Acta vet. scand. 1984, 25, 577—592.

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COLONISATION PATTERN OF THE RESPIRATORY TRACT OF CALVES BY MYCOPLASMA DISPAR*

By

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TANSKANEN, RAILI: *Colonisation pattern of the respiratory tract of calves by Mycoplasma dispar.* Acta vet. scand. 1984, 25, 577—592. — Eight conventionally reared, 1- to 11-week old Ayrshire calves were naturally infected by a strain of Mycoplasma dispar (M. dispar). The colonisation was quantitatively followed by nasal swab samples, transtracheal aspiration samples and by the examination of the whole of the respiratory tract for mycoplasmas at slaughter after a follow-up period of 7—10 months.

The fairly uniform pattern of the colonisation by M. dispar was revealed: A high degree of colonisation, measuring 10^5—10^8 colour change units (ccu) per nasal sample, lasted for a period of 2—5 months and was followed by a slow decrease in titres. Seven of the calves still harboured M. dispar in their respiratory tracts at slaughter. Intermittently obtained transtracheal aspiration samples were all positive for M. dispar and the titres were regularly higher than those for the simultaneously taken nasal samples indicating a high ability of M. dispar to continuously colonize the more distal parts of the respiratory tract. It was demonstrated that the sensitivity of nasal swabbing in the detection of M. dispar infection largely depended on the phase of colonisation: The method was good for the detection of a fairly recent infection of M. dispar, but inadequate for detection of low grade carriers.

In various phases, the calves also became infected by Mycoplasma bovirhinis and Acholeplasma laidlawii. Their ability to colonize the whole respiratory tract was lower than that of M. dispar.

Mycoplasma infections; cattle; respiratory tract infections; epidemiology.

Mycoplasma dispar (M. dispar) was first isolated and characterized in England (*Gourlay* 1969, *Gourlay & Leach* 1970). It has since been one of the most commonly isolated mycoplasmas from pneumonic lungs of calves in several countries, England,

* This work was supported by grants from The Finnish Academy and The Finnish Veterinary Science Foundation.

Australia, Denmark, Japan, and USA among others (*Gourlay et al.* 1970, *St. George et al.* 1973, *Bitsch et al.* 1976, *Kuniyasu et al.* 1977, *Munster et al.* 1979). The pathogenicity of M. dispar for the respiratory tract of calves has been established by studies in gnotobiotic (*Howard et al.* 1976, *Gourlay et al.* 1979) and colostrum-deprived calves (*Friis* 1980) and in vitro in experiments on explant cultures of bovine trachea (*Thomas & Howard* 1974).

The basic findings of wide-spread occurrence and potential pathogenicity notwithstanding, the epidemiology of M. dispar infection and its various determinants have not received closer attention in the form of more thorough experimental or field studies. The aim of the present studies has been to throw light on the ability of and the pattern by M. dispar to colonize the respiratory tract of calves. The duration, level and localisation of the colonisation were investigated in conventionally reared calves infected by natural routes under experimental conditions. The colonisation was quantitatively followed by nasal swab samples and transtracheal aspiration samples. Some results on the present subject have been reported earlier in connection with first report on the isolation of M. dispar in Finland (*Tanskanen* 1983).

MATERIAL AND METHODS

Calves and facilities

Eight conventionally reared, 1- to 11-week old Ayrshire calves (nos. 1—8) were included in the studies carried out in 1982—1983 at the experimental facilities of the Department of Microbiology and Epizootology at the College of Veterinary Medicine in Helsinki.

Seven of the calves originated from two other Departments of the Veterinary College and calf 8 was obtained from the State Veterinary Medical Institute in Helsinki. The calves of the original stock including the experimental calves were found to be free of the infection of M. dispar when repeatedly tested by nasal isolation. Calves 5 and 7 were found to harbour a mild Mycoplasma bovirhinis (M. bovirhinis) infection (10^2—10^3 ccu/nasal sample).

The calves were included in the study and brought to an isolation unit one by one. Five calves from the earlier colonisation study (*Tanskanen* 1983) were at first kept in the same isolation unit and moved away during the course of the study. The calves

were also subjected to transmission experiments (to be reported later) and were therefore first kept in separate individual boxes and thereafter placed in common pens, 2—3 calves to a pen. After a period of approx. 7 months the calves were brought into contact with the other calves in the facilities as well.

Four of the calves (nos. 1—3 and 6) were splenectomized for the purpose of parallel use in experiments with Eperytrozoon tuomii (*Tuomi* 1966) infections. However, only calves 1 and 8 were used for these studies.

Infection

The strain of M. dispar involved in the present study has been briefly described earlier (*Tanskanen* 1983).

The calves were infected by varying degrees of contact with donor calves. At exposure, the calves were aged 59, 56, 82, 10, 24, 27, 81 and 7 days in order of both numbering and sequence of infection, respectively. The first calves received the infection from the calves of the earlier colonisation study (*Tanskanen* 1983).

The calves were also unintentionally exposed to M. bovirhinis and Acholeplasma laidlawii (A. laidlawii) when brought into contact with the calves of the earlier colonisation study, as well as with the other calves at the facilities in the later period of the study.

Sampling

Nasal samples were taken with rayon-tipped, polystyrene swabs (Culturette®, Marion Scientific Co.). The tip of a swab was moistened thoroughly against mucosal surfaces in the nasal cavity and immediately placed in the mycoplasma broth (1.8 ml). On the basis of absorption tests in vitro, it was estimated that the thoroughly wet tip of the swab adsorded approx. 0.2 ml of secrete.

For securing transtracheal aspiration samples, intravenous catheters (Intracath® nos. 3162, 3174, 3184, Deseret Co.) were used. The samples were taken under xylazin (Rompun®, Bayer) analgesia. Unnecessary lifting of a calf's head was carefully avoided in order to prevent possible aspiration of saliva. A catheter was inserted aseptically into the trachea as distally as possible so that the tracheobronchial tree of calves was judged

to have been sampled. Phosphate balanced salt solution in the amount of 5—10 ml was injected through the catheter and the subsequent aspiration was accomplished by means of a tight syringe. As an inoculum for culturing 0.2 ml of the aspirate was used. Nasal sampling was performed at approx. 3 day intervals during the first 3 weeks after finding the calf to have been infected, and afterwards at an average of 10 day intervals (Figs. 1 and 2). Transtracheal aspiration samples were taken at irregular intervals (Fig. 2).

The calves were slaughtered after 210—306 days from the detection of the infection by M. dispar. Immediately after removal from the thorax, the larynx, trachea and lungs were put into plastic bags, avoiding contamination, and sampled within 1—2 h. The larynx, upper and lower parts of the trachea, and the right apical bronchi were sampled with swabs. Two grams of the right apical lobus, the caudal margin of the diaphragmatic lobus, and pneumonic lesions were cut into pieces with scissors and a 10 % tissue-mycoplasma broth suspension was prepared for further processing.

Clinical observations

Rectal temperatures and observations of coughing and nasal discharge were recorded twice a day. If signs of clinical illness were detected the calves were subjected to a closer observation of various signs.

Media

For the culturing of M. dispar, a glucose calf-serum (GS) broth described by *Gourlay & Leach* (1970) was used with the following modifications. Hartely's digest broth and foetal calf serum in the medium of *Gourlay & Leach* were replaced by Bacto PPLO Broth w/v CU (Difco) and newborn calf serum (Gibco). In addition, the medium was supplemented with fresh yeast extract (8 %) prepared according to the method by *Hers & Masurel* (1967). For the purpose of selective isolation of M. dispar, the modified GS-broth with an added rabbit antiserum against M. bovirhinis (5 ml/100 ml) according to *Friis* (1979) was tried. The selective GS-broth was used particularly in the later stage of the colonisation by M. dispar, when the numbers of M. dispar in the sample were small and when M. bovirhinis was expected to be present in the same sample.

The conventional mycoplasma medium of the Hayflick's type described by *Chanock et al.* (1962) with the supplementation of calf thymus DNA (Sigma-highly polymerixed) (0.002 %), glucose (1 %) and fresh yeast (8 %) was usually used in parallel with GS-broth. The pH was adjusted to 7.8 with IN-NaOH. The conventional medium was supplemented with L-arginine (1 %) instead of glucose and pH was adjusted to 7.2 for culturing the antigen of M. arginini for immunization.

In both media, thallium acetate was used at the final concentration of 1:4000, Phenol Red at the concentration of 1:5000 and penicillin was replaced by ampicillin (Ampicin, Leiras) (0.5 mg per ml). Solid media consisted of the appropriate liquid broth with the addition of 0.8 % purified Bacto-Agar (Difco).

Culturing methods

The sample broths were agitated thouroughly on a Vortex mixer and preincubated for 30—45 min at 37°C. The swabs were then discarded and a series of tenfold dilutions up to 10^9 was prepared from preincubated broths. Preincubated broths and broth dilutions were incubated at 37°C for 3 weeks before the titre of the growth was determined. Growth in the undiluted sample broth was regarded as 10^1 ccu or cfu per sample.

Passages on solid media were carried out when a color change in the broth was noticed, or blindly from the undiluted broth and first 2 dilutions in days 3 and 7 of the incubation. Broth to broth passages were similarly carried out from undiluted broth and first 2 dilutions if there appeared no growth of M. dispar within the first 5—7 days. The incubation of GS broths were still continued after initial subculturing on the solid media and subcultured again for the isolation of M. dispar from the sample broths with mixed cultures of M. bovirhinis and M. dispar. Cultures on solid media were incubated at 37°C in a humidified atmosphere in air with increased CO_2 (approx. 3 %) for 10 days.

Identification

A careful watch was also kept for occurrence of mycoplasmas other than M. dispar.

The preliminary characterization of isolated mycoplasmas depended on growth characteristics, colony morphology and the results of the digitonin test made according to *Freundt et al.*

(1973). The epi-immunofluorescence method (epi-IF) was principally applied for identification proper. A few strains selected on the basis of the results of the epi-IF test were studied after purification by cloning using the growth inhibition (GI) test made according to *Clyde* (1963).

Antisera were prepared in rabbits against the following strains: M. arginini G 230, M. bovigenitalium PG 11, M. bovirhinis PG 43, M. bovis Donetta PG 45, M. dispar 462/2, A. axanthum S743, A. laidlawii PG8 and A. modicum PG 49. The above-mentioned strains as well as the strains of M. alkalescens D 12 PG 51, M. bovoculi M 165/69, M. gallinarum PG 16, M. gateae CS, M. verecundum 107, Bovine group 7 (*Leach*) PG 50 and Bovine group L (*Al-Aubaidi*) B144P and corresponding control antisera were obtained from the FAO/WHO Collaborative Centre for Animal Mycoplasmas, Aarhus, Denmark.

The preparation of antigen for immunization was made according to instructions by *Stone* (1976) with the modification that M. dispar was grown in GS broth and the other mycoplasmas in conventional Hayflick broth.

For immunization of rabbits, 1 injection of the antigen mixed with equal parts of complete Freund adjuvant (3 ml in all) or 3 similar weekly injections (1 ml) were given both i.m. and s.c. in the first phase. Rabbits were then boostered by i.v. injections of increasing amounts of antigen without adjuvant (0.2—2 ml), at first 3 times at a few days' intervals and then approx. every tenth day until the potency of antisera in the GI test was judged to be good enough.

For preparation of conjugates precipitation of globulins with $(NH_4)_2SO_4$ and conjugation with FITCH was carried out according to instructions by *Stone* (1976). Globulin concentration and FITCH/mg protein ratio were adjusted to 1—2 % and 1:50, respectively.

The direct IF-test was applied in the form of an epi-IF test according to *Rosendahl & Black* (1972) with slight modifications. The strains to be identified were grown on solid GS medium. The strains of M. dispar were passaged several times from broth to broth before subculturing on solid GS medium for attaining colonies attached to the agar. Agar blocks with colonies were fastened to the slides by mixture of paraffin wax and vaseline. The washing was carried out on a slowly revolving shaker either twice for 30 min in PBS or only once, in which case the slides

with agar blocks were left in sterile distilled water overnight (4°C).

The undiluted conjugates were checked against the reference strains of various species of interest in the present study, in order to secure the specificity of the conjugates. The specificity of the staining was also occasionally controlled by inhibiting the reaction with a preceding application of an unconjugated immune serum. The conjugates were routinely used as dilutions of 1:1—1:10 in PBS.

RESULTS

The colonisation by Mycoplasma dispar

The calves were found to be infected 1—19 days after exposure. Fig. 1 shows the appearance of M. dispar after exposure and

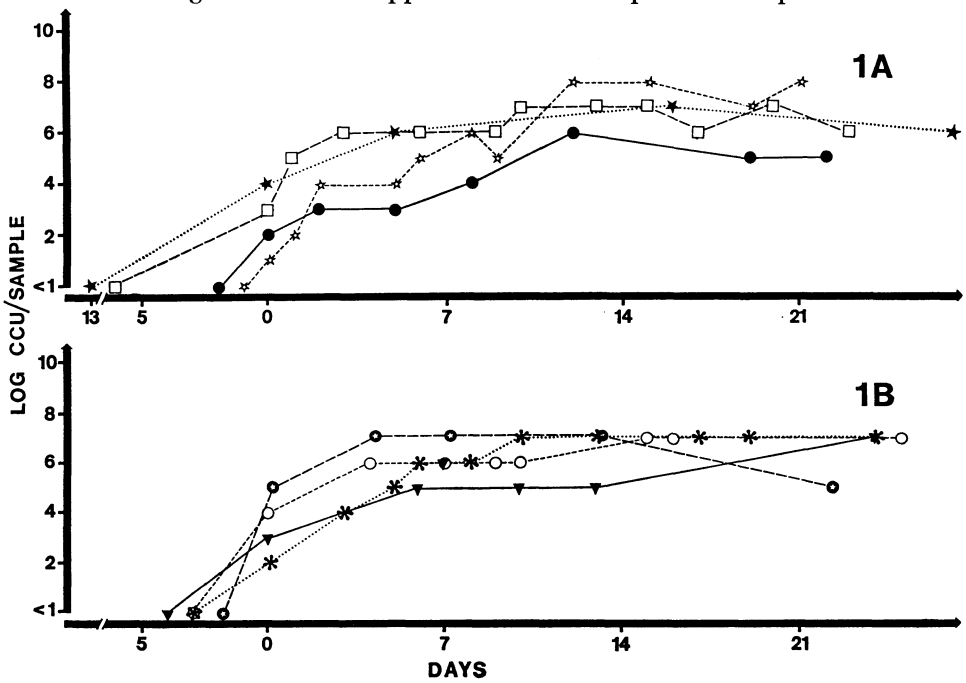

Figure 1. The early phase of colonisation of the respiratory tract of 8 calves by M. dispar measured by nasal swab samples.

A. non-splenectomized calves: calf 4□, 5●, 7☆, 8★.

B. Splenectomized calves: calves 1✪, 2○, 3▼, 6✻.

Symbols represent the titres of single or duplicate daily samples. Calves 5 and 7 harboured an infection of M. bovirhinis at the time of exposure to M. dispar. ccu = colour change unit.

the subsequent rise in colonisation titres measured by nasal swab samples. The number of M. dispar organisms in the first positive nasal samples varied from 10^1 to 10^5 ccu and increased to 10^5 ccu or higher in all calves during the first 12 days. The slowest rises in titres, extending to 7 and 12 days respectively, were recorded in the two calves that carried a M. bovirhinis infection when exposed to M. dispar.

As shown in Fig. 2, the phase of highest degree of colonisation, measuring 10^5—10^8 ccu per sample, lasted for a period of 2—5 months and was followed by a phase of slow decrease in titres to the level of 10^2—10^4 ccu. This phase with regular findings of continued colonisation extended to days 100 to 299 post recognition of infection, after which negative results were intermittently recorded. Calf 4 displayed an exceptional continuation

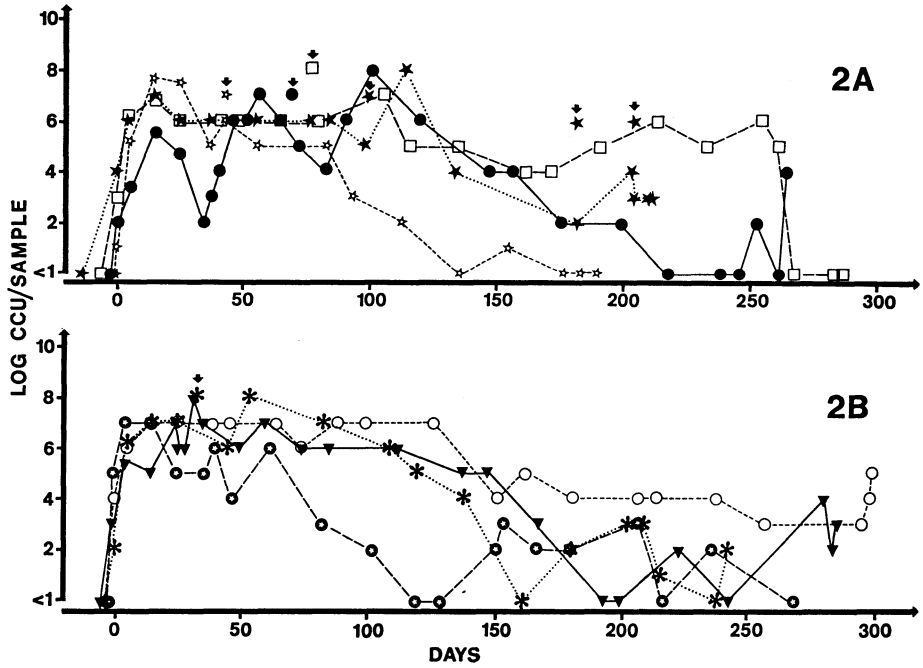

Figure 2. Colonisation of the respiratory tract of 8 calves by M. dispar measured by nasal swab samples and transtracheal aspiration samples. A. Non-splenectomized calves. B. Splenectomized calves. Three symbols after the first positive nasal swab sample (on the day 0) represent the mean titre of samples from the period of 10 days. Other symbols indicate the titres of single or duplicate daily samples. ⬇ Transtracheal aspiration sample. Other symbols: see Fig. 1.

of the high titre level for 8.5 months, up to the last month of the follow-up period (Figs. 2 and 3).

When the titres dropped to 10^1—10^3 ccu, it was usually necessary to use broth to broth passages for the isolation of M. dispar. The prozone phenomenon was common finding in this phase of colonisation and the presence of M. bovirhinis and A. laidlawii sometimes hampered the isolation of M. dispar. However, the experiments of selective isolation of M. dispar by modified GS-broth with antiserum against M. bovirhinis added did not unequivocally produce higher titres than the broth without antiserum.

Fig. 2 demonstrates that transtracheal aspiration samples irregularly obtained during days 32 to 204 after recognition of infection were all positive for M. dispar. The titres for the trans-

Table 1. Mycoplasma isolations from the respiratory tract of 8 calves after the follow-up period of the colonisation by M. dispar.

Calf no.	Mycoplasma species	Isolation site						Days after the first nasal isolation of M. dispar
		Larynx	Upper trachea	Lower trachea	Bronchus apic. dx.	Lobus apic. dx.	Lobus diaphr.	
1***	M. dispar	4*	0	0	nd	0	0	291
	M. bovirhinis	5	0	0		0	1	
2***	M. dispar	4	4	nd	4	0	3**	306
	M. bovirhinis	5	0		0	0	0	
3***	M. dispar	4	5	4	4	0	0	285
	M. bovirhinis	7	0	0	0	0	0	
4	M. dispar	0	0	0	0	0	0	287
	M. bovirhinis	6	3	0	0	0	0	
5	M. dispar	7	7	8	6	1	1**	265
	M. bovirhinis	7	0	0	0	1	0	
6***	M. dispar	6	6	2	3	0	0	242
	M. bovirhinis	6	0	0	0	0	0	
7	M. dispar	3	1	3	1	0	0	232
	M. bovirhinis	4	0	0	0	0	0	
8	M. dispar	7	7	6	5	0	1	210
	M. bovirhinis	6	3	0	0	0	0	

* Figures indicate the $\log_{10}$ of colour change units per swab sample or 0.2 ml of 10 % lung suspension.

** Sample was taken from the pneumonic lesions; otherwise the caudal margin of Lb. diaphr. was sampled.

*** A splenectomized calf.

nd = not done.

trachal aspiration samples of 0.2 ml were regularly higher than those for the concurrent nasal samples.

M. dispar was still present and found to colonize the respiratory tract from larynx to apical bronchi in 6 of the 8 calves examined at slaughter (Table 1). In only 3 of the calves, the lung parenchyma was also found to be infected; in 2 of the cases in the areas of pneumonic lesions. Only from calf 4 could M. dispar not be isolated at all.

Other mycoplasmas

In Fig. 3 examples are given of simultaneous infections by M. dispar, M. bovirhinis and A. laidlawii. All 8 calves became infected by M. bovirhinis also, and seven of them additional by A. laidlawii. As mentioned above, calves 5 and 7 were infected by M.

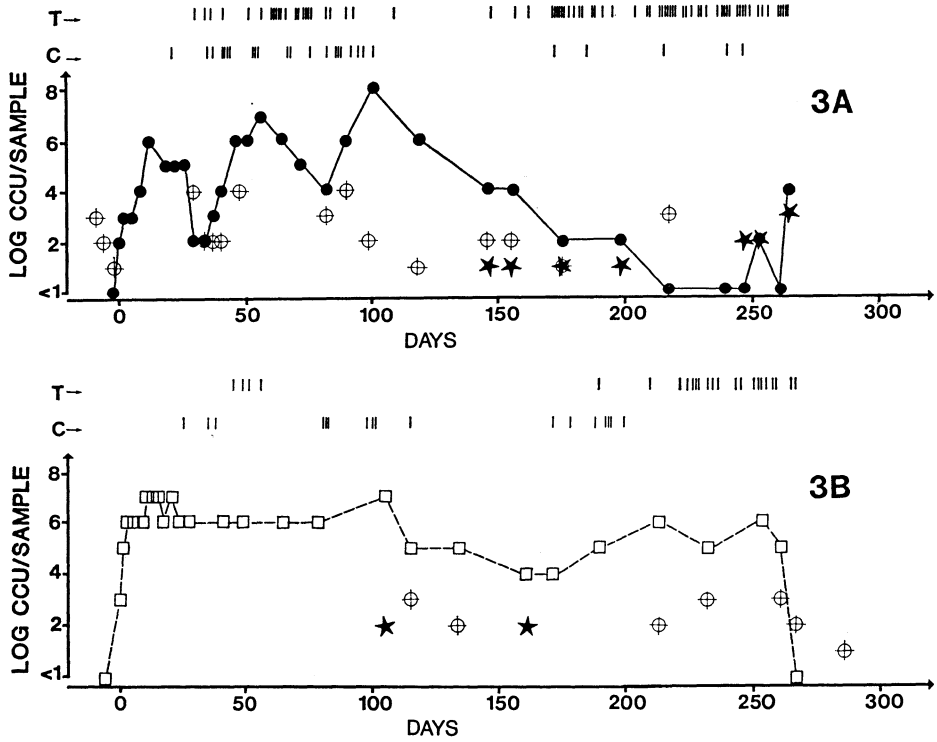

Figure 3. The simultaneous colonisation of the respiratory tract of 2 calves by M. dispar ● and M. bovirhinis ⊕ and A. laidlawii ✶ and some clinical findings during the colonisation. A. Calf 5. B. Calf 4.
C (|): Coughing recorded in daily observation.
T (|): Rectal temperatures over 39.0°C recorded.

bovirhinis even before exposure to M. dispar. The rest were found to be infected by M. bovirhinis for the first time at 50 to 181 days.

The strains identified as M. bovirhinis were not identical with the reference strain. The intensity of fluorescence at the epi-IF test was not as strong as by the reference strain, and only slight inhibition of growth, if at all, was obtained with the reference antiserum in the GI-test. Because none of all the other antisera included in the study inhibited the growth of these strains in the GI-test, they were classified ad hoc as M. bovirhinis on the basis of the results by the immunofluorescence test.

In general, except during the end phase of the observation period, the titres for M. bovirhinis were lower than those for M. dispar and the former was also isolated less regularly, suggesting false negative results.

In some cases there was a decrease in the titres of M. dispar coinciding with and probably caused by an concurrent, and usually recently acquired, infection of M. bovirhinis. The phenomenon was apparent during days 29—40 in calf 5 and at the end of the follow-up period in calf 4, as shown in Fig. 3.

M. bovirhinis was isolated from the transtracheal aspiration samples of calf 7 on day 43 and of calf 8 on days 181 and 204. On examination at slaughter M. bovirhinis was isolated in 7 of the calves from the upper respiratory tract only (Table 1).

A. laidlawii was occasionally isolated from nasal samples but seldom in several successive samples and the titres were low (10^1—10^3 ccu per sample).

Clinical findings

Calf 1 showed no evidence of a respiratory illness. Calves 2 and 3 irregularly experienced a few short and mild temperature elevations. In calves 6 and 7, mild coughing occurring intermittently was recorded. Calf 8 repeatedly experienced slight temperature rises and was also affected by intermittent coughing and nasal discharge 53—189 days after recognition of infection. The most definite cases of chronic respiratory illness with coughing and slightly elevated temperatures were recorded in calves 5 and 4. The phases during which the clinical findings of these two calves were most conspicuous are presented in Fig. 3.

Macroscopic pneumonic consolidation at post-mortem examination was detected in the diaphragmatic lobes of the lungs of calves 2 and 5.

DISCUSSION

The present longitudinal study demonstrated a fairly uniform pattern of a long-standing but gradually fading colonisation of the respiratory tract by M. dispar. Constant high titres for nasal swab samples during a period of several months were suggestive of a high degree of mucosal colonisation with abundant shedding of organisms. Consequently, during that period of colonisation, nasal swabbing proved to be a reliable method in the detection of a M. dispar infection. However, in the later phase of colonisation the inadequacy of the nasal swab method became apparent. The results agree with and complement the earlier, more sketchy findings by the author (*Tanskanen* 1983).

Findings on M. dispar colonisation, reported in the literature, are based on cross-sectional studies mainly of post mortem material of healthy or sick calves (*Gourlay et al.* 1970, *Thomas & Smith* 1972, *Pirie & Allan* 1975, *Bitsch et al.* 1976, *Munster et al.* 1979, *Friis & Krogh* 1983). However, the conclusions drawn from the age distribution agree largely with the present results.

Colonisation persisted throughout the follow-up period of 7—10 months. The one negative finding (calf 4) at slaughter is probably a false negative one, explained by interference by the preceding fresh infection of M. bovirhinis. In the earlier work (*Tanskanen* 1983), M. dispar was isolated from nasal swab samples for a period of 9 months from all 6 calves studied and the last successful nasal isolation was in month 13 from 1 of those calves. However, M. dispar was still isolated from the larynx and the upper part of trachea of 3 splenectomized calves slaughtered after the follow-up periods of 13, 15 and 17 months, respectively. The combined results suggest that the infection of M. dispar caught in early calfhood is generally still present in the yearlings and may persist in a more latent form for a considerably longer period.

The results indicate that M. dispar typically and long-standingly also colonize the tracheobronchial tree and not only the upper parts of the respiratory tract. In addition, the generally higher titres in transtracheal aspiration samples than in concurrent nasal samples, and the negative findings or the low titres in nasal swab samples in the later phase of the colonisation contrasted with an overt colonisation of the distal parts apparent at slaughter suggest that M. dispar to some degree favours the tracheobronchial tree to the upper parts of the respiratory tract as

its natural focus. However, in the earlier study of a longer follow-up period (*Tanskanen* 1983) a colonisation restricted in the end to the larynx and the upper part of the trachea was discovered.

The results showed that the sensitivity of nasal swabbing in the detection of an infection by M. dispar is largely dependent on the colonisation phase. The nasal swab method proved to be a reliable diagnostic technique in the detection of a fairly recent infection, but not adequate for detecting carriers in the later phase of colonisation, which could be recognised by examination of the larynx and often also from the more distal parts of the respiratory tracts.

In the later phase of the colonisation, the presence of other mycoplasmas and the prozone phenomenon interfered with the detection of small numbers of M. dispar. The use of broth to broth passages were found to be very important in this phase of colonisation.

The present data on the colonisation pattern resulted from experiments with only 1 strain of M. dispar. Differences in the colonisation properties between various natural strains of the organism may, of course, exist. The relatively high ability to colonize the respiratory pathway of calves by M. dispar has also been suggested by the results of short term experimental studies by others (*Howard et al.* 1976, *Gourlay et al.* 1979, *Friis* 1980).

The results did not indicate that splenectomizing affected susceptibility to infection by M. dispar. Neither were any age differences suggested within the rather narrow range studied.

The relatively long-standing colonisation demonstrated above with abundant shedding consitutes an effective way of maintaining the agent of M. dispar in larger populations of calves. A chronic colonisation with the distal location revealed in the present studies also provides a better understanding of the factors on the known property of potential pathogenicity of M. dispar. The present clinical and pathological data, though still somewhat equivocal, was suggestive of this kind of chronic pathogenic effect by M. dispar.

The colonisation patterns for other mycoplasmas inferred from the results remain only tentative but present clear cut differences with those of M. dispar. The strains of M. bovirhinis found to infect the calves showed a lesser ability to colonize the respiratory tract than M. dispar. The titres were constantly lower, nasal samples were irregularly positive and the isolations at

slaughter were largely restricted to the upper parts of the respiratory tract.

Some indication was found of a suppressive effect of a recent infection by M. bovirhinis on the infection by M. dispar. For example the failure to isolate M. dispar from the last nasal samples and at slaughter of calf 4, in contrast with high titres in the preceding nasal samples, could have such an explanation. A specific type of antagonistic effect between 2 of the avian mycoplasmas has been reported earlier (*Taylor-Robinson & Cherry* 1972).

The findings on A. laidlawii implied only a weak ability of this mycoplasma to colonize the respiratory tract of calves. This finding supports the general conception of non-pathogenic role for, and a more saprophyte nature of this mycoplasma (*Gourlay & Howard* 1979).

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SAMMANFATTNING

Mycoplasma dispars kolonisationsmönster i andningsvägarna hos kalv.

Åtta konventionellt uppfödda 1 till 11 veckor gamla Ayrshire kalvar besmittades på naturlig väg med en stam av Mycoplasma dispar.

Den kvantitativa kolonisationen studeras genom odling av nässekret, transtrakeala aspirationsprov och genom undersöknig av hela andningsvägarna med avseende på förekomst av mycoplasmer vid slakten efter en uppföljningsperiod på 7—10 månader.

M. dispar uppvisade ett rätt regelbundet mönster vid kolonisationen. En höggradig kolonisation, vid vilken 10^5—10^8 colour change units per näsprov uppmättes, varade i 2—5 månader och åtföljdes av en långsam titersänkning. Sju av kalvarna hyste fortfarande M. dispar i andningsvägarna vid slakten.

Suoradiska transtrakeala aspirationsprov var alla positiva med avseende på M. dispar, och titrarna var i regel högre än i de prov som samtidigt tagits från näsan, vilken tyder på en kontinuerlig kolonisation av M. dispar i de lägre delarna av andningsvägarna. Tillförlitligheten av nässekretodlingar vid M. dispar infektioner visade sig till stor del bero på kolonisationsfasen. Metoden var bra för att påvisa en rätt färsk infektion av M. dispar, men olämplig för att påvisa långvariga infektioner.

Kalvarna infekterades också vid olika tillfällen med Mycoplasma bovirhinis och Acholeplasma laidlawii. Deras förmåga att kolonisera hela andningsvägarna var sämre än M. dispar.

(Received September 17, 1984).

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