J. Vet. Med. B **46,** 693–700 (1999)
© 1999 Blackwell Wissenschafts-Verlag, Berlin
ISSN 0931–1793

Danish Veterinary Laboratory, Bülowsvej 27, Copenhagen, Denmark

Pathological and Microbiological Studies on Pneumonic Lungs from Danish Calves

C. TEGTMEIER[1,3], Aa. UTTENTHAL[1], N. F. FRIIS[1], N. E. JENSEN[1] and H. E. JENSEN[2]

Addresses of authors: [1]Danish Veterinary Laboratory, Bülowsvej 27, DK-1790 Copenhagen V, Denmark; [2]The Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Frederiksberg C, Denmark; [3]Corresponding author

With 2 tables

(Received for publication 2 November, 1998)

Summary

During 1 year, the association between microbiological and pathological findings in 72 lungs from calves submitted to the Danish Veterinary Laboratory for diagnostic purposes was studied. All cases were evaluated pathologically and bacteriologically, whereas only 68 cases were examined for the presence of bovine respiratory syncytial virus (BRSV), parainfluenza-3 virus (PI-3 virus) and bovine coronavirus, 62 cases for bovine viral diarrhoea virus (BVD), 45 cases for bovine adenovirus and 51 cases for mycoplasmas. Based on histopathological examination, the cases were diagnosed as fibrinous and/or necrotizing bronchopneumonia, suppurative bronchopneumonia, embolic pneumonia and others. The diagnoses were based on the dominating and most severe lesions in each lung. *Haemophilus somnus*, *Pasteurella multocida*, *Actinomyces pyogenes, P. haemolytica* and BRSV were the most commonly found bacterial and viral lung pathogens, respectively. *Pasteurella* spp. and *H. somnus* were often associated with the more severe fibrinonecrotizing type of bronchopneumonia, whereas BRSV was primarily detected in cases of suppurative bronchopneumonia. *Mycoplasma bovis* was isolated from one case only, whereas *M. dispar, M. bovirhinis* and *Ureaplasma diversum* were present, often concomitantly, in the majority of cases. *Aspergillus fumigatus* was isolated from one case.

Introduction

Respiratory tract infection is one of the most common diseases affecting calves in Denmark (Blom, 1981; Madsen, 1984). Thus, ≈ 20 % of the bovine material submitted to the Danish Veterinary Laboratory (DVL) for diagnostic purposes consists of lungs from calves with an anamnestic history of respiratory illness, often originating from herds with severe outbreaks of pneumonia.

A variety of micro-organisms is known to be involved in the development of calf pneumonia, either as a monoinfection or in combination with others (Bitsch et al., 1976; Ishino et al., 1979; Krogh et al., 1986; Binder et al., 1990; Orr, 1992, Watts et al., 1994; Uttenthal et al., 1996). However, the results of either viral and/or mycoplasmal examination are often not available in such diagnostic studies.

The aim of the present study was to gain information on the frequency and pattern of pathogenic micro-organisms involved in calf pneumonia, and to thoroughly evaluate the association between the pathological and microbiological findings, including all relevant pathogens, under Danish conditions. Thus, in this study, lung tissue from 72 calves was evaluated pathologically and analysed for bacterial, viral, mycoplasmal and fungal pathogens.

U. S. Copyright Clearance Center Code Statement: 0931-1793/99/4610-0693$14.00/0

Materials and Methods

Samples of lung tissues

During the period October 1993 to September 1994 lung tissue from 72 calves from 68 different herds submitted to the DVL for diagnostic purposes was examined. The material represented all apparently non-autolysed lungs received at the DVL during this period.

Pathology

At necropsy, macroscopic lesions were recorded, and two to five samples of lung tissue were collected for histopathology. The samples were obtained from two to three sites, i.e. from areas with inflammation, from the borderline between inflamed and normal tissue, and when available, also from normal areas. The specimens were fixed in 10 % buffered formalin, embedded in paraffin wax, cut in 3–4 μm thick sections, and mounted on Super Frost slides (Hounisen, Denmark).

All sections were stained with haematoxylin and eosin (HE) for histopathological interpretation. From each lung, two samples, i.e. from an inflamed area and from the borderline between inflamed and normal tissue, were stained with Grocott's methenamine silver method for visualization of fungi including *Pneumocystis carinii*. Selected cases were stained with Mallory's phosphotungstic acid haematoxylin (PTAH) for demonstration of fibrin. Connective tissue was demonstrated with the van Gieson–Hansen stain.

Based on the histopathological examination, the cases were diagnosed as fibrinous and/or necrotizing bronchopneumonia, suppurative bronchopneumonia, embolic pneumonia and other pneumonic lesions. The diagnoses were based on the dominating and most severe lesions in each lung, i.e. areas with suppuration were interpreted as concomitant when found in cases with fibrinous/necrotizing lesions.

Bacterial cultivation and identification

All cases were examined bacteriologically by standard laboratory methods. In brief, tissue from one pneumonic area of the lungs was streaked on to four blood agar plates (Columbia agarbase, Oxoid, Unipath Ltd, Basingstoke, UK; all supplemented with 5 % sterile calf blood) and further onto one plate containing brom thymolblue, 1 % saccarose and 1 % lactose (Drigalsky agar plate). Two of the blood agar plates contained polymyxin B-sulphate 25 units/ml (Sigma, St Louis, USA; P1004). After inoculation, one plate with and one without polymyxin B-sulphate and the Drigalsky plate were incubated in a normal atmosphere at 37°C, whereas the remaining two blood agar plates were incubated in an atmosphere of 10 % CO_2 and 90 % air at 37°C. All plates were inspected for growth after 16 h and 40 h. A tentative identification of *Haemophilus somnus* was based on the growth of tiny white or yellowish-white colonies on plates without polymyxin B-sulphate incubated in 10 % CO_2, and on no growth in normal air. A final identification was based on negative Gram staining, a positive oxidase reaction, a negative catalase reaction, production of indol and fermentation of glucose. A preliminary identification of *Pasteurella multocida* and *P. haemolytica* was based on colony morphology, colour, and possible haemolysis on the plates incubated in a normal atmosphere, negative Gram staining and positive oxidase and catalase reactions. The final identification was performed as proposed by Casals and Pringler (1993), using Rosco diagnostic tablets (Rosco Diagnostica, Taastrup, Denmark). The identification of *Actinomyces pyogenes* was based on colony morphology and haemolysis on plates incubated in a normal as well as in a CO_2-enriched atmosphere (regardless of the content of polymyxin B-sulphate), morphology by positive Gram staining and a negative catalase reaction. Blue colonies on Drigalsky plates were suspected as S*almonella* spp., a final confirmation being based on conventional *Salmonella* serotyping (Popoff and Le Minor, 1992). The identification of *Streptococci* and *Staphylococcus aureus* was performed in accordance with Barrow and Feltham (1993).

An examination for bacterial inhibitors (antibiotics/chemotherapeutics) in the lungs was performed by placing $\approx$ 1 g of lung tissue on a 5 % blood agar plate (Columbia agar base, Oxoid) seeded with a *Sarcina lutea* culture. The development of an inhibition zone around the lung tissue after incubation at 37°C overnight was interpreted as the presence of antibacterial substances.

In a few cases, cultivation for fungi was carried out on a Sabouraud glucose agar with 50 IE/ml penicillin and 0.05 mg/ml dihydrostreptomycin and incubation at 30°C. The species identification was based on morphological criteria according to Larone (1995).

Virus examination

The majority of the cases were examined for common pulmonary viral pathogens (Table 1). The presence of bovine respiratory syncytial virus (BRSV), parainfluenza-3 virus (PI-3), bovine coronavirus and bovine adenovirus was examined by indirect sandwich-enzyme-linked immunosorbent assay (ELISA) systems as previously described (Uttenthal et al., 1996). In brief, a $\approx 1 \times 1 \times 1$ cm piece of inflamed lung tissue close to the borderline between macroscopically inflamed and normal parenchyma was minced in phosphate-buffered saline (PBS) buffer, homogenized in a Stomacher and centrifuged. The supernatant was used for the detection of virus antigens; in 68 cases for BRSV, PI-3 virus and bovine coronavirus, and additionally, in 45 randomly selected cases for bovine adenovirus. Antigens of BRSV and PI-3 virus were detected using hyperimmune calf sera and of bovine coronavirus by using immunserum from a rabbit, immunized with a rabbit cell culture-adapted strain of bovine coronavirus (Meyling, 1982). Bovine adenovirus was detected using rabbit antibodies to hexons of *Hepatitis contagiosa canis* (HCC) adenovirus of dogs. For all ELISA systems, microtitre plates were coated using the specific detection sera. The supernatant from homogenized lung tissue was subsequently applied followed by incubation with biotinylated specific antibodies towards the viral antigen. The binding of peroxidase-conjugated avidin (DAKO P347, Glostrup, Denmark) was visualized by orthophenylenediamine substrate (OPD) (Kem-En-Tec, Copenhagen, Denmark). Threshold levels have been described previously (Uttenthal et al., 1996). In 62 cases, lung tissue was also tested for the presence of bovine viral diarrhoea virus (BVD) by cultivation on bovine turbinate cells followed by specific staining after the principles described by Meyling (1984).

Virus detection did not include infectious bovine rhinotracheitis (IBR) virus which is considered eradicated in Denmark.

Mycoplasma examination

Cultivation for mycoplasmas and ureaplasmas was performed in 51 cases (Table 1). Isolation and identification were performed according to the principles described by Friis and Krogh (1983). However, the antibacterial compound, thallium acetate, was substituted by the antibiotic cycloserine and the volume of Hanks' balanced salt solution was increased as described previously (Friis et al., 1991; Kobisch and Friis, 1996). The classical mycoplasmas were identified by the disc growth inhibition test (DGI) on solid medium (Clyde, 1983) by using type strain antisera. Ureaplasmas were identified by the ability to degrade urea and from the colour and morphology of colonies on solid medium in accordance with the descriptions of Razin (1983).

Results

Gross pathology

The great majority of the lungs (n = 69, 96 %) contained pneumonic lesions which involved the cranioventral parts of the lung. In most cases with catarrhal, mucopurulent or purulent exudate, a distinct lobular pattern of distribution could be observed, in contrary to most

Table 1. Number and type of microbiological examinations of 72 pneumonic calf lungs evaluated pathologically

Bacteriological cultivation	72
Viral examination (ELISA)	
BRSV	68
PI-3 virus	68
Coronavirus	68
Adenovirus	45
Viral examination (cultivation)	
BVD virus	62
Mycoplasma cultivation	51

ELISA, enzyme-linked immunosorbent assay; BRSV, bovine respiratory syncytial virus; PI-3 virus, parainfluenza-3 virus; BVD virus, bovine viral diarrhoea virus.

fibrinous and necrotizing bronchopneumonias where the lobular texture was indistinct. One and the same lung frequently contained distinct areas with different lesions, for example fibrinous and necrotizing bronchopneumonias often contained areas with suppuration. Likewise, lungs with a catarrhal exudate and emphysema on the borderline between normal and inflamed tissue, often contained areas dominated by suppuration in the more distal parts.

In eight cases fibrinous pleuritis was seen along with the pneumonia.

Histopathology

The histopathological diagnosis and microbiological findings are presented in Table 2. The fibrino-necrotizing bronchopneumonias (n = 31, 43 %) were characterized by various degrees of exudation of fibrin and/or necrosis, some lungs being dominated by fibrinous lesions (n = 10), whereas others mostly contained necrotic areas (n = 21). In lungs with primarily fibrinous lesions, the dominating picture was alveolar lumina filled with fibrin, often concomitant with a mixed population of inflammatory cells composed of neutrophils and macrophages. In most cases, distinct areas of coagulation necrosis surrounded by a demarcation zone was observed. Sometimes also areas with haemorrhage into the alveolar spaces were present. In the necrotizing bronchopneumonias, the necrotic changes were either present within the bronchiolar epithelium and/or within the walls of the alveoli. The lumina of bronchioles and large areas of the surrounding alveolar parenchyma were often heavily infiltrated with a mixed population of inflammatory cells, oat-shaped cells being frequent. *Pasteurella* spp. were often isolated from bronchopneumonias dominated by exudation of fibrin, whereas *H. somnus* was a common isolate from the bronchopneumonias dominated by necrosis.

The catarrhal to suppurative bronchopneumonias (n = 36, 50 %) were characterized by the migration of neutrophils and macrophages into bronchioli and alveolar lumina without

Table 2. Histopathological diagnoses and microbiological findings in 72 cases of pneumonic calf lungs submitted to the Danish Veterinary Laboratory, October 1993 to September 1994

Histopathological diagnosis (number/total)	Bacteriological examination (number/total)	Viral examination (number/total)	Mycoplasma/ureaplasma examination (number/total)
Fibrino-necrotizing bronchopneumonia (31/72)	*H. somnus* (10/11) *P. multocida* (5/10) *P. haemolytica* (4/4) *A. pyogenes* (5/9) *S. dublin* (1/2)	BRSV (2/18) Coronavirus (1/1)	*U. diversum* (16/35) *M. dispar* (13/31) *M. bovirhinis* (7/17)
Suppurative bronchopneumonia (36/72)	*P. multocida* (4/10) *A. pyogenes* (4/9) *H. somnus* (1/11) *Strep. uberis* (1/1) *E. coli* (1/2)	BRSV (16/18) BVD virus (4/4) PI-3 virus (1/1)	*U. diversum* (17/35) *M. dispar* (15/31) *M. bovirhinis* (10/17) *M. bovis* (1/1)
Embolic pneumonia (4/72)	*P. multocida* (1/10) *S. dublin* (1/2) *E. coli* (1/2) *Staph. aureus* (1/1)		*M. dispar* (3/31) *U. diversum* (2/35)
Other lesions (1/72)	*A. fumigatus* (1/1)		

H. somnus, *Haemophilus somnus*; *P. multocida*, *Pasteurella multocida*; *P. haemolytica*, *Pasteurella haemolytica*; *A. pyogenes*, *Actinomyces pyogenes*; *S. dublin*, *Salmonella enterica* susp. *enterica* serovar *dublin*; *E. coli*, *Escherichia coli*; *Strep. uberis*, *Streptococcus uberis*; *Staph. aureus*, *Staphylococcus aureus*; *A. fumigatus*, *Aspergillus fumigatus*; BRSV, bovine respiratory syncytial virus; BVD virus, bovine viral diarrhoea virus; PI-3 virus, parainfluenza-3 virus; *M. dispar*, *Mycoplasma dispar*; *M. bovirhinis*, *Mycoplasma bovirhinis*; *M. bovis*, *Mycoplasma bovis*; *U. diversum*, *Ureaplasma diversum*.

necrosis or marked exudation of fibrin. Alveolar fetalization, the formation of syncytial cells and interstitial emphysema were prominent features in a number of cases in which BRSV antigen was often detected.

In a number of cases the concomitant formation of abscesses was occasionally observed from which *A. pyogenes* was often isolated.

Four cases (5.5 %) were diagnosed as embolic pneumonias whereas one case (1.5 %) remained unclassified (dissiminated mycotic pneumonia from which *Aspergillus fumigatus* was isolated).

Microbiological findings

The microbiological findings correlated to the histopathological diagnoses are presented in Table 2.

Among the 72 examined lungs, bacterial pathogens were isolated from 35 lungs (49 %); *H. somnus* (n = 11), *P. multocida* (n = 10), *A. pyogenes* (n = 9), *P. haemolytica* (n = 4), *Salmonella enterica* susp. *enterica* serovar *dublin* (*S. dublin*) (n = 2), *Eschericia coli* (n = 2), *Staphylococcus aureus* (n = 1) and *Streptococcus uberis* (n = 1). In most lungs only one bacterial species was isolated, except for four lungs dually infected with *P. multocida* and *A. pyogenes*, and one case dually infected with *H. somnus* and *S. dublin*. Antibacterial substances were detected in 23 (32 %) cases, most of which (n = 18, 78 %) were found to be negative by bacteriological cultivation.

Viral examination revealed BRSV antigen in 18 cases, BVD virus in four cases and corona and PI-3 virus antigen in one case each. BRSV antigen was detected concomitantly with BVD virus in one lung. Adenovirus was not detected.

In the examination of the 51 lungs for mycoplasmas, 43 (84 %) yielded one or more species (*M. dispar*, *M. bovirhinis* and *U. diversum*), whereas *M. bovis* was only found in one case.

Aspergillus fumigatus was isolated from one case.

Discussion

The complexity of lesions among and especially within the same lung, makes a complete classification of the lungs into distinct groups difficult and often impossible. However, it seems reasonable to use the histopathological classification made in the present survey, where the dominating lesion was determined and used as a guideline for grouping the cases.

The present survey showed that *H. somnus*, *P. multocida*, *A. pyogenes* and *P. haemolytica* are the most common bacteria associated with severe outbreaks of calf pneumonia in Denmark. This distribution is in agreement with a previous survey performed in Denmark (Krogh et al., 1986), and surveys performed in other countries (Binder et al., 1990; Orr, 1992; Watts et al., 1994).

The *Pasteurella* spp. (nine of 14) and *H. somnus* (10 of 11) were often associated with the severe fibrino-necrotizing type of bronchopneumonia, which is in accordance with former studies (Schiefer et al., 1978; Andrews et al., 1985; Bryson et al., 1990). *Pasteurella multocida* was frequently isolated in combination with other bacterial or viral pathogens which might indicate that this bacterium is usually not capable of inducing such severe lesions unless another pathogen acts concomitantly. This supports the general opinion that *P. multocida* tends to cause milder, less fulminating pneumonias as compared with *P. haemolytica* (Schiefer et al., 1978; Dungworth, 1993). *Actinomyces pyogenes* was often isolated concomitantly with other pathogens, presumably acting as a secondary complicating organism, as described by Dungworth (1993).

In agreement with a recent study (Uttenthal et al., 1996), the present results show that BRSV has become the dominating viral pathogen associated with severe outbreaks of calf pneumonia in Denmark, whereas PI-3, BVD virus, and coronavirus seem to be of more limited importance. BRSV (16 of 18 detected) was often detected in cases of suppurative bronchopneumonia, in which a macroscopically visible rim of emphysema was often present on the borderline between inflamed and normal tissue, and formation of syncytial cells and alveolar fetalization were observed by histopathology.

Mycoplasma dispar, M. bovirhinis and *U. diversum* were isolated, either alone or concomitantly,

from 43 of the 51 lungs examined, without preference for any of the types of pneumonia. Thus, they appear to be widespread among the Danish cattle population. These mycoplasmas are likely to take part in the development of initial lesions, i.e. by decreasing the mucociliary efficiency (Almeida and Rosenbusch, 1994), while their pathogenic importance during the following phases of disease seems uncertain and further studies should be performed in order to elucidate the pattern of pathogenicity of these mycoplasmas. *Mycoplasma bovis* is usually regarded as the most pathogenic of the mycoplasmas isolated. Gourlay et al. (1989) has demonstrated the organism in areas with coagulation necrosis in exudative bronchopneumonias. The organism is widespread in other European countries (Binder et al., 1990; Laak et al., 1992), the USA (Knudtson et al., 1986; Adegboye et al., 1995) and Canada (Donkersgoed et al., 1993) where it is considered one of the most important bovine pulmonary pathogens. In this study, *M. bovis* was found in only one case, thus indicating a low prevalence in Denmark (Friis and Krogh, 1983). Only one lung contained fungi (*A. fumigatus*). *Pneumocystis carinii* has previously been demonstrated in Danish calf lungs (Settnes and Henriksen, 1989), but the organism was not found in this study, which indicates that it does not seem to be involved in outbreaks of severe pneumonia in Danish calves.

In general, bacteria were often associated with a fibrinous necrotizing type of bronchopneumonia and BRSV with a suppurative bronchopneumonia accompanied by interstitial emphysema. A clear correlation between pathological changes and aetiology could, however, not be established. The reasons for this may be many: intensive antibiotic therapy, the immune status of the calf, variation in virulence between different strains of the same micro-organisms, concomitant infections, false-negative microbiological diagnosis, etc. One likely explanation of major importance for the often negative outcome of bacterial cultivation attempts is probably the frequent appearance of antibacterial substances within the lungs (32 %), reflecting recent antibiotic therapy. Therefore, more work should be carried out in order to enhance the sensitivity of routine screening for bacterial pathogens, for example by the application of concomitant diagnostic tools such as polymerase chain reaction (PCR) techniques, the detection of bacterial antigens in tissue extracts, or by immunohistochemistry. In a recent study, the results of the application of a peroxidase–anti-peroxidase technique for the demonstration of *H. somnus* antigen in pneumonic calf lungs in Denmark revealed a distinct higher rate of infection as compared with cultivation results (Tegtmeier et al., 1995).

In conclusion, this study demonstrates that some degree of pathological classification of pneumonic calf lungs can be performed, when based on the dominating and the most severe lesions. Also, different pathogens can be associated with the different types of pneumonias, i.e *P. haemolytica* and *H. somnus* with fibrinous necrotizing types of bronchopneumonia and BRSV with suppurative changes accompanied by interstitial emphysema on the borderline between inflamed and macroscopically normal tissue.

In Denmark, multiple pathogens are involved in severe outbreaks of calf pneumonia: *P. multocida*, *H. somnus*, *P. haemolytica*, *A. pyogenes* and BRSV being the most common. The mycoplasmas *M. bovirhinis*, *M. dispar* and *Ureaplasma* can be considered widespread among Danish calves, whereas *M. bovis* is only rarely isolated.

Acknowledgements

Annie R. Pedersen, Ulla L. Andreasen and Jannie Pedersen are gratefully acknowledged for their excellent technical assistance. This work was partly supported by the Research Secretariat of the Ministry of Food, Agriculture and Fisheries (grant no. SUN95-SVS-6).

References

Adegboye, D. S., P. G. Halbur, D. L. Cavanaugh, R. E. Werdin, C. C. L. Chase, D. W. Miskimins, and R. F. Rosenbusch, 1995: Immunohistochemical and pathological study of *Mycoplasma bovis*-associated lung abscesses in calves. J. Vet. Diagn. Invest. **7,** 333–337.

Almeida, R. A., and R. F. Rosenbusch, 1994: Impaired tracheobronchial clearance of bacteria in calves infected with *Mycoplasma dispar*. J. Vet. Med. B. **41,** 473–482.

Andrews, J. J., T. D. Anderson, L. N. Slife, and G. W. Stevenson, 1985: Microscopic lesions associated with the isolation of *Haemophilus somnus* from pneumonic bovine lungs. Vet. Pathol. **22,** 131–136.

Barrow, G. I., and R. K. A. Feltham, 1993: Cowan and Steel's Manual for the Identification of Medical Bacteria, 3rd edn. Cambridge University Press, Cambridge.

Binder, A., G. Amtsberg, S. Dose, W. Fischer, H. Scholz, and H. Kirchhoff, 1990: Untersuchung von Rindern mit respiratorischen Erkrankungen auf Mykoplasmen und bakterielle Bronchopneumonieerreger. J. Vet. Med. B. **37,** 430–435.

Bitsch, V., N. F. Friis, and H. V. Krogh, 1976: A microbiological study of pneumonic calf lungs. Acta. Vet. Scand. **17,** 32–42.

Blom, J. Y., 1981: Enzootic pneumonia in calves. PhD Thesis, The Royal Veterinary and Agricultural University, Denmark.

Bryson, D. G., H. J. Ball, M. McAliskey, W. McConnell, and S. J. McCullough, 1990: Pathological, immunocytochemical and microbiological findings in calf pneumonias associated with *Haemophilus somnus* infection. J. Comp. Path. **103,** 433–445.

Casals, J. B., and N. Pringler, 1993: The Zym-kits and Identification Tablets for Bacteria and Yeast. Rosco Diagnostica, Taastrup.

Clyde, W. A., 1983: Growth inhibition tests. In: Razin, S. and J. G. Tully (eds), Methods in Mycoplasmology, pp. 405–410. Academic Press, New York.

Donkersgoed, J. V., C. S. Ribble, L. G. Boyer, and H. G. G. Townsend, 1993: Epidemiological study of enzootic pneumonia in dairy calves in Saskatchewan. Can. J. Vet. Res. **57,** 247–254.

Dungworth, D. L., 1993: The respiratory system. In: Jubb, K. V. F., P. C. Kennedy, and N. Palmer (eds), Pathology of Domestic Animals, 4th edn. Academic Press, San Diego.

Friis, N. F., P. Ahrens, and H. Larsen, 1991: *Mycoplasma hyosynoviae* isolation from the upper respiratory tract and tonsils of pigs. Acta. Vet. Scand. **32,** 425–429.

Friis, N. F., and H. V. Krogh, 1983: Isolation of mycoplasmas from Danish cattle. Nord Vet-Med. **35,** 74–81.

Gourlay, R. N., L. H. Thomas, and S. G. Wyld, 1989: Increased severity of calf pneumonia associated with the appearance of *Mycoplasma bovis* in a rearing herd. Vet. Rec. **124,** 420–422.

Ishino, S., M. Oka, S. Terui, and S. Ikeda, 1979: Pathological and microbiological studies on calf pneumonia occurring in mass rearing facilities. Nat. Inst. Anim. Hlth Qu. **19,** 91–103.

Knudtson, W. U., D. E. Reed, and G. Daniels, 1986: Identification of mycoplasmatales in pneumonic calf lungs. Vet. Microbiol. **11,** 79–91.

Kobisch, M., and N. F. Friis, 1996: Swine mycoplasmoses. Rev. Sci. Technical Off. Int. Epiz. **15**(4)**,** 1569–1605.

Krogh, H. V., K. B. Pedersen, and N. F. Friis, 1986: Pneumonia in calves associated with *Haemophilus somnus*. In: Proceedings of the 14th International Congress on Diseases of Cattle, Dublin, pp. 585–589. Irish Cattle Veterinary Association, Dublin.

Laak, E. A. T., J. H. Noordergraaf, and R. P. J. W. Dieltjes, 1992: Prevalence of mycoplasmas in the respiratory tracts of pneumonic calves. J. Vet. Med. B. **39,** 553–562.

Larone, D. H., 1995: Medically Important Fungi, a Guide to Identification, 3rd edn. ASM Press, Washington.

Madsen, E. B., 1984: Morbidity of calves in specialized fattening calf production. PhD Thesis, The Royal Veterinary and Agricultural University, Copenhagen.

Meyling, A., 1982: ELISA for detection of *Bovine coronavirus* in faeces and intestinal contents. Curr. Top. Vet. Med. Anim. Sci. **22,** 161–169.

Meyling, A., 1984: Detection of BVD virus in viremic cattle by an indirect immunoperoxidase technique. Curr. Top. Vet. Med. Anim. Sci. **29,** 37–46.

Orr, J. P., 1992: *Haemophilus somnus* infection: a retrospective analysis of cattle necropsied at the Western College of Veterinary Medicine from 1970 to 1990. Can. Vet. J. **33,** 719–722.

Popoff, Y., and L. Le Minor, 1992: Antigenic Formulas of the *Salmonella* Serovars. Institut Pasteur, Paris.

Razin, S., 1983: Urea hydrolysis. In: Razin, S., and J. G. Tully (eds), Methods in Mycoplasmology, pp. 351–353. Academic Press, New York.

Schiefer, B., G. E. Ward, and R. E. Moffatt, 1978: Correlation of microbiological and histological findings in bovine fibrinous pneumonia. Vet. Pathol. **15,** 313–321.

Settnes, O. P., and S. A. Henriksen, 1989: *Pneumocystis carinii* in large domestic animals in Denmark. A preliminary report. Acta. Vet. Scand. **30,** 437–440.

Tegtmeier, C., N. E. Jensen, and H. E. Jensen, 1995: Development of a peroxidase-antiperoxidase (PAP) technique for the identification of *Haemophilus somnus* in pneumonic calf lungs in Denmark. APMIS **103,** 540–547.

Uttenthal, A., N. P. B. Jensen, and J. Y. Blom, 1996: Viral aetiology of enzootic pneumonia in Danish dairy herds: Diagnostic tools and epidemiology. Vet. Rec. **139,** 114–117.

Watts, J. L., R. J. Yancey, J. R. Sarah, S. A. Salmon, and C. A. Case, 1994: A 4-year survey of antimicrobial susceptibility trends for isolates from cattle with bovine respiratory disease in North America. J. Clin. Microbiol. **32,** 725–731.