient diagnostic methods on a worldwide scale and to move forward on the road to a vaccine.

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