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REGULAR ARTICLES

Survey of *Anaplasmataceae* bacteria in sheep from Senegal

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Abstract
Purpose The authors studied the role of bacteria belonging to *Anaplasmataceae* family as the causes of acute illnesses of sheep in West Africa.
Methods We examined and sampled 120 febrile sheep in two regions of Senegal for this study. The DNA extracted from these blood samples was tested by PCR using two pairs of primers (*groEL*-based and 16S rRNA gene-based).
Results In 52/120 samples, the microscopic examination revealed intraerythrocytic and/or intraphagocytic spherical inclusions. In 48/52 cases, we succeeded in identifying the bacterial agent: in 38 cases, it was *Anaplasma ovis*; in six cases, it was *Ehrlichia ruminantium*; in two cases, *Anaplasma phagocytophilum*; in one case, *Anaplasma platys*; and in one case, a yet uncultured *Anaplasma* sp. closely related to *A. phagocytophilum*.
Conclusions Our studies demonstrated the great variety of pathogenic bacteria from the *Anaplasmataceae* family in the blood of clinically ill sheep. *A. ovis* was identified unexpectedly often. For the first time, *A. phagocytophilum* was found in sub-Saharan Africa, and its further epidemiology may be now reconsidered. The roles of canine pathogen, *A. platys*, and yet undescribed *Anaplasma* sp. "Badiouré" in ovine pathology should be more closely studied.

Keyword Sheep · Anaplasmosis · Ehrlichiosis · Ixodid ticks · Senegal

Introduction

Anaplasmataceae is one of three officially recognized families of the order *Rickettsiales* of α-*Proteobacteria*. (Dumler et al. 2001). All representatives of these genera are obligate intracellular parasites of vertebrates and invertebrates, and some of them are etiological agents of arthropod-borne diseases of mammals.

Anaplasma ovis, *Anaplasma marginale*, and *Anaplasma centrale* are intraerythrocytic rickettsial pathogens of ruminants, including sheep and goats (Friedhoff 1997). *A. ovis* is moderately pathogenic in small ruminants. Ovine anaplasmosis has been observed in domestic and wild animals worldwide, including Europe (Ioannou et al. 2011), China (Liu et al. 2012), and the USA (de la Fuente et al. 2006). Interestingly, a variant of *A. ovis* was recently implicated in human pathology in Cyprus (Chochlakis et al. 2010). *Anaplasma platys* is considered pathogenic almost exclusively in canines (Dumler et al. 2001). *Anaplasma phagocytophilum* is the agent of an emerging tick (*Ixodes* spp.)-transmitted disease, human granulocytic anaplasmosis (Dumler et al. 2001), as well as canine and equine anaplasmoses. It has been described in the USA, Europe, the Asiatic part of Russia, and North Africa. It causes persistent infection in deer, mice and wood rats, and sheep, which may represent a reservoir for this infection (Yu and Walker 2006).

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Ehrlichia ruminantium is an obligate intracellular bacterium that causes the heartwater disease, an illness of African and Caribbean ruminants (including sheep) that is transmitted by ticks of the *Amblyomma* genus.

Due to intensive animal production systems in enzootic areas, the economic impact of these diseases has become increasingly severe, especially in cases of heartwater disease; however, current knowledge of these diseases in West Africa is limited. The objective of this study was to identify the causative agents of anaplasmosis and ehrlichiosis in sheep at two different sites in Senegal.

Materials and methods

The study was performed in two areas: the Niayes (two locations) and the Casamance (Ziguinchor) (one location) (Table 1). The Niayes area is a coastal strip some 10 km wide and is located in the northwest Senegal between the Dakar and Saint Louis regions. The breeding of cattle and small ruminants (sheep and goats) is one of the traditional activities of the area. The Casamance (Ziguinchor) is a transition zone between the dry northern and wet southern parts of West Africa. Farming is one of the main activities of the local people, and the livestock consists exclusively of cattle and small ruminants. Sheep farming in all sites is based on individual owners of a sheep herds (five to 50 in a herd). In each of the three sites, 150–200 sheep were included in the study for a total of 520 animals (Table 1). Over the course of 2 years (February 2009–January 2011), all animals were regularly screened by veterinarians. All animals with the following clinical signs of acute infection were included in the study: hyperthermia, anemia (pallor of mucosa, tachypnea, and tachycardia), rapid weight loss, staggering, and paresis of the hind limbs. Blood samples from all ill animals were collected and examined prior to storage in liquid nitrogen for further molecular studies. Blood smears were made from all sheep samples, and staining was performed as previously described using the RAL 555 kit (RAL Diagnostics, Martillac, France). The smears were then observed under light microscopy.

Genomic DNA from blood was extracted using the QIAamp® DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. We used the primers EHR16SR and EHR16SD to amplify a portion of the 16S rRNA gene of the most representatives of the *Anaplasmataceae* family (Parola et al. 2003). Using GenBank sequences of the *groEL* gene, we designed degenerate primers to amplify bacteria from the *Anaplasma* genus (ANgroElf 5′- GAG GCC ATC ACA GAT GG A5G-3′ and ANgroElr 5′-CGG AAC TGC ATA TCA CCR TCA GT-3′). Amplification reactions were performed with a DNA thermal cycler as described elsewhere (Parola et al. 2003). The obtained sequences were assembled (ChromasPro 1.49, Technelysium Pty Ltd., Tewantin, Australia) and aligned (BioEdit) (Hall 1999). A total of 306 bp of the16S rRNA gene was used for construction of a phylogenetic tree. The evolutionary history was inferred using Bayesian phylogenetic analysis (Ronquist and Huelsenbeck 2003) by TOPALi 2.5 software (Biomathematics and Statistics Scotland) with an integrated MrBayes application (http://mrbayes.csit.fsu.edu/) that uses a GTR substitution model with gamma regression.

Results

In total, we sampled 120 febrile animals during 2-year survey period of a group initially comprised 520 sheep (Table 1). Microscopic examination of only 52 of 120 blood smears of sheep revealed small (<1 μm), spherical intraerythrocytic and/or intraphagocytic inclusions that are suggestive of infection by *Anaplasma* or *Ehrlichia* spp. In 48/52 cases, we successfully obtained one or two amplicons from the blood samples (Table 2).

Nucleotide basic local alignment search tool analysis (http://blast.ncbi.nlm.nih.gov) of the obtained sequences of 16 s rRNA gene showed that in all 48 cases, the amplified genes belonged to bacteria of the *Anaplasmataceae* family. In ten cases, we were able to identify the species, because of

Table 1 Animals included in the study

Area	Sites	Geographic coordinates	Number of animals surveyed	Race	Number of animals included in the study	Male/female sex ratio
Niayes	Niague	14° 50′ 32″ N 17° 10′ 49″ E	200	Touabire, Peul peul, and Djallonké	60	20/40
	Keur Mbir Ndao	14° 54′ 53″ N 17° 3′ 59″ E	170	Touabire, Peul peul, and Djallonké	30	8/22
Ziguinchor	Badioure	12° 33′ 0″ N 16° 19′ 12″ E	150	Djallonké	30	11/19

Table 2 Results of the molecular identification of *Anaplasmataceae* bacteria in 48 samples. GenBank accession number of the matched sequence is indicated when applicable

Identified species	Number of samples	Localization	Maximum identity of the 306-bp portion of 16S rRNA gene (GenBank accession number)	Maximum identity of the 456-bp portion of *groEL* gene
Anaplasma ovis	8	KMND Niayes	*Anaplasma ovis*/*A. marginale*/*A. centrale* 100 %	*Anaplasma ovis* 100 %
	19	Niague Niayes	*A. ovis*/*A. marginale*/*A. centrale* 100 %	*A. ovis* 100 %
	11	Badiuoré Ziguinchor	*A. ovis*/*A. marginale*/*A. centrale* 100 %	*A. ovis* 100 %
Ehrlichia ruminantium	3	KMND Niayes	*Ehrlichia ruminantium* 100 % (U03776)	NA
	2	Badiuoré Ziguinchor	*E. ruminantium* 100 % (U03776)	NA
Anaplasma platys	1	Badiuoré Ziguinchor	*Anaplasma platys* 100 % (HQ585879)	NA
Anaplasma phagocytophilum	2	Badiuoré Ziguinchor	*Anaplasma phagocytophilum* 100 %	NA
Anaplasma sp.	1	Badiuoré Ziguinchor	Anaplasma sp. 100 % (GU556622)	NA

NA not amplified

the 100 % identity of the amplified gene with deposed in the GenBank sequences (Table 2). In six cases, it was *E. ruminantium*, and in two cases, *A. phagocytophilum*. In two other cases, it was *A. platys* and a yet uncultured *Anaplasma* sp. previously indentified in the blood of Korean water deer (*Hydropotes inermis argyropus*) and in *Haemaphysalis longicornis* ticks in Korea (Kang et al. 2011). The latter has also been identified in the blood of goats in China (Liu et al. 2011) and both white-tailed deer (*Odocoileus virginianus*) (Dawson et al. 1996) and *Amblyomma americanum* (U52514) ticks in Missouri, USA. *A. phagocytophilum* found in samples nos. 34 and 38 is identical to most of the *A. phagocytophilum* from Europe and Asia found in the GenBank database. Similarly, *A. platys* amplified from sample no. 44 is identical to most of *A. platys* found in the GenBank database. All identified *E. ruminantium* are identical to only the Omatjenne strain of *E. ruminantium* isolated in South Africa (U03776). In 38 cases (all identical among each other), the variability of the obtained portion of 16S rRNA gene was not enough to determine the species. In these cases, the identification was based on the comparison of the *groEL* gene sequences. In all 38 cases, it was *A. ovis*. The phylogenetic relationship of the identified bacteria and recognized species is presented in the Fig. 1.

Discussion

Very little is known about the repertoire of *Anaplasmataceae* pathogens and their geographical distribution in Africa. Most studies concern *E. ruminantium* and *A. marginale* infections in South Africa (Allsopp et al. 2007). In a few other studies, the identification of the species was either morphological or serologically based (Bell-Sakyi et al. 2004; Ngeranwa et al. 2008), so the role of other *Anaplasmataceae* bacteria in animal pathology in Africa was not clear. In the present study, we tried to identify all macroscopically identified bacteria associated with a febrile disease in sheep.

E. ruminantium was the causative agent in only 6/48 (12.5 %) of cases. In the Casamance region, situated just to the south of Gambia, the proportion of *E. ruminantium* among identified *Anaplasmataceae* bacteria was higher (3/18, 16.7 %) compared with the northern Niayes region (3/30, 10 %). The Niayes region probably corresponds to the northern limit of distribution of *Amblyomma variegatum* tick vectors that may explain this finding.

Surprisingly, few identified *E. ruminantium* may be explained by the dominating role of *A. ovis* (38/48, 79 %) in the study area. In Africa, this pathogen is rarely detected, so our data are the first to suggest the leading role of *A. ovis* in sheep pathology. The geographical distribution and vectors of *A. ovis* in Senegal are yet to be determined.

A. platys was previously described as an agent of infectious thrombocytopenia in dogs. It is regularly found in ticks, usually those of the *Rhipicephalus* genus in Africa (Marie et al. 2009) and especially in the region of Cape Verde closest to Senegal (Gotsch et al. 2009). To the best of our knowledge, the only previous report of this bacterium in sheep was in Cyprus (Chochlakis et al. 2009). The identification of this primarily canine pathogen in the blood of ill sheep expands the known pool of natural hosts and susceptible animals. The role of *A. platys* as the pathogen of sheep has yet to be evaluated.

A. phagocytophilum, a widely distributed tick-borne pathogen of veterinary and medical importance, was never identified in sub-Saharan Africa before, where all its known vectors are absent (Walker et al. 2003). The only evidence of this bacterium in African ticks was reported from Poland, where it was identified by PCR in *Amblyomma flavomaculatum* ticks collected from the imported African

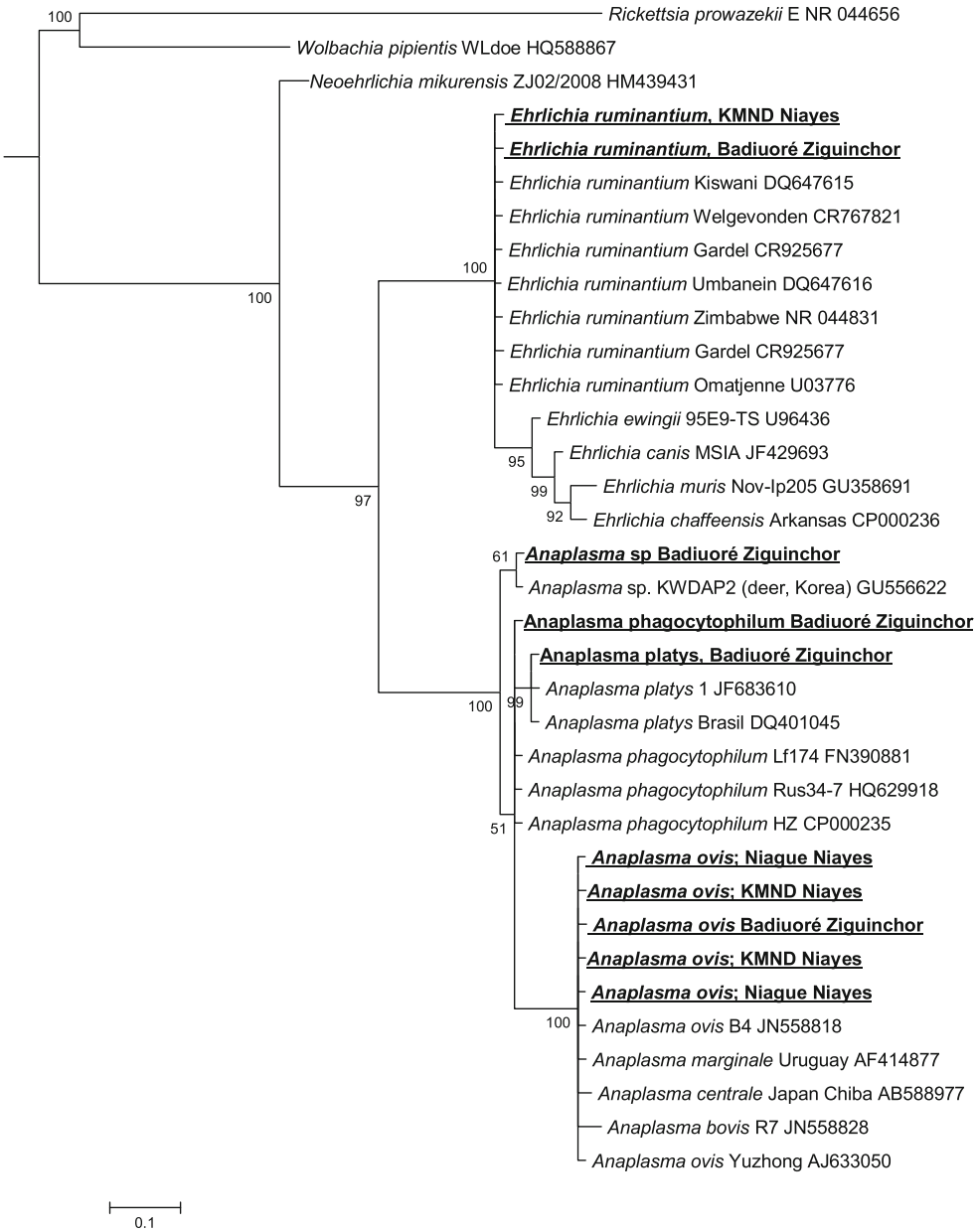

Fig. 1 Phylogenetic tree of bacteria in the family *Anaplasmataceae*. Bacteria identified in the present study are *underlined* and *bolded*

lizard *Varanus exanthematicus* (Nowak et al. 2010). On the other hand, Inokuma et al. (2005) reported the case of *Anaplasma* sp. occurring in South African dogs. This new species was most closely related (98.5 %) to *A. phagocytophilum* (Inokuma et al. 2005). In our study, we identified *A. phagocytophilum* and closely related *Anaplasma* sp. in the blood of three clinically ill sheep. These results suggest that *A. phagocytophilum* circulates in natural foci in sub-Saharan Africa, where the typical vectors of *A. phagocytophilum* are absent. This finding may be very important since granulocytic anaplasmosis of humans and animals has not been previously reported in Africa.

In conclusion, our studies identified a great variety of pathogenic bacteria from the *Anaplasmataceae* family in the blood of clinically ill sheep in Senegal. Additional studies are required, including the isolation of these bacteria from pure culture, assessment of the pathologic role of these bacteria in humans, the development of new treatments, and the implementation of preventive measures.

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