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Looking in ticks for human bacterial pathogens

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ABSTRACT

Ticks are considered to be second worldwide to mosquitoes as vectors of human diseases and the most important vectors of disease-causing pathogens in domestic and wild animals. A number of emerging tick-borne pathogens are already discovered; however, the proportion of undiagnosed infectious diseases, especially in tropical regions, may suggest that there are still more pathogens associated with ticks. Moreover, the identification of bacteria associated with ticks may provide new tool for the control of ticks and tick-borne diseases. Described here molecular methods of screening of ticks, extensive use of modern culturomics approach, newly developed artificial media and different cell line cultures may significantly improve our knowledge about the ticks as the agents of human and animal pathology.

1. Introduction

Ticks are small arthropods in the order *Ixodida*, subclass *Acarina*. All ticks are ectoparasites highly specialized on hematophagy. Mammals, birds, reptiles and amphibians' blood is the only source of nutritional support of tick's body for both sexes and all developmental stages. Order *Ixodida* comprise three families: *Ixodidae* (hard ticks) with more than 700 officially recognized species [1], *Argasidae* (soft ticks) comprising roughly 200 species and *Nuttalliellidae* with a single species [2].

They occur worldwide and are capable of transmitting a broad range of human and animal pathogens including viruses, bacteria, protozoa and helminths, of which most have a life cycle that requires passage through the vertebrate host. Some of these pathogens are of exceptional importance because of high morbidity and mortality (in both humans and animals), long-lasting or permanent neuropsychiatric sequelae and impact on animal production [3,4].

2. Identification of ticks

The identification of tick species is a primary task in epidemiological studies of tick-borne diseases (Fig. 1). Morphological identification using standard taxonomic keys for endemic species in specific geographic regions is the oldest and very reliable approach. Since the birth of acarology, taxonomic keys were developed for specific regions of the world [5], hosts [6] and taxonomic phyla [7]. However, damaged ticks and immature stages may be very difficult to identify: the typical example is the ticks of *Rhipicephalus* (*Boophilus*) genus whose identification is based on the counting of the number of columns of the hypostomal teeth. Once the attached tick is removed leaving its hypostome inside the animal's skin, the morphological identification may be very difficult.

Molecular methods were also developed to identify arthropods, including ticks. Mitochondrial markers (mitochondrial 12S and 16S ribosomal DNAs, cytochrome oxidase subunit 1 [COX1], cytochrome b) are usually easy to amplify and sequence, but the degree of intraspecies difference is rarely enough to distinguish the phylogenetically close species [8,9]. Nuclear markers (18S, 28S, internal transcribed spacers 1 and 2) have also been used, but only entire mitochondrial genome seems to be enough for phylogeneitc studies [10,11]. In addition to the technical and logistical drawbacks of PCR assays, this approach is further limited by the availability of gene sequences in genetic databases.

Protein profiling by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is now increasingly common for the routine identification of microorganisms in clinical microbiology [12]. In entomology and acarology, the MALDI-TOF MS approach was first applied to arthropods for the differentiation of *Drosophila* species [13]. Nowadays, this method,

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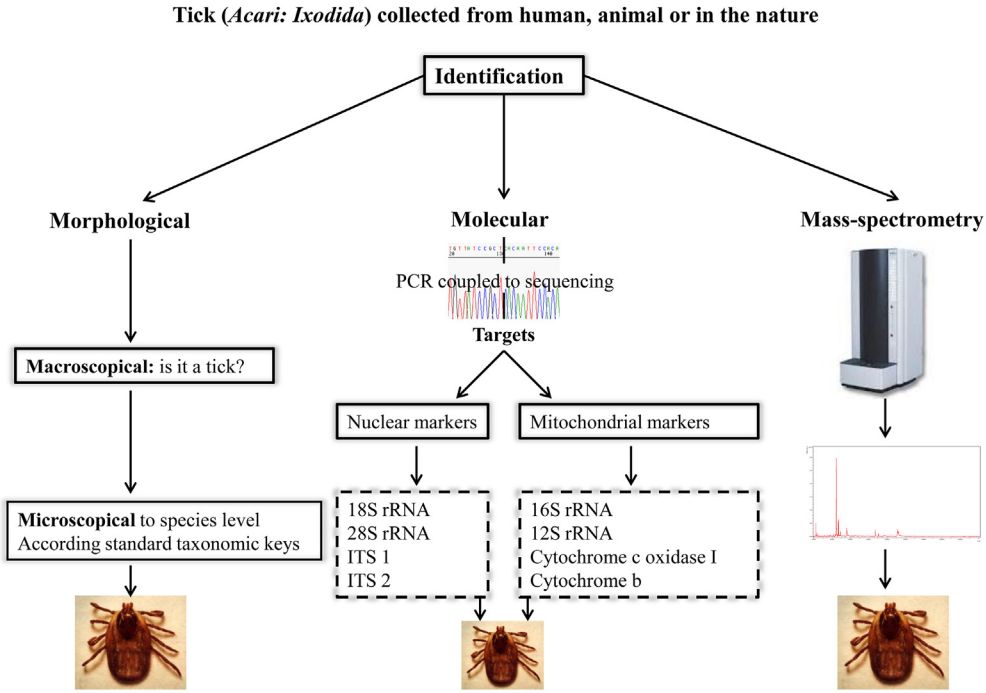

Fig. 1. Schema of identification of the tick species.

still developing, is quite promising as a cheap, rapid and quite reliable alternative for the identification of ticks [14,15].

3. Research of pathogenic bacteria in ticks

Identification of pathogenic bacteria in ticks using the approaches of molecular biology is one of the most often used and important tools in researches in infectious diseases. Sometimes, the search of certain pathogens in a tick may be important in clinical diagnostics, for instance, when the tick is removed from the patient. Identification of pathogenic bacteria (for instance, Lyme disease causing *borreliae*) in the ticks removed from humans may be necessary for the prophylactic antibiotic treatment to prevent Lyme disease [16,17].

However, mostly the research of pathogenic bacteria in ticks is performed in order to discover or precise the epidemiology of tick-borne diseases including the identification of vectors and risk areas for certain bacteria. The identification of tick vectors may play important role in preventive measures against tick-borne diseases.

4. Molecular studies: general approach

The first step of the identification of bacterial pathogens in ticks is usually based on PCR screening (Fig. 2). The choice of the primers used in the PCR entirely depends on the goals. In rare cases when the bacterial pathogen is well known and phylogenetically isolated as *Coxiella burnetii*, the screening with specific primers may be of use [18]. In many cases a bacterial genus contains many pathogens and the researcher may not know which exactly is the species that he or she may find in ticks. In such cases the approach include the screening with conventional or real-time PCR (qPCR) using the primers with broad specificity capable to produce the positive results in case of presence of all known (and even potentially unknown) species of the certain genus. It is the case of, for example genera *Rickettsia*, *Borrelia* and *Bartonella*. Further identification of the exact species relies on either following species-specific (q)PCR or the amplification of the relatively large portion of a gene, its sequencing and the search for its identity in one of the genetic databases.

5. Group-specific and degenerate primers

Standard PCR coupled with amplicon sequencing provide an invaluable tool for detection and identification of bacterial pathogens in any kind of sample, including ticks. Specificity of the reaction depends on complementarities of primers used in the PCR to the actual gene sequence of the researched bacteria. The specificity of primer(s) is a variable value that may be changed according to the goal of the research.

Depending on the goals, sets of primers for conventional or qPCR previously reported may be used or may be designed. The scheme (Fig. 3) shows the common stages for the development of the broad range set of primers for the PCR. Briefly, the target gene should be quite conservative and be present in all organism of researched group (usually, housekeeping genes are good candidates). No genes with documented lateral gene transfers are accepted in order to evade the false positive results. The gene should contain the highly conservative sites in order to give the possibility to design the primers capable to hybridize with all or majority of bacteria from the group. However, a certain degree of variability is necessary; otherwise the BLAST search of the obtained sequence may not successfully identify the species. Optionally, the existing of the large database is quite important; otherwise the researcher should, however, identify and depose the sequences of most important species in the group in the genetic database. Growing number of the bacterial genome sequences available significantly facilitate the identification of bacteria.

Once the gene is selected, the available sequences (often extracted from the genomes) are to be aligned with appropriate

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