Case report: First description and evidence of *Anaplasma ovis* infection in the sheep in Croatia (Eastern coast of Adriatic Sea)

TOMISLAV ŠARIĆ - DEPARTMENT OF ECOLOGY, AGRONOMY AND AQUACULTURE, UNIVERSITY OF ZADAR, ZADAR CROATIA

RELJA BECK - LABORATORY FOR PARASITOLOGY, CROATIAN VETERINARY INSTITUTE, ZAGREB, CROATIA

Intoduction

Anaplasma ovis

- Gram negative rickettsial bacteria
- Infects red blood cells in small ruminants
- Transmitted by different tick species
- Usually it causes only mild clinical symptoms in small ruminants
- Acute diseases are described to be associated with stress factors like co-infection, hot weather, vaccination, deworming, heavy tick infestation, long-distance transportation and animal movement

Infections with ***A. ovis*** occur in certain areas in Europe:

- Portugal, France, Italy, Greece, Turkey, Bulgaria, Southeast Romania and Hungary

So far it has not been proven on the Eastern coast of the Adriatic Sea

Case report

Ram (1) with simptoms:

- Fever (41,5 °C)
- Loss of appetite
- Apathy
- Anemia
- Weight loss

➡ Clinical signs of piroplasmosis?

We took the blood sample of the ram and sent it to the lab.

- Blood was analysed with PCR for Babesia/Theileria species as well as for Anaplasma sp.

Field study

Frequent occurrence of clinical signs that correspond to the sheep piroplasmosis, in the last years in Zadar County, induced us to conducted field study

At beginning we collect anamnestic data from veterinary practitioners

Anamnestic data

The most common disease affects animal older than 5 to 6 months and younger than 18 months

It is interesting that they never observed cases of disease in young lambs aged up to five months. There are very rare cases of diseases in older sheep

Disease mostly affects the introduced sheep breeds

Disease occurs more frequently in males than in females animals (ratio 1: 5)

The disease usually occurs in the late spring and early summer

One or more attached ticks are usually found in sick animals

Clinical signs

Animal stays behind the herd (usually walk last), do not graze or not to consume other foods

Body temperature is higher than 41 ° C, sometimes even 42 ° C

The testicles of rams are in a lower position than usual (as further away from the body)

➡ There are no other specific symptoms at the beginning of the disease

Three to four days after the beginning of the disease, the visible mucosa becomes lighter and yellowish

As the disease lasts longer the animals are walking harder, slowing down, and the owners can easily catch them among other animals

Usually four to five days after the beginning of the disease, the animals lie down and cant get up

- Such animals in most cases do not respond to treatment and die through 1-2 days

Field study

We collected new samples:

- Blood sample from **another ram (2)** presenting identical clinical signs such as fever, apathy, anemia and icterus
- Blood samples from **all 13 sheep in the herd** (without any signs of disease) at the same time as the ram with signs of disease, and repeated blood sample after 7 months
- Ticks from same area

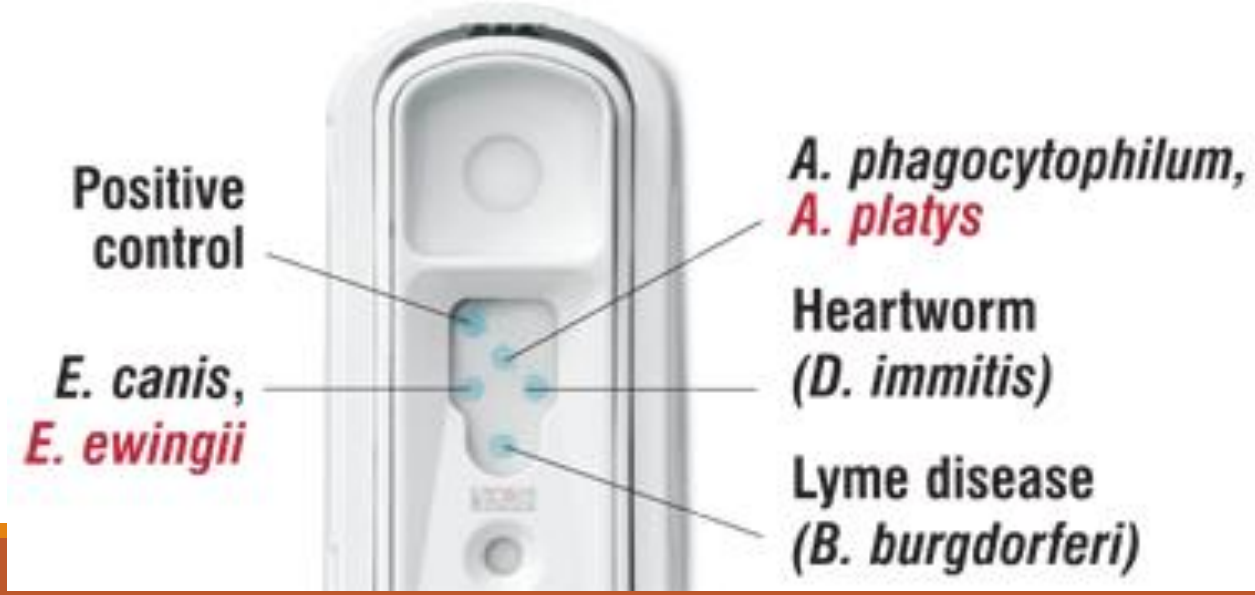
Rams were treated with long-acting oxytetracycline and after few days they fully recovered!

Results

Before the PCR results were known **SNAP 4Dx Plus Test** (screen for six vector-borne diseases) was also done

Tests were negative for *Anaplasma phagocytophilum* but positive for *Ehrlichia canis*!

After that, we were very curious…

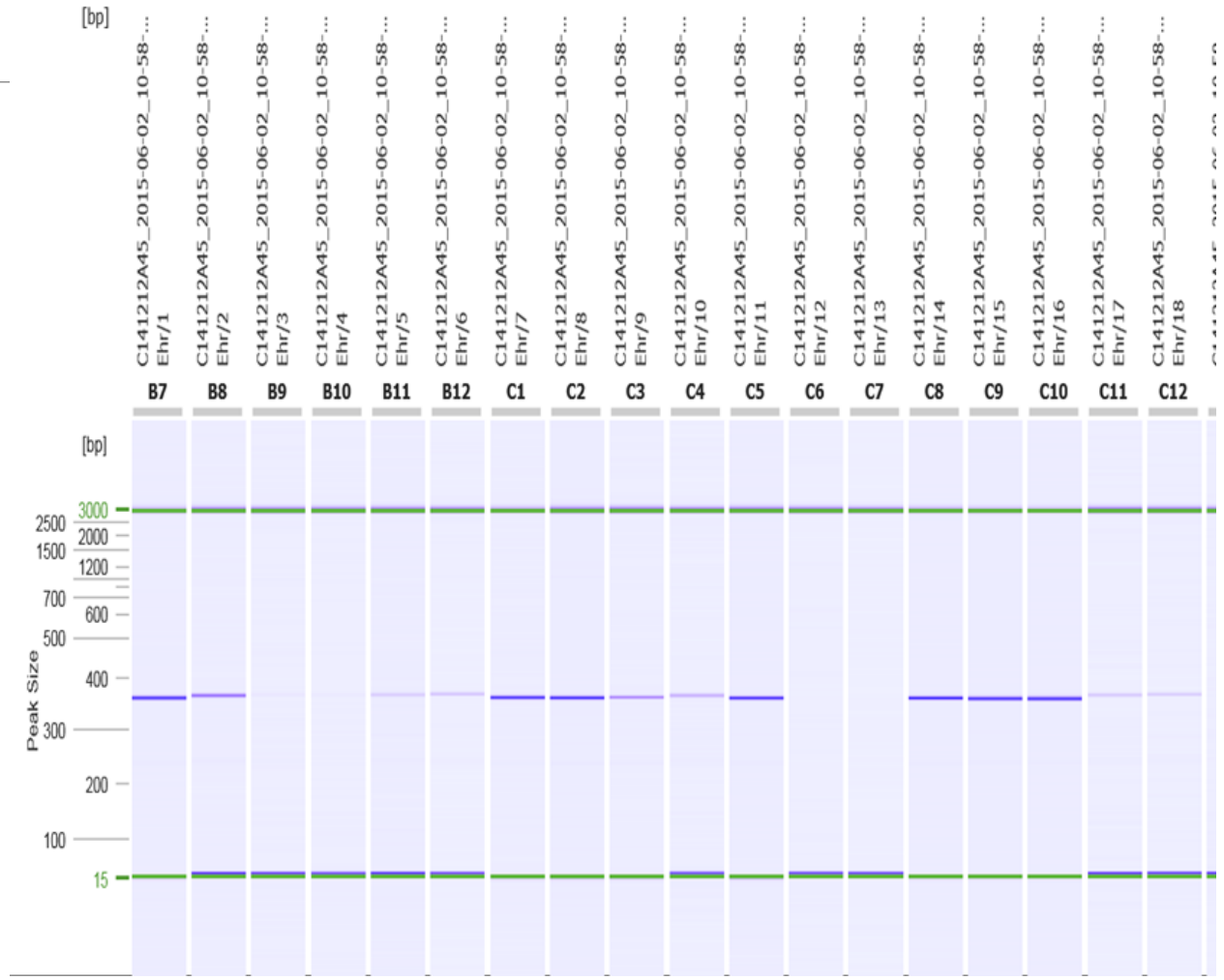


Results

PCR and subsequent sequencing of 16 S rRNA and groEL showed 100% similarity with ***A. ovis*** in both rams with clinical signs of disease

Piroplasms were not found in the blood of diseased rams

Infection with *A. ovis* was found in 92% of healthy sheep from the same herd



Results

Repeated blood sampling and molecular analysis from same animals after 7 months showed:

- persistent infection of *A. ovis* in all examined sheep
- in the blood of all sheep the piroplasm species *Theileria ovis* was also found

Identical sequences from *A. ovis* and *T. ovis* were also detected in *Rhipicephalus bursa* ticks from same region

Conclusion

Despite the fact that sheep anaplasmosis is considered as “tropical” disease and global climatic changes could affected the distribution of vectors and possible spread of this pathogen we believe that *A. ovis* present an “autochthonous“ species in this region

The presented data in this research support this theory, as usually rams exhausted during the breeding season and other immunosuppressed animals develop disease

This study presents the first evidence of asymptomatic infection in sheep and clinical signs in rams from Croatia

Thank you for your attention!