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Contagious bovine pleuropneumonia: humoral and pathological events in cattle infected by endotracheal intubation or by exposure to infected animals

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Summary

Results of trials in which cattle were infected by endotracheal intubation of *Mycoplasma mycoides* var. *mycoides* small colony (*Mmm*SC) cultures or by contact exposure to animals affected by contagious bovine pleuropneumonia (CBPP) are numerous. However, an analysis of the effects of the two different routes of infection on disease outcome is lacking. This study analyses the disease outcome in cattle infected by the two methodologies. Data originate from two controlled trials conducted in Namibia under field conditions. Intubation appears to be responsible for chronic evolution of the disease while in-contact infected animals develop more severe infection inducing mortality. Our results seem to suggest that the mode of infection may condition the disease outcome and should be taken into consideration in studies on the pathogenesis of CBPP.

Keywords

Bovine, Cattle, CBPP, Contagious bovine pleuropneumonia, Intubation, *Mmm*SC, *Mycoplasma mycoides* var. *mycoides* small colony, Namibia.

Pleuropolmonite contagiosa bovina: patologia e risposta umorale in bovini infettati per intubazione endotracheale o per contatto con animali infetti

Riassunto

Numerosi sono gli studi sull'infezione di bovini per intubazione endotracheale del Mycoplasma mycoides *var.* mycoides *small colony (*Mmm*SC) o per contatto con animali infetti. Tuttavia manca un' analisi degli effetti dei due differenti metodi di infezione. Il presente studio analizza l'evoluzione della malattia in bovini infettati con le due metodiche. I dati riportati provengono da due sperimentazioni condotte sul campo, in condizioni controllate, in Namibia. L'intubazione endotracheale del mycoplasma sembra essere causa di una evoluzione della malattia a carattere cronico in assenza di mortalità mentre negli animali infettati per contatto l'evoluzione assume carattere acuto seguito da mortalità.I risultati sembrano suggerire che la metodica di infezione condizioni il manifestarsi della malattia e debba essere presa in considerazione negli studi sulla patogenesi della PPCB.*

Parole chiave

Bovino, Intubazione, *Mmm*SC, *Mycoplasma mycoides* var. *mycoides* small colony, Namibia, PPCB, Pleuropolmonite contagiosa bovina.

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Introduction

Contagious bovine pleuropneumonia (CBPP) caused by *Mmm*SC affects cattle and domestic buffalo and remains one of the most important animal diseases in Africa. It is the cause of heavy productivity losses and the outcome may result in the death of the animal (8, 10). Under field conditions, incubation can last several months and the course of disease may be acute, subacute or chronic (8). Infection results in severe pulmonary pathology, usually affecting only one lung.

On a few occasions, the aetiological agent has been isolated from small ruminants showing signs of mastitis or pneumonia (2, 12); their role in the epidemiology of the disease is questionable.

In the second half of the 20th century, CBPP reappeared in southern Europe, namely: Italy, Portugal and Spain; the disease was eradicated using a policy of disease surveillance, cattle movement controls and stamping-out.

In Africa, where stamping-out and control of animal movements is difficult to implement, vaccination is the method of choice used to control the disease.

Current vaccines are produced using the T1/44 attenuated vaccine strain which has limited efficacy (13) and reveals residual pathogenicity when administered to cattle via the endo-tracheal route (6).

The role of innate or acquired cell-mediated and humoral immunity in conferring protection has not yet been elucidated (9, 14). However, the pathological lesions observed following infection appear to be conducive to an immunological process (4, 5).

Since no laboratory animal model is available, *in vivo* studies on CBPP have been conducted in cattle that may have been infected by endotracheal intubation or by contact with infected/diseased animals. While the former method is used more frequently, the latter simulates natural infection.

Since past experience has indicated that the disease outcome varies in accordance with the method of infection (7, 11), it was decided to analyse clinical, serological and pathological events made available from controlled field trials conducted in Namibia, on two separate occasions (in 2004 and 2005). The impact that the mode of infection may have on the pathogenesis of the disease is unknown.

Materials and methods

Data refer to two separate field experiments identified as Okavango 02 and Caprivi 03, performed in Namibia within the northern disease restricted area bordering Angola, beyond the veterinary cordon fence at the Mashare experimental farm of the Ministry of Agriculture, Water and Forestry.

Mycoplasmas

The Okavango strain was isolated from a natural disease outbreak that occurred in the Okavango region in early 2002; a second passage culture with a titre of $10^{9.0}$ colony-forming units (cfu)/ml was used.

Caprivi pleural fluid was collected from a cow during a CBPP outbreak in the Caprivi region in 2003. The fluid was checked for purity and was stored at –30°C. At the time of intubation, the *Mmm*Sc titre was 10^{10} cfu/ml.

Both pathogens were identified by growth inhibition, biochemical tests and polymerase chain reaction (PCR).

Cattle

Cattle originating from the southern commercial farming area that was free of CBPP (1) were used for the trials. Animals aged two years or more were screened serologically to exclude antibody for CBPP, brucellosis, bovine viral diarrhoea (BVD), bovine leucosis virus (BLV), bovine respiratory syncytial virus (BRSV), parainfluenza 3 virus (PI3) and infectious bovine rhinotracheitis (IBR).

Trials were conducted in accordance with European legislation (3) in regard to the protection of animals used for experimental purposes.

Infection in cattle

Infection by endotracheal intubation

Endo-tracheal intubation was performed by inserting a horse stomach tube into the trachea with the aid of a bronchoscope, until

bifurcation. Each animal was infected with 10 ml of Okavango 02 mycoplasma cultures or Caprivi 03 pleural fluid, mixed with 25 ml of Thiaucourt medium containing 2% agarose, broken up by gentle homogenisation. Finally, 40 ml of medium were used to flush the inoculum to the target site.

Twelve cattle intubated with the Okavango 02 preparation were monitored for 84 days post infection when they were slaughtered. The nine animals intubated with the Caprivi 03 preparation were monitored for 73 days and then slaughtered.

Infection by contact

In the in-contact infection trials, a mortality rate ≥40%.was the criterion arbitrarily used for slaughtering surviving animals.

In the Okavango 02 trial, 12 naive animals were exposed to the 12 animals infected by incubation 48 h earlier. At the time of contact, the health of the intubated cattle was considered good. Cattle were observed for 147 days post exposure.

In the Caprivi 03 trial, 10 naive animals were exposed to the 9 cows infected by intubation 26 days earlier. At the time of contact, intubated cattle had reacted showing antibody response, pyrexia and clinical symptoms. Animals were monitored 84 days post exposure.

Recording of clinical signs and sampling

In all instances, cattle were examined for respiratory distress, temperatures were taken every morning and only those exceeding 39°C were considered febrile. Bleedings were performed at daily intervals for the first 20 days post infection and thereafter at weekly intervals. Lung, mediastinal and peribronchial lymph nodes collected from animals that had died or slaughtered were stored at –20°C pending examination.

Tests

Serological tests

The modified Campbell and Turner complement fixation (CF) test was performed in accordance with the World Organisation for Animal Health (OIE: *Office International des Épizooties*) *Manual of diagnostic tests and vaccines for terrestrial animals* (15) on serum samples that had been stored at –20°C. Titres are expressed as the reciprocal of serum dilution.

Bacteriological tests

Purity and identification of isolates were performed according to the *Manual* (15).

Necropsy

Necropsy was performed on all animals at death or at slaughter.

The type of lesions and percentage of affected parenchyma were recorded in *ad hoc* forms. Lesions observed in the thoracic cavity were classified as follows:

- acute lesions: hepatisation at different stages, thickening of interlobular septa, presence of pleural fluid in the thoracic cavity, fibrinous pleurisy (omelette)
- subacute lesions: adhesion between visceral and parietal pleura, necrotic area in lung parenchyma
- chronic lesions: encapsulated sequestra at different stage of organisation or liquefaction.

Results

Respiratory distress was characterised by coughing, laboured breathing, dilated nostrils and ptialism.

Cattle infection by intubation

No undesirable reactions were recorded following manipulation related to intubation procedures.

Okavango 02 trial

In the 12 intubated cattle, no mortality was recorded over the observation period that lasted 84 days.

Clinical symptoms

Body temperatures were first recorded between days 7 and 15 post infection with an average time of 11 days. Persistence of pyrexia varied from 2 to 16 days. Respiratory distress of variable intensity was recorded in the animals.

Serology

The average time for detecting serological response was 10 days post infection, with the earliest reactions recorded on day 7 and the latest on day 15. Peak CF titres varied between 320 and 10 240 and were detected between days 15 and 28; animals were still reactive at slaughter.

Necropsy

Chronic lesions were observed in all 12 cattle at slaughter. In all instances, CBPP was confirmed by isolation of *Mmm*SC.

In eight cattle, seroconversion preceded pyrexia, whereas in four, the contrary was observed.

Caprivi 03 trial

Of the nine intubated animals, only one died on day 21 post infection, due to an acute form of CBPP.

Clinical symptoms

In eight animals pyrexia was first recorded between days 9 and 18 post infection, with an average time of 12 days. Persistence of pyrexia varied from days 8 to 14.

All eight animals showed respiratory distress of variable intensity. The only animal that died in the course of the trial had shown pyrexia, serological response and severe clinical symptoms the day before death.

In one animal, neither pyrexia nor clinical signs were recorded, despite a peak antibody titre of 1 280 between days 25 and 32 post infection.

Serology

The average time for detecting serological response in all animals was 12 days; the earliest reactions were recorded on day 7 post infection and the latest on day 13. Peak CF titres of 160 and 10 240 were detected between days 13 and 24; the eight surviving animals were serologically reactive at slaughter on day 73.

Necropsy

Acute lesions were observed only in the animal that had died on day 21 post infection, whereas chronic changes were recorded in the remaining eight. In all instances, CBPP was confirmed by isolation of *Mmm*SC.

In seven animals, seroconversion preceded or was recorded in the total absence of pyrexia, whereas in two, the contrary was observed.

Data are summarised in Table I.

Table I
Events following infection of cattle by intubation with *Mycoplasma mycoides* var. *mycoides* small colony

Recorded events	Okavango 02 Day post infection	Caprivi 03 Day post infection
	No. of cattle	
	12	9
Pyrexia		
First recording	7	9
Last recording	15	18
Average time	11	12
Serology		
First recording	7	7
Last recording	15	13
Average time	10	12
Lesions	Chronic	8 chronic, 1 acute

Cattle infected by contact

Okavango 02 trial

Of the 12 cattle exposed to animals intubated two days earlier, five (41%) had died during the observation period that lasted 147 days on days 119, 127, 134, 138 and 141 post exposure.

Clinical symptoms

No fever was recorded in two animals, one of which had died on day 119 post exposure. When present, temperatures were first recorded between days 88 and 138 post exposure, with an average time of 120 days and maximum persistence of 14 days. Respiratory signs were observed in all animals.

Serology

The CF antibody response was first detected on day 63 post exposure and all animals gave positive serological results by day 133, with an average seroconversion time of 103 days. Peak CF titres ranging from 2 560 and 5 120 were recorded between days 133 and 147. High antibody levels persisted over the entire observation period.

Necropsy

Acute or sub-acute pathological changes were recorded in five animals at death and in four at slaughter, whereas chronic lesions were observed in the remaining three cattle. *Mmm*SC was isolated from all 12 cattle.

In 10 animals, antibody response preceded or was recorded in total absence of pyrexia.

Caprivi 03 trial

Of the 10 cattle exposed to animals intubated 26 days earlier, four (40%) died in the course of the observation period of 84 days.

Clinical symptoms

Febrile reactions were not recorded in two animals despite seroconversion and respiratory distress. In the remaining eight, fever was first recorded between days 30 and 75 post exposure with an average period of 45 days and a persistence varying between 7 and 13 days. Respiratory distress was recorded in all animals.

Serology

The earliest serological reactions were recorded in two animals on day 14 post exposure and on day 42 in the remaining animals; the average seroconversion time was 39 days. Peak titres ranging from 640 to 10 240 were recorded between days 28 and 70. In the surviving animals, serological reactivity was still evident at slaughter.

Necropsy

In the four animals that died during the trial, severe and extensive acute lesions were recorded. In the five surviving cattle, sequestra with thick capsules, involving 30%-40% of the lung, were observed, whereas in the remaining animal, chronic and acute lesions were simultaneously present. In all instances, the CBPP diagnosis was confirmed by isolation of *Mmm*SC.

In nine animals, antibody response preceded or was recorded in the total absence of pyrexia; the contrary was recorded in only one animal.

Data are summarised in Table II.

Table II
Events following infection of cattle by contact with *Mycoplasma mycoides* var. *mycoides* small colony

Recorded events	Okavango 02 Day post exposure	Caprivi 03 Day post infection
	No. of cattle	
	12	10
Pyrexia		
First recording	88	30
Last recording	138	75
Average time	120	45
Serology		
First recording	63	14
Last recording	133	77
Average time	103	37
Lesions	9 acute/ subacute, 3 chronic	4 acute, 5 chronic, 1 mixed

Conclusions

The two groups of cattle infected by intubation with *Mmm*SC Okavango 02 and *Mmm*SC Caprivi 03, reacted similarly despite differences in the two preparations, i.e. cultures versus pleural fluid and titres expressed in cfu. Pyrexia was detectable after an average incubation period lasting 11 days with the former and 12 days with the latter, serological reactions were detected after an average of 10 and 12 days, respectively. In both cases, mortality was a rare event, one animal out of a total of 21. Pathological lesions at slaughter were chronic in 20 animals. Acute pathological changes were only observed in the animal that died during the observation period.

In 15 of the 21 animals, seroconversion either preceded or was recorded in the total absence of pyrexia.

On the contrary, in cattle infected by contact, the mortality rate, within the observation period, slightly exceeded 40% in both trials, five of 12 head in the Okavango 02 trial and four out of 10 in the Caprivi 03 trial. Irrespective of the different intervals between intubation and exposure (2 days versus 26), mortality occurred after an average of 27 days

from the appearance of antibody response with the former and 29 days with the latter.

In 19 out of 22 animals exposed to infection, antibody response was the first indication that the animals had become infected and it preceded pyrexia or was recorded in the total absence of pyrexia; only in the three remaining animals did pyrexia precede antibody response.

Whether these observations support the hypothesis that CBPP pathology is a result of an immunological process (4, 5) remains debatable.

In the conditions of the trials, the two modes of infection showed the following differences:

- in animals intubated endotracheally with *Mmm*SC, mortality was a rare event in contrast to observations recorded in cattle infected by contact exposure
- in intubated animals, chronic lesions were the predominant pathological findings, whereas in animals infected by contact, irrespective of the inoculum used, disease evolved indifferently in an acute form, followed by death, or in a chronic form followed by survival
- in cattle infected by contact, increased temperature is a reaction that most often follows serological response or is totally absent despite high antibody response.

In conclusion, the mode of infection, intubation versus contact exposure, appears to affect the outcome of disease and consequently this should be taken into consideration in studies on the pathogenesis of CBPP.

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