Macedonian Veterinary Review 27, 1/2 p.35-39 (1998)
UDC: 619:636.3]:616.98:579.887.111(497.7)

MYCOPLASMAS OF SMALL RUMINANTS AND THEIR RELEVANCE TO MACEDONIA

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Proceedings of the "6th International Conference on Sheep and Goat Production and 4th Symposium on the Reproduction of Domestic Animals" September 9-11, 1998 Ohrid, Macedonia

Mycoplasmas are small fastidious wall-less bacteria which cause cause disease in all major species of animals including man. In small ruminants they can cause respiratory disease, mastitis, arthritis, genital disease and eye lesions. The most important of these diseases are contagious caprine pleuropneumonia (CCPP) and contagious agalactia (CA) which are designated by the Office of International Epizooties as List B diseases because of their economic impact on the farming industry. Some of the mycoplasmas causing CCPP and CA are members of the "*Mycoplasma mycoides*" cluster which consists of six mycoplasmas, all of which are important pathogens of small and large ruminants.

Contagious caprine pleuropneumonia

Table 1 lists the pathogenic mycoplasmas so far isolated from sheep and goats and the diseases with which they have been linked. CCPP caused by *M.capricolum* subsp. *capripneumoniae* (formerly *Mycoplasma* F38) has been known for many years and results in major losses in goat herds in at least 30 countries in Africa and Asia including Turkey. To date, however, the mycoplasma has only been isolated in 12 countries because of difficulties in recovering the organism from clinical material (table 2). Research into the control of CCPP has been hampered by confusion into the exact cause of the disease. Two other "*M. mycoides* cluster" members, *M. m. mycoides* LC and *M. m. capri*, were for some time implicated in the aetiology of the disease because they can cause a pleuropneumonia in small ruminants that resembles CCPP. This confusion was compounded by the difficulty of isolating and growing the highly fastidious F38 *in vitro*. True CCPP is characterised by its ready contagious nature to susceptible goats and, histo-pathologically, by an interstitial intralobular oedema of the lung rather than thickening of the interlobular septa which is seen with *M. m. mycoides* LC and *M. m. capri*. Improved media formulation and molecular biological tools are now available to isolate and identify *M. c. capripneumoniae*. A major development was the set of polymerase chain reaction (PCR) tests by Bashiruddin *et al.* (1994) which could detect all members of the *M. mycoides* cluster and distinguish *M. m. mycoides* LC and *M. m. capri* from *M.c.capripneumoniae* and *M.c.capricolum*. Later Bascunana *et al.* (1994) developed a PCR based on the 16sRNA gene which could specifically identify *M.c. capripneumoniae* following a restriction enzyme digestion step.

These PCRs were used recently to identify *M. c. capripneumoniae* in lung material from clinically affected goats in Eritrea. Because of the fragility of these mycoplasmas during transport, lung samples were freezedried before despatch to the UK. At CVL, they were resuspended and then grown in Mycoplasma Experience Medium (Reigate, UK). The PCR was carried out at an early passage because of the risk of overgrowth by *M. ovipneumoniae*, a ubiquitous mycoplasma lacking the typical "centred" morphology and which has been shown to be a cause of pneumonia usually in association with other bacterial pathogens. The PCR products gave three bands following digestion with *Pst*I and represented the first identification of CCPP in Eritrea.

Serological detection of CCPP can be achieved by traditional tests such as the complement fixation test (CFT) or more robustly by a latex agglutination test which is available commercially from Dr Shompole, Kenya (Rurangirwa *et al.*, 1987). This test can performed in minutes in the laboratory on serum or in the field on whole blood and in our experience compares favourably with the CFT in terms of sensitivity and specificity (table 3).

As Turkey is the closest CCPP affected country, veterinary officers in Macedonia should be aware of the clinical manifestations of the disease in order to prevent the introduction and spread of this devastating disease which is invariably imported with clinically normal carrier animals. Naive herds can suffer losses of up to 80% mortality and 100% morbidity when exposed for the first time. The threat of CCPP may become of more concern as the population of goats in Macedonia increases.

Like most mycoplasma infections, CCPP is generally refractory to many commonly used antibiotics like penicilin and streptomycin. Those such as the tetracyclines and tilmicosin are only effective if administered early in infection. An inactivated vaccine has been shown to be useful but immunity is generally short-lived. A genetically modified vaccine is due to undergo field trials shortly (March *et al.*, 1998).

Contagious agalactia

M. m. mycoides LC is also one of the causative mycoplasmas of contagious agalactia, a serious disease syndrome of small ruminants which is characterised by mastitis, arthritis, keratoconjuncytivitis and, occasionally, abortion. *M. agalactiae* is, however, the main cause of the disease in sheep and goats, but *M. m. mycoides* LC, *M. capricolum* subsp. *capricolum*, and *M. putrefaciens* also contribute significantly to losses, particularly in goats. The disease has been estimated to cause annual losses of as much as $30 million in European countries around the Mediterranean, mainly as a result of milk production losses but mortality and abortions can also occur. Several Balkan countries, including Croatia, Bulgaria, Greece and Albania, have reported clinical CA and as Macedonia shares many open borders with these countries, it is almost certain that it has or will shortly become infected with the disease. Indeed joint serological work by CVL and the Veterinary Institute, Skopje found serological evidence of CA and recent reports of clinical CA on the Albanian border will soon be investigated.

CA is predominantly a disease of milking sheep and goats. It often appears in a herd in the spring soon after lactation begins as a result of the activation of latent infection. The young ruminants become infected directly at suckling while the adults are contaminated via the milker's hands, milking machines or by bedding which often provides a rich source of mycoplasmas. Mastitis then a marked drop or complete cessation of milk production are

the first indications of disease. The udder becomes inflamed then can undergo complete fibrosis resulting in permanent agalactia. Debilitating but transient arthritis is often seen particularly in young animals while keratoconjuctivitis occasionally leading to blindness may follow in animals of any age. *M. agalactiae* tends to cause a chronic disease resulting in few deaths; flocks generally show only arthritis and eye lesions. The other mycoplasmas can cause a more severe acute disease resulting in high levels of morbidity and mortality.

The infection of healthy flocks occurs mainly throught the introduction of carriers or by contact with other flocks. Investigating 250 cases of CA over a 10 year period in France, Bergonnier *et al.* (1996) showed that over 70% were caused by contact with affected flocks, 11% involved the purchase of affected animals and 11% following disease relapse in previously affected flocks. It follows, therefore, therefore that CA will persist in areas where there is high concentration of susceptible animals, where there is uncontrolled exchange of animals and common grazing. Poor hygiene and long milking periods will exacerbate the condition.

Clinical signs are rarely characteristic so laboratory testing is essential to confirm disease outbreaks. The most suitable suitable samples include milk, mastitic secretions, joint fluid or eye swabs. The ear canal, perhaps surprisingly, has also been found to be a rewarding site for mycoplasmas including pathogenic ones (Cottew and Yeats, 1981). This finding has suggested the possible role of ear mites in the transmission of the mycoplasmas. Confirmation of CA may be achieved by isolating the causative mycoplasmas, all of which are not intrinsically difficult to grow *in vitro* if suitable media are used and laboratory conditions are satisfactory (Nicholas and Baker, 1998). Mycoplasmas can be identified by growth inhibition or fluorescent antibody tests using hyperimmune specific sera. The PCR offers advantages in time, specificity and sensitivity over culture which can sometimes take up to 2-3 weeks Several PCRs have been reported but the use of the "mycoides cluster" PCR (Bashiruddin *et al.*, 1994) together with a specific one for *M. agalactiae* (Johansson *et al.*, 1996) should ensure maximum chance for detection. Many ELISAs, including commercial kits, have been described for the serological detection of the disease and are used today in favour of CFTs because of their sensitivity and ease for large scale testing.

As for CCPP and most mycoplasma infections, antibiotic control is generally ineffective for CA tending to reduce clinicals signs but promoting the carrier state. In an *in vitro* study of Sicilian isolates only enrofloxacin and tetracyclines gave acceptable levels of sensitivity (Loria *et al.*, 1998). There was evidence of resistance to florfenicol and tilmicosin. In Europe both live and inactivated vaccines have been used with mixed success. Some have provided protection from clinical disease and have been useful in endemic areas but the problems of encouraging the carrier state still apply. Generally the duration of immunity is short. New live vaccines are essential to produce longer lasting immunity to enable the control of CA. In endemic areas of France the prevalence of the disease has been effectively reduced by a long campaign involving testing and slaughtering without the use of vaccine which confuses the testing process (Bergonnier *et al.*, 1996). This approach is cost effective where disease is restricted to small manageable areas but where disease is more widespread and transhumance is involved, test and slaughter is impractical. The type of control strategy used must be based on a sound knowledge of the extent and distribution of the disease which can only be achieved by intensive surveillance using the appropriate serological test backed by laboratory facilities to confirm the disease.

***Table 1**: Pathogenic mycoplasmas of sheep and goats*

Mycoplasma	Host	Distribution	Disease
M. agalactiae	Sheep/goats	Worldwide	CA
M. c. capripneumoniae	Goats (sheep)	Middle East, Africa, Asia	CCPP
M. c. capricolum	Goats (sheep)	Worldwide	Pneumonia, CA
M. conjunctivae	Sheep/goats	Worldwide	Keratoconjunctivitis
M. m. mycoides LC	Goats (sheep)	Worldwide	Pneumonia, CA
M. m. capri	Goats	Worldwide	Pneumonia
M. ovipneumoniae	Sheep/goats	Worldwide	Pneumonia
M. putrefaciens	Goats (sheep)	USA, Europe	Mastitis/arthritis/CA

***Table 2**: Countries which have isolated M. c. capripneumoniae (CCPP) from cases of CCPP*

Africa	Chad, Ethiopia, Kenya, Niger, Sudan, Tunisia, Uganda, Eritrea
Asia	Oman, United Arab Emirates, Turkey, Yemen

***Table 3:** Clinical findings and serological testing of Eritrean small ruminant herds affected by CCPP*

Herd	% Morbidity	% Mortality	% Positive by LAT	%positive by CFT
A	90	65	55	47
B	58	47	31	20

REFERENCES

1. Bascunana, C. R., Mattsson, J. G., Bolske, G & Johansson, K. E. (1994) Characterisation of the 16sRNA genes from Mycoplasma sp. strain F38 and development of an identification system based on PCR. J. Bacteriol 176, 2577-2586

2. Bashiruddin, J. B. Taylor, T. K. & Gould, A. R. (1994) A PCR based test for the specific identification of *M. mycoides* subsp. *mycoides* SC. J. Vet. Diagn. Invest. 6, 428-434

3. Bergonnier, D, Berthelot, X. and Poumarat, F. (1996) Agalactiae contagieuse des petit ruminants: epidemiologie diagnostic et controle. Rev. sci. tech. Off. int. Epiz. 15, 1431-1476

4. Johansson , K E, Berg, L O, Bolske, G. *et al.* (1996) Specific PCR systems based on the 16sRNA genes for Mycoplasma agalactiae and M. bovis. In : Mycoplasmas of ruminants. Edited by J Frey and K Sarris. EUR 16934 pp88-90

5. Jones, G. E. and Gilmour, J. S. (1983) Atypical pneumonia. In: Diseases of sheep. Edited by W. B. Martin. Blackwell Scientific Publications, Oxford pp17-23

6. Loria G. R., Worth D. R., Caracappa, S., Nicholas, R. A. J. (1998) Identification and antibiotic susceptibility of mycoplasmas associated with contagious agalactia in Sicily. In : Mycoplasmas of ruminants

Volume 3. Edited by L Stipkovitz and J Frey. In press

7. March, J B. and Jones, G. E (1998) Inhibitory effects of vaccines containing subunit fractions of *M. capricolum* subsp. *capripneumoniae*. In : Mycoplasmas of ruminants. Volume 2. Edited by G Leori, and J Frey. EUR 18018 pp44-49

8. Nicholas, R.A.J. & Baker, S. E. (1998) Recovery of mycoplasmas from animals. In: Mycoplasma Protocols. Edited by R.J. Miles and R.A.J. Nicholas, Humana Press, Totowa pp37-44.

9. Rurangirwa, F. R, McGuire, T., Musoke, A. J., Kibor, A. (1987) Differentiation of F38 mycoplasmas causing contagious caprine pleuropneumonia with a growth inhibiting monoclonal antibody. Infection and Immunity 55, 3219-3220.

10. Thiaucourt, F. & Bolske, G. (1996) Contagious caprine pleuropneumonia and other pulmonary mycoplasmoses of sheep and goats. Rev. sci. tech. Off. int. Epiz. 15, 1397-1409

11. Cottew, G.S. and Yeats, F.R. (1981) The ear canal as a culture site for mycoplasmas in clinically normal goats. Aust. Vet. J., 57, 52.

МИКОПЛАЗМИТЕ НА МАЛИТЕ ПРЕЖИВАРИ И НИВНОТО ЗНАЧЕЊЕ ЗА МАКЕДОНИЈА

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Микоплазмите се мали безѕидни организми што предизвикуваат заболувања кај сите поважни видови на животни вклучувајќи го и човекот. Кај малите преживари тие можат да предизвикаат респираторни болести, маститис, артритис, болести на гениталиите и лезии на очите. Најважната од овие болести е заразната козја плевропневмонија (ССРР) и заразната агалаксија (СА) кои се класифицирани од Канцеларијата за меѓународни епизоотии како болести од листата Б заради нивното економско влијание врз земјоделското стопанство. Некои од микоплазмите што ги предизвикуваат ССРР и СА припаѓаат на " *Mycoplasma mycoides*" која се состои од шест микоплазми, од кои сите се важни патогени причинители кај малите и големите преживари.