Experimental Contagious Caprine Pleuropneumonia: A Long Term Study on the Course of Infection and Pathology in a Flock of Goats Infected with *Mycoplasma capricolum* subsp. *capripneumoniae*

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[1]National Veterinary Research Centre, Kenya Agricultural Research Institute, Kikuyu, Kenya, [2]National Veterinary Institute, Uppsala, Sweden, [3]CIRAD-EMVT, Santé Animale, Montpellier, France, [4]Veterinary Laboratories, Private bag, Kabete, Kenya, and [5]Department of Biomedical Sciences and Veterinary Public Health, Division of Pathology, Pharmacology and Toxicology, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Wesonga HO, Bölske G, Thiaucourt F, Wanjohi C, Lindberg R: Experimental contagious caprine pleuropneumonia: A long term study on the course of infection and pathology in a flock of goats infected with *Mycoplasma capricolum* subsp. *capripneumoniae.* Acta vet. scand. 2004, 45, 167-179. – Contagious caprine pleuropneumonia (CCPP) is a major threat to goat farming in parts of Africa and Asia. It classically causes acute high morbidity and mortality early in infection, but little is known of its long term epizootiology and course. In this study, 10 goats were inoculated with *Mycoplasma capricolum* subsp. *capripneumoniae (M. capripneumoniae)* and then mixed with 15 goats for contact transmission. The disease course was monitored in each goat for 56-105 days, whereafter the goats were killed and necropsied. Varying features signifying infection occurred in altogether 17 goats (7 inoculated, 10 in-contact). Clinical signs were severe in 8 goats but no fatalities occurred. Only 6 goats had serum antibody titres against *M. capripneumoniae* in ELISA. Fourteen goats (5 inoculated, 9 in-contact) had chronic pleuropulmonary lesions compatible with CCPP at necropsy and 7 of those showed *M. capripneumoniae* antigen in the lung by immunohistochemistry. Neither cultivation nor PCR tests were positive for the agent in any goat. The results indicate that the clinical course of CCPP in a flock may be comparatively mild, *M. capripneumoniae*-associated lung lesions may be present at a late stage of infection, and chronic infection may occur without a significant serological response.

goat; Mycoplasma; contagious pleuropneumonia; ELISA; immunohistochemistry; serology; pathology.

Introduction

Contagious caprine pleuropneumonia (CCPP) is one of the most severe infectious diseases of goats, causing major economic losses in goat farming in Africa and Asia (*Jones* 1989). It is caused by *Mycoplasma capricolum* subsp. *capripneumoniae (M. capripneumoniae),* formerly *Mycoplasma* strain F38 (*Leach et al.* 1993, *MacOwan & Minnette* 1976). Clinical outbreaks in a flock often show a 100% morbidity and mortality rates of 60 to 70% with lesions of fibrinous pleuropneumonia in the acute stage (*Kaliner & MacOwan* 1976, *Msami et al.* 2001).

Long term survivors of acute disease may dis-

play chronic pleuropneumonia or chronic pleuritis (*Kaliner & MacOwan* 1976, *Rurangirwa et al.* 1991) but cultural recovery of the agent has not been demonstrated in such late stage pulmonary lesions (*MacOwan & Minnette* 1978, *Wesonga et al.* 1998). Still, negative results of cultivation of *M. capripneumoniae* is not proof of freedom of infection (*Thiaucourt & Bölske* 1996) and the use of complementary techniques for microbial identification is indicated. Especially so, since field observations indicate that outbreaks may follow the introduction of apparently healthy goats to a flock, suggesting that subclinical carriers may occur.

Most studies on CCPP have concentrated on vaccination trials and the stage of acute fulminant disease in flocks. There is an obvious need of further studies to monitor features of the long term course of infection, including possible persistence of the agent as well as serological responses and pulmonary pathology. The present study was designed to elucidate these matters in experimental *M. capripneumoniae* infection of a large flock of goats.

Materials and methods
Animals and husbandry
Thirty goats, 21 castrated males and 9 females, all of the Galla breed, were used. They originated from a large farmers´ cooperative, ranching mixed cattle, sheep and goats in the Eastern Province of Kenya with no history of CCPP. The goats were brought to the National Veterinary Research Centre at the age of 12-15 months. Polymerase chain reaction (PCR) tests and microbial cultivation on nasal, pharyngeal and ear canal swabs did not reveal *M. capripneumoniae* or other mycoplasmas in the ´*Mycoplasma mycoides* cluster´, but *Mycoplasma ovipneumoniae* and *Mycoplasma arginini* were in general cultivated. The goats were housed in pens with an adjoining fenced enclosure of approximately 20×30m in which they were freed

for feeding. They were dewormed with Nilzan plus cobalt® (Cooper, Nairobi, Kenya) directly upon arrival and 3 months later. They were fed on hay and mineral lick *ad libitum* and on concentrates (49.5% grain, 36.3% wheat and maize bran, 10.7% cotton seed cake, 3.5% mineral supplement) at 26g/kg bw. every second day.

Experimental design
The goats were observed for 3 months, during which no signs of disease were seen. Complement fixation tests for serum antibodies to *M. capripneumoniae (Muthomi & Rurangirwa* 1983) at arrival and 1 month before the start of the experiment, were negative in all goats (titers ≤1/16 at both occasions). They were then randomly allocated to either of 3 groups (A-C). Group A goats (n=10), housed approximately 1 km away from the other goats, were inoculated intratracheally (i.t.) with 20 ml of inoculum (see below) containing a mixture of a freshly ground suspension (5 ml) of an infected lung and 15 ml of *M. capripneumoniae* culture. Seven goats were inoculated on day 0 and 3 goats on day 17. Group B goats (n=15) were mixed with the A goats on day 18 for contact transmission, and group C goats (n=5) were non-exposed controls. The course of infection was monitored by clinical examinations, serology and microbiology. Starting day 74, two group A, three group B and one of group C goats were killed by electrocution and exsanguination and necropsied each of 5 consecutive weeks.

Microbiological culture, PCR and serology procedures
Inoculum. The lung suspension was from a pneumonic lung of a goat surviving a field outbreak of CCPP in Western Kenya, and was positive for *M. capripneumoniae* in PCR test up to dilution 10^4 but on culture only when undiluted. *M. ovipneumoniae* was also found, but no bac-

teria or other mycoplasmas. As the goats harboured this mycoplasma in their normal flora, no attempt was made to exclude it from the inoculum.The culture given to the first 7 goats was passage 6 of a field isolate and that given to the additional 3 goats was passage 2 of another field isolate of *M. capripneumoniae.* Both cultures contained 10^9 color changing units (c.c.u.) of *M. capripneumoniae* per ml.

Sampling and procedures for microbial isolation. Nasal, pharyngeal and ear canal swab samples for culture of Mycoplasma and bacteria, and for PCR, were taken from each animal once every 2 weeks of the experiment and on the day of killing, tracheal lavage samples were taken immediately after killing, and lung samples were taken at necropsy at sites also sampled for histopathology (see below). *M. capripneumoniae* was cultured on modified Newing´s broth (*Gourlay* 1964) with 0.2% sodium pyruvate (*Thiaucourt et al.* 1992) and on Medium F with 0.2% sodium pyruvate (*Bölske* 1988, 1995). Mycoplasma isolates were identified with PCR, growth inhibition (GI) (*Black* 1973) and immunofluorescence (IF) (*Rosendal & Black* 1972) tests. The GI and IF tests employed polyclonal rabbit antisera to the type strains of *M. arginini, M. capricolum* subsp. *capricolum (M. capricolum), M. capripneumoniae, M. mycoides* subsp. *capri, M. mycoides* subsp. *mycoides* LC and *M. ovipneumoniae.* Polymerase chain reaction and restriction enzyme analysis (REA) procedures were carried out according to *Bölske et al.* (1996). Bacterial culture was carried out on blood agar plates with sheep red blood cells.

Serology. Blood samples during the trial were investigated at the National Veterinary Institute for serum antibodies to *M. capripneumoniae* by a modification of an i-ELISA test used in *M. mycoides* subsp. *mycoides* SC infection (*Bölske*

et al. 1995b). Samples were taken fortnightly in all goats but due to storage problems collected pre-infection sera were only rarely available for testing with ELISA and a few goats could not be tested throughout the entire period of infection. The test employed a sodium dodecyl sulfate (SDS)-solubilized antigen of *M. capripneumoniae* (strain F38), grown in modified F-medium with gamma globulin-free horse serum and 0.2% sodium pyruvate (*Bölske* 1995, *Olsson et al.* 1990). The sera were tested at dilution 1:100 and applied in duplicate wells in the microtiter plates (M129A, Dynatech, Chantilly, USA). The conjugate was a rabbit anti-goat Ig-HRP (P160, Dako, Glostrup, Denmark). Positive control sera were from one goat immunized at CIRAD-EMVT and from a pool of sera from an outbreak of CCPP in Uganda (*Bölske et al.* 1995a). A cut off optical density (OD) value of 0.8 was established from the mean +3 SD of sera of 140 goats from Sweden or New Zealand. Blocking ELISA (b-ELISA), employing a cut off value at 20% inhibition (*Thiaucourt et al.* 1994), was carried out to check the validity of the i-ELISA results in 15 of the goats.

Clinical examination
Rectal temperature, cough, and respiratory rates and sounds were recorded daily between 8.00 and 10.00 am throughout the study period. Clinically normal goats showed rectal temperatures of 37.0 °C to 39.3 °C. Fever was recorded when the temperature was 39.5 °C and above.

Necropsy and histopathology
A complete necropsy was performed on all goats and tissue samples were fixed in 10% phosphate buffered formalin. Samples were taken from lung lobes with and without gross lesions, and from the heart, liver, kidney, spleen, thoracic lymph nodes and small intestine. Paraffin sections were stained with hema-

Table 1. Goats with signs of *M. capripneumoniae* infection (n=17). Data include serology (i-ELISA), immunohistochemistry (IHC) of lungs for *M. capripneumoniae,* pleuropulmonary lesions at necropsy, time periods of clinical signs, and timepoint of necropsy.

Group	Goat no.	i-ELISA[1]	IHC[2]	Lesions[3]	Time period of fever[4]	Time period(s) of respiratory signs[4]	Timepoint of necropsy[4]
A	130	-	+	+	-	28	80
A	131	+	-	-	27-30	27-34	75
A	139*	+	-	+	5-6	5-11	82
A	142	-	+	+	16-18	16-79	80
A	145*	-	-	+	-	11	88
A	174	+	-	-	-	2	88
A	188	+	+	+	28-32	28-73	74
B	124	-	-	+	-	-	56
B	127	-	-	+	-	40	63
B	129	-	+	+	-	16-67	70
B	133	-	-	+	15	14-15, 50	70
B	134	-	+	+	-	11-59	81
B	144	-	-	-	13-18	34, 53	63
B	151	+	+	+	-	6-7, 36-39	87
B	160	-	+	+	-	44-74	81
B	172	-	-	+	36-37	6-7, 36-39	87
B	191	+	-	+	21-26	19-56	56

[1]Pertinent antibody titre present: +, not present: -. [2]Antigen present: +, not present: -.
[3]Lesions compatible with *M. capripneumoniae* infection: +, no lesions: -.
[4]Group A: day(s) post inoculation, group B: day(s) post time-point of contact transmission; no symptoms: -.
* Inoculated day 17

toxylin and eosin (H&E) and, when appropriate, also with Periodic acid-Schiff (PAS), Masson´s trichrome connective tissue stain and Martius-Scarlet-Blue (MSB) for fibrin.

Immunohistochemistry
The paraffin-embedded lung tissues were investigated for *M. capripneumoniae* antigen by immunohistochemistry, employing polyclonal anti-*M. capripneumoniae* antibodies.

Preparation of antibodies. A whole-cell antigen was used to inoculate rabbits for antibody production. *M. capripneumoniae* (strain F38), grown in rabbit meat infusion broth with 20% rabbit serum, 4% yeast extract, 40% Hanks´ balanced salt solution and 0.2% sodium pyruvate, was harvested in late log phase growth, centrifuged at 12,000g for 30 min and washed with PBS 3 times. Inoculation of rabbits was performed according to *Olsson et al.* (1990). The rabbit antiserum was tested in GI (*Black* 1973) and IF (*Rosendal & Black* 1972) tests against type strains of *Mycoplasma sp.* Group 7, *M. arginini, M. capricolum* subsp. *capricolum, M. capripneumoniae, M. mycoides subsp. capri, M. mycoides* subsp. *mycoides* LC and *M. ovipneumoniae.* In both tests, it reacted strongly against *M. capripneumoniae* and *Mycoplasma* sp. Group 7, but no reaction against the other mycoplasmas was observed.

Immunohistochemical method. The streptavidin biotin complex-horse radish perox-
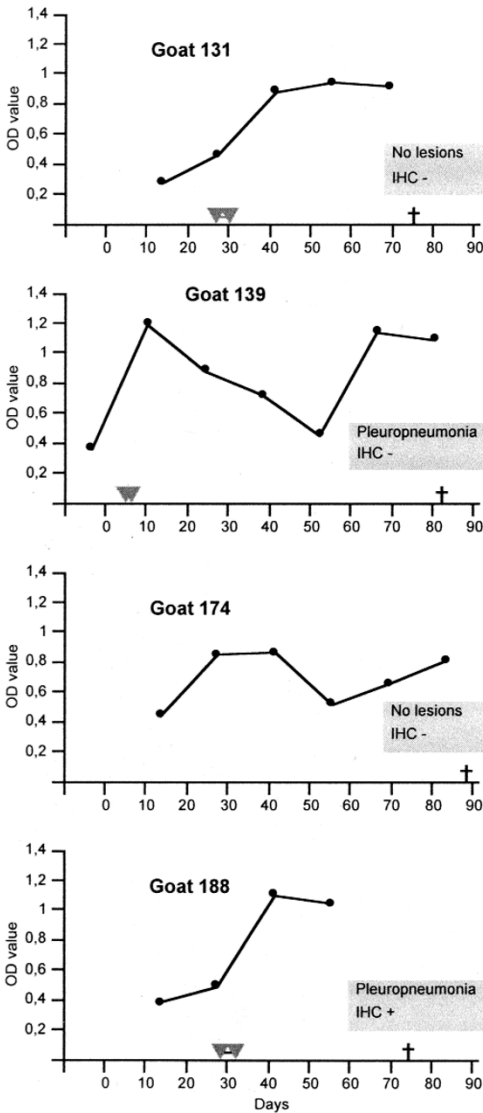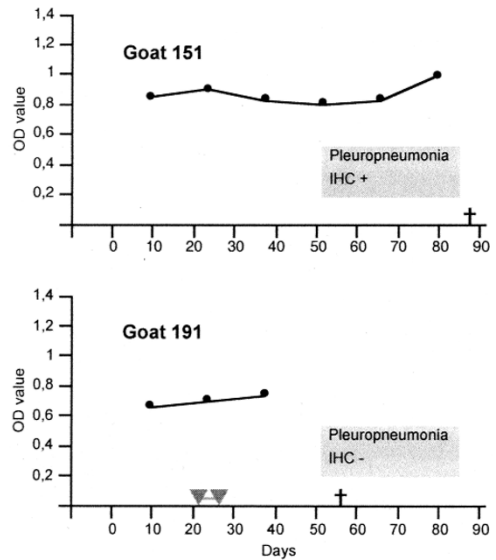
Figure 1. Serum antibody response (OD value of i-ELISA) in relation to period of fever and pleuropulmonary lesions and immunohistochemistry (IHC) for *M. capripneumoniae* at necropsy in sero-reactive group A (inoculated) goats. Days = days post inoculation. ▽-▽ : Period of fever; † : Timepoint of necropsy.

Figure 2. Serum antibody response (OD value of i-ELISA) in relation to period of fever and pleuropulmonary lesions and immunohistochemistry (IHC) for *M. capripneumoniae* at necropsy in sero-reactive group B (in-contact exposed) goats. Days = days post initial contact exposure. ▽-▽ : Period of fever; † : Timepoint of necropsy.

idase method (StreptABC/HRP) was used. Non-specific protein binding was minimized by pre-treatment with 20% normal swine serum (Dako, Glostrup, Denmark) and endogenous peroxidase was quenched with 0.3% hydrogen peroxide. The primary antibody was diluted 1:100, and biotinylated swine-anti-rabbit immunoglobulins 1:200. The chromogen was amino-ethyl-carbazole, the counterstain hematoxylin, and tris-buffered saline (TBS), pH 7.6, was used for dilutions.

Specificity and methodological controls. Paraffin sections of the lungs of 6 goats that died with acute fibrinous pneumonia in the early phase of CCPP and with *M. capripneumoniae* confirmed on culture (*Wesonga et al.*

Table 2. Gross and light microscopic characterization of pulmonary lesions in goats with thoracic pathology at necropsy (n= 14).

| Goat, group and no. | Gross pathology | | | | Light microscopy | | | | | | | |
	Consolidation	Pleural adhesions	Pleural fibrin deposits	Distribution of gross changes[1]	Bronchitis/bronchiolitis	Alveolar exudate	Alveolar fibrosis	Septal/peribronchial fibrosis	Abscessation	Lymphoid cell cuffs at airways	Acute fibrinous pleuritis	Chronic fibrous pleuritis
A 130	+	-	-	Mf	+	+	-	+	-	+	-	+
A 139	+	-	-	Mf	+	+	-	+	-	+	-	+
A 142	+	+	-	Ext	+	+	+	+	-	+	-	-
A 145	+	-	-	Mf	-	+	-	+	-	+	-	-
A 188	+	+	-	Ext	+	+	+	-	-	+	-	+
B 124	+	-	-	F	+	+	+	+	-	+	-	-
B 127	-	+	-	Ext	-	-	-	+	-	-	-	+
B 129	+	+	+	Mf	+	+	-	+	-	+	-	+
B 133	-	+	-	Ext	+	+	-	+	-	+	-	+
B 134	+	+	-	Ext	+	+	-	+	-	+	-	-
B 151	-	+	-	F	-	+	-	+	-	+	-	+
B 160	+	-	-	F	+	+	+	+	-	-	-	+
B 172	-	+	-	Mf	-	+	-	-	-	+	-	+
B 191	+	-	+	Ext	-	-	-	+	+	+	+	+

[1]Ext = extensive; F = focal; Mf = multifocal.

1993, 1998) were positive control tissues. For control of antigenic specificity of the primary antiserum, the following procedures were performed: the antiserum was (i) absorbed with *M. capripneumoniae* antigen (2 ml antiserum mixed with 0.2 mg antigen, kept at +4°C overnight and centrifuged) and then applied diluted 1:100 and 1:200; (ii) substituted by normal rabbit serum (Dako); (iii) applied on sections of pneumonic goat lungs with *M. ovipneumoniae, M. capricolum, M. mycoides* subsp. *mycoides* (LC) or *Pasteurella multocida* infection on culture; (iv) applied on sections of goat lung with schistosome egg granulomas, i.e., a completely irrelevant infection. Sections were incubated with TBS instead of primary antiserum for method controls.

Results

Clinical signs
See Table 1. Eight goats showed an episode of fever (40.0-41.4°C), as a rule accompanied by intense cough and harsh respirations. Respiratory signs only, in general with intermittent cough, at times with harsh breathing, were observed in some goats. In addition, all i.t.-inoculated goats showed a rise in temperature on days 1 and 2 after inoculation only. Group C goats remained healthy throughout the study period.

Serology
On i-ELISA (Table 1), 4 group A goats showed clear-cut seroconversion to *M. capripneumoniae* infection. One goat of group B had OD values above the cut-off value and another showed an antibody rise approaching the cut-off value. The OD values of the above goats are shown together with other variables of infection in Figs. 1 and 2. Three seropositive goats (nos. 131 and 188 of group A, and no. 151 of group B) were confirmed seropositive on b-ELISA, with inhibition values of 33%, 22% and 34%, respectively, and goat no. 139 had inhibition value 17%, just below cut-off. The other goats
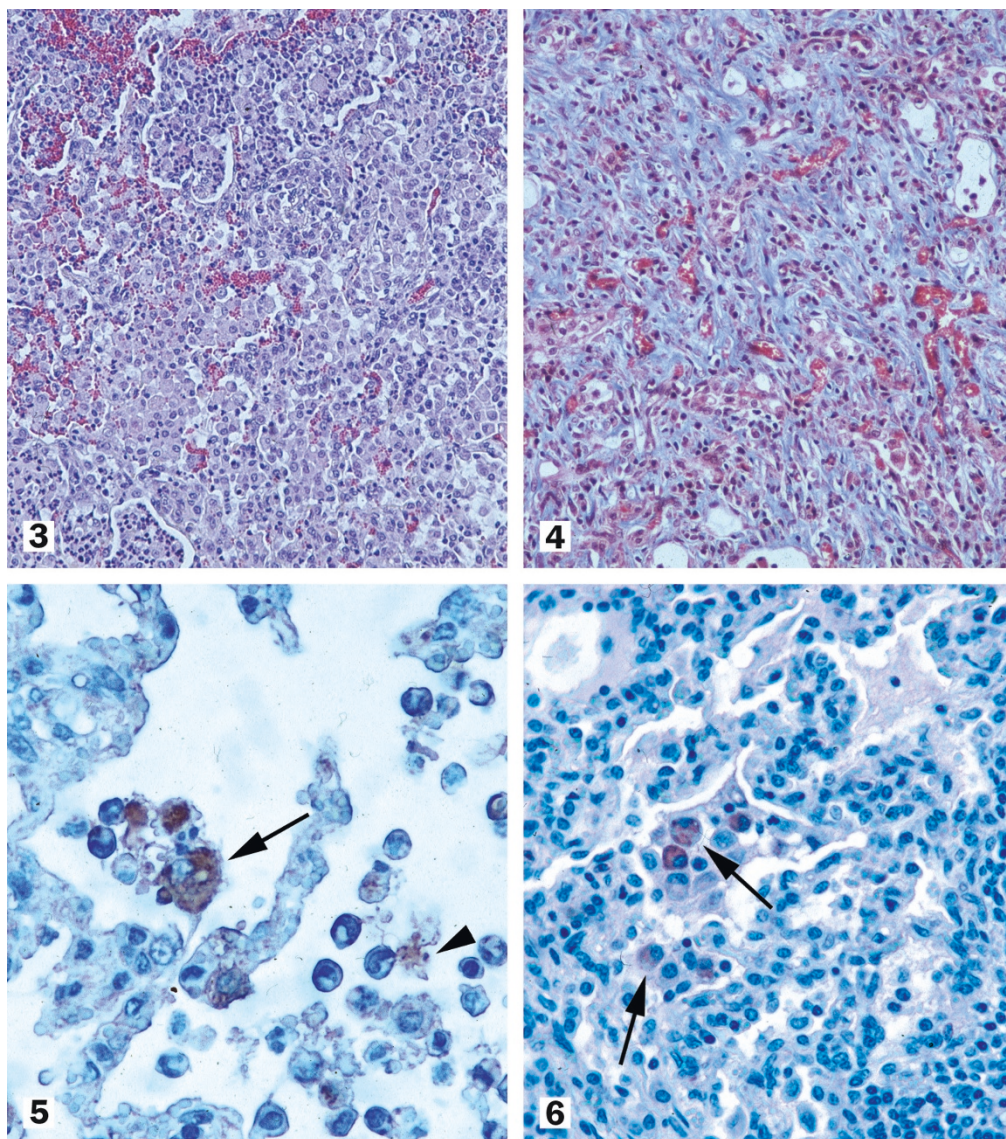
Figure 3. Alveolar exudate with prominence of alveolar macrophages, admixed with neutrophils. Goat no. 160. H&E, x123.

Figure 4. Collagen-rich granulation tissue in the alveolar tissue. Goat no. 142. Masson´s trichrome, ×123.

Figure 5. Immunostained *M. capripneumoniae* antigen in alveolar macrophages (one is arrowed) and free in alveoli (arrow-head). Control goat (culture positive) for immunohistochemistry. StreptABC/HRP, ×504.

Figure 6. Immunostained *M. capripneumoniae* antigen in alveolar macrophages (arrows) of an inoculated goat (no. 188). StreptABC/HRP, ×318.

tested with b-ELISA were negative. Seroconversion was not observed in any goat of group C (OD values 0.17-0.62, mean 0.33).

Gross pathology
Non-exposed goats. No gross changes were observed in the non-exposed goats.

Goats exposed to M. capripneumoniae. Gross lesions of lung and pleura (see Table 2) were observed in 14 (5 group A and 9 group B) goats. Unilateral lung and pleural involvement was the rule; bilateral affection was seen only in 2 goats. Lesions of the lungs were classified as focal, multifocal, or extensive (affecting a substantial part of each lobe of one lung). Goats showing pulmonary consolidation also showed firm enlargement of regional lymph nodes. Goats no. 142, 188 and 191 had marked accumulation of serous fluid in one of the pleural cavities.

Histopathology
Non-exposed goats. Slight infiltrates of macrophages and lymphocytes in the alveolar septa were regularly observed.

Goats exposed to *M. capripneumoniae.* Only goats with gross lesions showed significant histological changes (Table 2). Most of them had pneumonia characterized by mucopurulent to fibrinopurulent exudate, sometimes with necrosis, in dilated hyperplastic bronchi, alveolar exudates dominated by macrophages and with a variable component of neutrophils (Fig. 3), and pulmonary fibrosis. Alveolar fibrin deposits were found in occasional goats. Fibrosis was most often septal and peribronchial and was sometimes marked. Large strands of fibrous granulation tissue in alveoli (Fig. 4) was seen in some cases, and chronic fibrous pleuritis was often prominent. In a few goats, chronic pleuritis and septal fibrosis predominated markedly, whereas active inflammation of airways was not evident. Lymphoid nodules and follicles (cuffs) around airways were abundant in almost all goats. Sometimes, when combined with mononuclear alveolitis, such changes were compatible with bronchointerstitial pneumonia. Grossly enlarged bronchial lymph nodes displayed lymphoid hyperplasia with markedly enlarged follicle germinal centres. All other organs examined were normal. Goats without gross lesions showed pulmonary histology similar to controls.

Culture and PCR for mycoplasma and bacteria
M. capripneumoniae was not isolated on culture and was neither identified on PCR in any sample of any one goat. Nasal swabs during the course of the experiment yielded *M. ovipneumoniae* in 8 goats (nos. 128, 140, 156 and 188 of group A and nos. 126, 134, 144 and 160 of group B). This microorganism was isolated from the lungs and/or tracheal lavage samples at necropsy of all the above goats except no.160, and in addition in 1 group C goat and in goat 145 of group A. Pharyngeal swabs yielded *M. arginini* in 2 group A (nos. 140 and 188) and 2 group B (nos. 129 and 144) goats. From lung samples, non-haemolytic streptococci were grown in 4 group A (nos. 130, 139, 142 and 145) and 2 group B goats (nos. 134 and 160) goats and *Staphylococcus* sp. in 1 group A (no. 145) and 3 group B goats (nos. 134, 143 and 172).

Immunohistochemistry
Control materials. The positive control lung tissues showed distinct immunostaining as granular material in free form in fibrinous deposits and granular or large, dense material in the cytoplasm of alveolar macrophages (Fig. 5). No staining was seen with antigen-absorbed antiserum, normal sera or TBS. Lung tissue with *M. mycoides* subsp. *mycoides* LC infection

showed weak immunoreactivity. Lungs in which other mycoplasmas were isolated, or with schistosome or bacterial infection, were all negative.

Non-exposed goats. No immunostaining was detected in the lung of any goat.

Goats exposed to *M. capripneumoniae.* Immunostaining was seen in 3 group A and 4 group B goats (Table 1). In all cases, immunoreactivity was distinct and only intracellular, appearing in the cytoplasm of alveolar macrophages (Fig. 6). Cells with positive staining appeared multifocally, sometimes in abundance.

Discussion

Contagious caprine pleuropneumonia (CCPP) is generally recognized as a disease with a fulminant clinical course and high mortality from fibrinous pleuropneumonia shortly after infection (*McMartin et al.* 1980). The present experimental reproduction of CCPP displayed another pattern with a milder course and no acute mortality. *March et al.* (2002) described a similar course in a study which however comprised only a few goats. In our study, true reproduction of infection was indicated by cases with seroconversion and *M. capripneumoniae* antigen in the lungs on immunohistochemistry (IHC). However, neither cultural nor molecular identification of the agent was successful and only a minority of goats seroconverted. Altogether, these features suggest that the diagnosis of CCPP also under field conditions may not be straightforward.

One possible reason to consider for the mild disease course is a low virulence of the organisms in the inoculum, since repeated subcultures of laboratory strains may lower their virulence. Further, the lung suspension with *M. capripneumoniae* used was derived from a sur-

viving goat at the end of a CCPP outbreak. It is argued that *M. mycoides* (SC) strains isolated at the end of contagious bovine pleuropneumonia (CBPP) outbreaks are less virulent than those from early cases (*Provost et al.* 1987) and this possibly applies to CCPP as well.

Severe CCPP is characterized by appearance of fever at the end of the incubation period (*Mac Owan & Minnette* 1977). The wide variation in the incubation period of inoculated goats in our study was a striking finding. Fever was generally delayed, up to 28 days post inoculation (p.i.). This surprising lack of early clinical signs was the reason for the later inoculation of additionally 3 goats with another strain, which actually produced in one goat an episode of fever starting within the commonly observed 3-9 days p.i. (*MacOwan & Minnette* 1977). In-contact goats became febrile within a previously recorded time range of 11-41 days (*MacOwan & Minnette* 1977). The observed fever on days 1 and 2 p.i. was possibly a response to components of the inoculation medium since goats receiving medium only (*Wesonga,* unpublished data) also responded in this way.

In the great majority of goats with lesions at necropsy, the lungs showed fibrosis combined with fibrinopurulent or mucopurulent airway exudates and active alveolar inflammation with macrophages predominating over neutrophils. These features are collectively consistent with chronic fibrinous bronchopneumonia (*Dungworth* 1993, *López* 2001) and thus compatible with poor resolution of pneumonia from *M. capripneumoniae* infection. However, the agent was not grown from the lungs, in accordance with previous experiences from attempts to cultivate *M. capripneumoniae* from pulmonary or pleural lesions late in experimental infection (*MacOwan & Minnette* 1978, *Rurangirwa et al.* 1991, *Wesonga et al.* 1998). In our study, cultivation was negative despite use of pyruvate-containing media known to markedly support

growth (*Bölske et al.* 1995a, *Msami et al.* 2001). This makes it likely that living organisms were truly not present in the lungs.

On i-ELISA, clear-cut seroconversion was observed in 40% of the inoculated (A) goats. In most seroreactive A goats (Fig. 1), titres rose from about week 3 p.i., similar to previous findings (*MacOwan & Minnette* 1978, *March et al.* 2002). Goat no. 131 showed fever, further confirming infection that, however, was overcome at the timepoint of necropsy. The slow development of the disease in this goat and goat no.188 was reflected by a slow and steady rise of antibody titres. In comparison, goats no. 139 and 174 showed early signs and a biphasic pattern of the antibody response after a steep rise to the initial peak. The biphasic pattern is similar to that observed in the antibody response to *M. capripneumoniae* capsular polysaccharide antigen by *March et al.* (2002), who explained it as a possible effect of an 'eclipse' of antibodies by excess antigen in circulation. It can also be speculated that our goats with this pattern may have mounted a humoral response that was not protective to a first wave of infection, resulting in a second antibody response following intrapulmonary mycoplasmal proliferation. Though goat no. 151 of the in contact-exposed group showed steadily significant antibody titres after infection, both on i-ELISA and b-ELISA, it was consistently afebrile and at necropsy 3 months post exposure *M. capripneumoniae* antigen was present in a focus of residual chronic pleuropneumonia. These features altogether suggests a low-grade chronic infection in this goat.

Whereas serum antibody levels indicating infection were observed in only 24% of the exposed goats, 56% had lesions compatible with *M. capripneumoniae* infection at necropsy, and 20% were serologically negative but positive for *M. capripneumoniae* antigen in lesions. Though local antibody secretion into the lungs and airways was not investigated, this may indicate a poor activation of antigen-specific Th 2 immune responses in many goats.

This is apparently the first report on the use of IHC to detect *M. capripneumoniae.* However, IHC has been applied for some other mycoplasmas in ruminants, including *M. mycoides* subsp. *mycoides* (SC) in CBPP, where it was considered useful for etiological diagnosis (*Ferronha et al.* 1990, *Scanziani et al.* 1997, *Scanziani* 1998). In our study, *M. capripneumoniae* antigen was demonstrated in 7 of the 14 goats with lung lesions, but in none of the goats which lacked pulmonary pathology at necropsy. The results provide strong evidence that *M. capripneumoniae* antigen is associated with late stage lesions of CCPP. Whereas in positive control cases from an acute CCPP outbreak the antigen was both in free form in alveolar spaces and in alveolar macrophages, similar to findings in MmmSC infection (*Ferronha et al.* 1990), the goats of our study displayed antigen only in alveolar macrophages.

The presence of antigen in alveolar macrophages indicates the importance of these cells in the defense of the goat lung against *M. capripneumoniae.* In vitro studies have indicated antibody-mediated phagocytosis of mycoplasmas by alveolar macrophages as an effective mechanism of defense against *Mycoplasma pneumoniae* as well as *Mycoplasma pulmonis* (*Powell & Clyde* 1975, *Taylor & Howard* 1981). In mice with *M. pulmonis,* involvement of innate host defenses, such as surfactant protein A-dependent killing of organisms by IFNγ-activated alveolar macrophages, appears critical for the pulmonary defense (*Cartner et al.* 1998, *Hickman-Davis et al.* 1998), whereas humoral responses seem particularly important against systemic dissemination of the agent (*Cartner et al.* 1998).

Surprisingly, the PCR did not detect any *M. capripneumoniae* DNA even in lungs amply displaying microbial antigen on IHC. This

might be due to insufficient sensitivity or inhibition of the PCR reaction by substances in the samples (*Wilson* 1997) and warrants studies on optimization of sample pretreatment and use of internal amplification control for this PCR method.

M. ovipneumoniae, considered an ubiquitous mycoplasma of small ruminants world-wide (Jones 1989), was isolated from the lungs or trachea of 8 experimental goats with or without pulmonary pathology, and one control goat. The organism was a common inhabitant of the upper respiratory tract of the goats prior to infection and was also cultivated from the lung suspension used as part of the inoculum. The possible contribution to lung disease by this agent is difficult to ascertain, but caprine *M. ovipneumoniae* infection is one possible cause of cuffing pneumonia (*Mohan et al.* 1992), which commonly was a part of the pulmonary histopathology observed in our goats.

This study of long-term *M. capripneumoniae* infection has shown that the disease pattern and infection biology of CCPP may be a complex matter. Any chronic carrier state was not demonstrable. Our results indicate the need of further studies especially on immune mechanisms and interactions of *M. capripneumoniae* with host tissue cells in CCPP.

Acknowledgements

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Sammanfattning

Experimentell kontagiös pleuropneumoni hos get: En långtidsstudie över infektionsförlopp och patologi hos en flock getter infekterade med Mycoplasma capricolum subsp. capripneumoniae.

Kontagiös kaprin pleuropneumoni (CCPP) utgör ett viktigt hot mot gethållningen i delar av Afrika och Asien. Sjukdomen är i sin typiska form förenad med hög akut morbiditet och mortalitet i tidig infektionsfas, men litet är känt om dess förlopp och epizootologi vid långvarig infektion. I denna studie inokulerades 10 getter med *Mycoplasma capricolum* subsp. *capripneumoniae (M. capripneumoniae)* och de blandades sedan med 15 getter för kontaktsmitta. Sjukdomsförloppet studerades hos varje get under 56 - 105 dagar, varefter getterna avlivades och obducerades. Varierande tecken på infektion uppträdde hos totalt 17 getter (7 inokulerade, 10 kontaktsmittade). Åtta visade markanta kliniska symptom men inga dödsfall uppträdde. Endast 6 getter hade antikroppstitrar i serum mot *M. capripneumoniae* i indirekt ELISA. Fjorton getter (5 inokulerade, 9 kontaktsmittade) hade kroniska pleuropulmonära lesioner förenliga med CCPP vid obduktion och 7 av dessa visade *M. capripneumoniae*-antigen i lungorna vid immunohistokemisk undersökning. Varken vid mykoplasmaodling eller PCR-test kunde smittämnet påvisas. Resultaten visar att det kliniska förloppet vid CCPP kan vara jämförelsevis milt, att lunglesioner associerade med *M. capripneumoniae* kan förekomma i sent stadium av infektion i en flock, och att sjukdomen kan uppträda utan signifikant serologiskt antikroppssvar.