DEVELOPMENTS IN VETERINARY SCIENCE

THE ETIOLOGY OF ENZOOTIC PNEUMONIA OF CALVES

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Introduction

The development and selection of appropriate vaccines to prevent or minimize losses from enzootic pneumonia demand accurate information about the etiological agents. Similarly, current concepts of rational chemotherapy are based on the selective destruction of infectious agents. One of the goals of clinical and laboratory diagnosis, therefore, is the etiologic diagnosis of disease. When the cause (single or multiple) cannot be identified, treatment becomes, at best, empirical and at worst, contraindicated or harmful.

Enzootic pneumonia, i.e. a non-parasitic pneumonia of variable or multiple etiology most frequently diagnosed in calves between three weeks and four months of age (7, 11, 14, 70, 86), is used to define the form of pneumonia without implying a specific etiology. Although terms such as "virus pneumonia" may correctly be applied to certain outbreaks, it is now clear that viruses are not the only primary cause of pneumonia in calves.

As a result, prevention and treatment are often, if not usually, based on an inaccurate, incomplete, or a misinterpreted etiologic diagnosis. It is the purpose of this review to describe briefly the signs and gross pathology of enzootic pneumonia, and to review in detail the results of numerous studies of the etiology of this disease. In addition, suggestions are made for the satisfactory submission of samples for laboratory diagnosis.

Signs and Gross Pathology

Symptoms of early uncomplicated cases of enzootic pneumonia include pyrexia, non-productive pneumonia, dyspnea and a dry cough. Diarrhea may or may not be observed (11) and toxemia is not a characteristic sign of the early disease. These symptoms may be transient, or may rapidly progress to those of productive bronchopneumonia with severe dyspnea and toxemia. The disease may be subclinical, chronic or acute; morbidity and mortality rates may approach 100% (11, 48, 86) and survivors may remain unthrifty (86).

On post-mortem examination lung lesions are remarkably similar in animals from different outbreaks, and most are characterised by patchy and confluent dark red areas of lung collapse (atelectasis) or consolidation. In early cases these lesions are primarily seen in the ventral aspects of anterior lobes of the lung, but later may extend deeply into all lobes and extensive lesions are usually accompanied by emphysema of the little remaining "normal" lung tissue. The cut surfaces of affected areas from early or uncomplicated cases of the disease are usually moist and plum red in colour. Cut bronchioles ooze a clear fluid exudate and in some cases bronchioles may be surrounded by a thin white protruding cuff produced by accumulated lymphoreticular cells. In protracted cases bacterial invasion may compound lesions to produce a variegated picture inclusive of purulent bronchopneumonia, lung necrosis, abcessation and perhaps pleurisy.

Clinical signs, epizootiology and macroscopic lung lesions are described in detail elsewhere (11, 51) and usually enable differentiation of enzootic pneumonia from some other calfhood respiratory diseases. In brief, aspiration pneumonia is of sporadic occurrence and is usually characterised by obvious necrosis of lung tissue. Pneumonic pasteurellosis (shipping fever) is rarely seen in housed calves younger than six months of age, but its signs include severe toxemia while the postmortem lung lesions are characterised by fibrinous pneumonia. Atypical pneumonia is usually characterised by severe emphysema, edema and a "meaty" consistency of lung tissue unaccompanied by consolidation unless atypical pneumonia has been superimposed on existing lesions. Parasitic pneumonia may be readily diagnosed if adult nematodes are visible in respiratory passages but differentiation from enzootic pneumonia may be difficult (11) and lesions of hematogenous origin (focal embolic pneumonia), including those caused by migrating lungworm larvae, are mainly apparent in dorsal rather than ventral areas of lung tissue.

However, while enzootic pneumonia may be diagnosed as a general respiratory syndrome

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on the basis of symptoms and gross pathology such characteristics do not allow identification of the cause of a specific outbreak.

Etiology

Early unsuccessful attempts to produce pneumonia in calves by the inoculation of bacteria isolated from naturally-occurring cases of calf pneumonia led to the conclusion that bacteria such as *Corynebacterium pyogenes, Actinobacillus actinoides, Staphylococcus aureus, Escherichia coli* or members of the Pasteurella or Hemophilus genus were probably not primary respiratory pathogens and often lung tissue from affected calves was bacteriologically sterile (54). However, in the early 1920's Carpenter and Gilman (14) produced pneumonia in calves intratracheally inoculated with saline washings from lungs of naturally-affected calves, and when this was repeated with lung washings from which recognised bacteria had been removed by filtration (4, 54), it was postulated that some calf pneumonias were produced by "filterable agents," but the "filterable agents" remained unidentified.

Bacteria[1]

1. *Chlamydia* (Psittacosis-lymphogranuloma venereum agents): About the first definitive association between a specific "filterable agent" and bovine pneumonia was made in the early 1950's when Chlamydia were isolated from cattle with acute fatal respiratory disease in Japan (61). Although these agents had previously been associated with sporadic bovine encephalomyelitis (63) and had been isolated from the feces of apparently normal animals (92), the Japanese isolate was shown to be capable of producing pneumonia in experimental calves (61). Since then Chlamydia have been associated with other natural outbreaks of respiratory disease in calves. Chlamydia, for example, were identified as one cause of widespread enzootic pneumonia of suckling calves in Hungary, and calves inoculated with the agents isolated from lung tissue of fatal field cases did develop symptoms of respiratory disease (74). Chlamydia were also isolated from the pneumonic lung tissue of four to six month old calves in England and this isolate, too, produced pneumonia in experimental calves (88). More recently, Chlamydia were isolated from the lungs of a calf with enzootic pneumonia in Ontario, and the agent was subsequently shown to produce extensive lung lesions in experimental calves (89). Similar results were also obtained by workers in England using a strain isolated from the feces of cattle with respiratory disease (72). The observation that Chlamydia cause enzootic pneumonia of sheep (36, 74), that strains isolated from pneumonic calves may produce pneumonia in sheep (74) and that Chlamydia are not particularly species specific agents (36) suggests that interspecies infection might occur under natural conditions.

2. *Mycoplasma (PPLO).* Contagious bovine pleuro-pneumonia (caused by *Mycoplasma mycoides*) is not seen in Canada, but the severity of this disease is a constant reminder of the potential respiratory pathogenicity of Mycoplasma. Interest in Mycoplasma is further heightened by the fact that these bacteria produce a pneumonia in man (67) and pigs (58) which is histologically very similar to a form of enzootic pneumonia seen in calves in various countries including Canada (17, 47, 51, 70, 86) and designated as "cuffing pneumonia" (47); however, the lesions of cuffing pneumonia may not be specifically associated with any one etiological agent (44, 49).

Mycoplasma (not *M. mycoides*) have been isolated from nasal exudate of adult cattle with respiratory disease in Canada (15) and England (38) and from lung tissue of calves with enzootic pneumonia (31, 37, 56) although definitive association between the isolates and respiratory disease was not established. However, it has been shown that Mycoplasma can rapidly disseminate within a calf population. and indirect evidence has indicated that such infection may exacerbate a virus-induced pneumonia of calves (31). Davis (33) suggested that although Mycoplasma were not uncommonly found in nasal swabs from "normal" calves they were more commonly found in lung tissue when the tissue was diseased, but it was not known if they were primary lung pathogens.

In a recent review of the importance of viruses and Mycoplasma as animal respiratory pathogens Darbyshire and Roberts (28) reiterated that some groups of Mycoplasma may be of more significance than others as respiratory pathogens. Thus, Mycoplasma of groups 2 and 8 may be found in bovine respiratory secretions but are not considered to be pathogenic, whereas those of groups 4 and 6 may be lung pathogens. It is obvious that the complexity of this bacterial genus dictates that clarification of the relative lung pathogenicity of the various Mycoplasma species will take some time,

[1](Chlamydia and Mycoplasma (PPLO) are classified as bacteria.)

but it seems reasonable to assume that some contribute directly or indirectly to the production of enzootic pneumonia in calves.

3. *Other bacteria.* The role of other bacteria in the production of enzootic pneumonia must not be summarily dismissed, although information on this subject is scant. There is little doubt that bacteria play an extremely important role as secondary pathogens of diseased lung tissue and there is considerable experimental evidence to show that normal clearance of bacteria from the lung may be reduced by microbiological, physical, or chemical factors which impede the normal lung physiology (52). Duncan (35) suggested that *Pasteurella hemolytica* could produce pneumonia in experimental calves if the homotypic antibody level in the calves was sufficiently low at the time of aerosol infection. Trapp *et al* (84) produced pneumonia in calves inoculated with *P. hemolytica* and *P. multocida* by the intratracheal route after they had been stressed by heating and cooling. Carter (16) produced pneumonia in calves inoculated with *P. hemolytica* but concluded that perhaps the pathogenicity of the bacteria had been enhanced by pre-existing asymptomatic lung disease. The fact that workers have failed to produce much evidence of respiratory disease in calves inoculated with *Pasteurella spp.* (6, 16, 20) suggests that perhaps these bacteria are not potent independent primary pathogens. Nevertheless it does seem that they may play a greater role in the production of respiratory disease in cattle than is presently recognised (21, 82), particularly if superimposed on pre-existing conditions of stress or lung damage.

Viruses

1. *Infectious bovine rhinotracheitis (IBR).* IBR is primarily a respiratory pathogen of adult or young adult cattle. Experimental or natural infection of calves with this herpes virus has resulted in disease referable to various systems including the respiratory tract (5, 22, 59) but evidence for including IBR as a lung pathogen is not strong. However, in its role as a pathogen of the upper respiratory tract IBR may contribute to aspiration pneumonia (5). It has been suggested that upper respiratory disease may contribute to infections of the lower respiratory tract because of the consequential continual exposure of the lung defence mechanisms to infectious agents in large numbers (91). The pathogenicity of the various strains of the virus appears to differ for experimental calves (59) but, as most clinical cases involve signs of disease of the upper respiratory tract, there is little justification for including IBR virus as a cause of enzootic pneumonia at this time.

2. *Parainfluenza 3 virus (PI-3).* First isolated from cattle with shipping fever in 1959 (73), PI-3 is now recognised as one of the most important of the known viral respiratory pathogens of both young and adult cattle. PI-3 virus has been associated with enzootic pneumonia of calves on serological (13, 31) and histological evidence (10, 50, 68, 80). Strains of PI-3 have produced clinical and histological (10, 29, 69) evidence of respiratory disease in experimental calves and although clinical disease was often mild and could easily be overlooked in the field (29) the macroscopic and microscopic lung lesions were usually severe.

There is little doubt that PI-3 plays a significant etiological role in enzootic pneumonia of calves, and the finding that over 80% of the cattle population may possess antibodies to PI-3 (13, 39) indicates the ubiquity of this virus. Furthermore, the observations that PI-3 may cause pneumonia of sheep, that PI-3 from sheep are serologically related to human PI-3 strains (41), and that bovine strains are serologically related to human strains (which can cause respiratory disease in man) (1, 18) raise interesting epizootiological questions.

3. *Reoviruses.* Although strains of the three mammalian reovirus serotypes have been isolated from calves and apparently may be very common in the cattle population (76, 77) their association with enzootic pneumonia is not clear. Experimental inoculation of calves with reovirus types 1, 2, or 3 has resulted in serological evidence of infection without symptoms (76), with mild symptoms of respiratory disease (83), or without symptoms but with the development of mild macroscopic and moderately severe microscopic lesions of pneumonia (55). Phillip *et al* (72) suggested that the importance of reovirus infection was that of a predisposing agent to bacterial infection, and reoviruses have been serologically incriminated as respiratory pathogens under field conditions (28).

Of interest here is that human and bovine reoviruses are serologically virtually indistinguishable (77) and respiratory disease in calves has been produced with both human (55, 83) and bovine (55) strains of reovirus type 1. The importance of cross infection between cattle and man is as yet unknown.

4. *Adenoviruses.* Of the six (9, 24, 53, 75) proposed bovine adenovirus serotypes, five

have been associated with respiratory disease in calves. Adenovirus type 1 has been isolated from normal cattle and has been associated with natural outbreaks of bovine respiratory disease (44, 53), and the same is true for types 2 and 3 (24, 27, 28, 53). Proposed types 4 and 5 have been incriminated as important pathogens of calves in Hungary, where they have been associated with widespread enzootic pneumonia and enteritis and where they have been shown to produce the disease in experimental calves (2, 8). An untyped adenovirus was isolated from the lung tissue of one of a group of calves with pneumonia in Australia (19) and serological evidence has associated bovine adenovirus with several outbreaks of respiratory disease in cattle (23, 25, 39).

Strains of adenovirus types 1, 2 and 3 have been shown to produce asymptomatic pneumonia in experimentally inoculated newborn colostrum-deprived calves, but lung lesions were severe and calves sometimes developed diarrhea (26, 27). However, other workers have not succeeded in producing pneumonia in older calves inoculated with these viruses (44, 53) or have produced only mild clinical evidence of disease (64). Thus, while virological and histological evidence exists to prove the pathogenicity of adenoviruses types 1, 2 and 3 for the respiratory tract of newborn colostrum-deprived calves, little evidence has yet been presented to demonstrate similar pathogenicity of these types for experimental calves of the age group in which enzootic pneumonia is commonly observed. Nevertheless, the evidence associating adenoviruses and bovine respiratory disease is strong, and it is possible that these viruses can contribute to the syndrome of enzootic pneumonia.

5. *Rhinoviruses.* Rhinoviruses have been isolated from calves in Germany (12, 62, 90), America (65) and England (45) but to date only one strain has been associated with lower respiratory disease in the calf (65). Recent work has shown that rhinovirus infection of calves may produce histologically severe focal erosive rhinitis, but inoculation of eight newborn colostrum-deprived calves by the intranasal and intratracheal routes with a bovine rhinovirus isolated in England (45) did not result in clinical disease, macroscopic or microscopic evidence of pneumonia (Ide, unpublished).[2]

Interest in this group of viruses is stimulated by the fact that human rhinoviruses cause the common cold in man (85). While it is now clear that bovine rhinoviruses will produce rhinitis in cattle, there is little to suggest that they are important pathogens of the lower respiratory tract.

6. *Enteroviruses.* Enteroviruses have been isolated from nasal washings and feces of calves on farms experiencing recurrent respiratory disease (43, 66). Similarly, "enterovirus-like" agents have been isolated from the lungs of calves with pneumonia, but their identity and respiratory pathogenicity was not determined (68). There have been unsuccessful attempts to produce pneumonia in calves by the intratracheal, intranasal or oral inoculation of bovine enteroviruses (12, 66), but pneumonia was observed when a bovine enterovirus was administered in conjunction with cortisone (66).

7. *Other viruses.* Hemadsorbing enteric (HADEN) virus, was shown to produce diarrhea and clinical evidence of respiratory disease in experimental calves (79). Respiratory syncytial virus is an important lung pathogen of children, and although this virus has not yet been isolated from cattle, serum neutralising substances thought to be specific antibody to it have been demonstrated in sera of cattle in England. Some of these sera exhibited a significant increase in titre after respiratory diseases of undetermined cause (34). Influenza type A has been isolated from calves with respiratory disease (68) although its significance is not established. A similar situation exists with bovine virus diarrhea virus. In the latter case coughing is sometimes observed as a symptom of the natural (3, 42) and experimental (3) disease, but pneumonia has not been recorded as a post-mortem finding (42, 51).

Multiple Causation

One of the most significant features of nearly all the virus-produced pneumonias in experimental calves has been that, even when macroscopic and microscopic evidence of severe pneumonia was present, calves seldom exhibited symptoms of respiratory disease. For this reason it seems logical to assume that the severity of natural outbreaks of enzootic pneumonia may be largely due to the combined action of two or more microbial agents, and evidence of this effect is presently available. Thus, pneumonia caused by a reovirus was more severe when calves were also inoculated with Chlamydia (72); and Chlamydia-induced pneumonia, in turn, was more severe when calves were secondarily infected with *Pasteurella hemolytica* (71). Calves inoculated with IBR virus and secondarily inoculated with

[2]PhD. Thesis, University of London. 1970.

Pasteurella hemolytica developed more severe symptoms of respiratory disease than did calves inoculated solely with the virus (20) and this association has been noted by other workers (59). Calves with existing lesions of one form of enzootic pneumonia in Ontario were more severely affected by experimental exposure to *Pasteurella hemolytica* than were calves without such lesions (16). In a group of calves with respiratory disease symptoms were more severe in those which exhibited significant serological response to PI-3 in conjunction with Mycoplasma infection (31). Calves exposed to PI-3 virus and then *Pasteurella multocida* or *hemolytica* 24 or 48 hours later generally developed more severe clinical signs of respiratory disease than those inoculated with virus alone, bacteria alone or virus and bacteria at the same time (6, 40).

Of interest here is the fact that mice infected with influenza A virus were found to be less capable of clearing staphylococci from their lung tissue, and that although virus replication was maximal at approximately two days after infection, the maximum defect in bacterial clearance from the lungs occurred at about a week after virus infection (78). That a similar effect may occur in calves in natural outbreaks of respiratory disease was suggested (but not proven) by the report that although PI-3 virus was isolated from 14 of 19 apparently healthy calves it could not be isolated from the same animals during the subsequent three weeks when several developed signs of severe respiratory disease (13). This also indicates the difficulty in interpretation of results of attempts to isolate the etiological agents of respiratory disease when symptoms are apparent. A synergistic effect of parainfluenza virus and hemophilus in pneumonia has been shown in mice, in which combined infections produced pneumonia and higher mortality than either agent alone, and immunisation with either agent prevented the synergistic effect (32). Certainly this field of research is bound to receive a great deal of attention in future investigations into calf respiratory diseases.

Laboratory Diagnosis and Sampling Procedures

The large number of agents which are potential respiratory pathogens of calves renders the diagnosis of the cause or causes of a single outbreak a very complex matter. The fact that relatively few outbreaks are etiologically diagnosed supports this conclusion. Generally speaking, determination of the microbiological cause of an outbreak of enzootic pneumonia may be based on histological or microbiological examination of diseased lung tissue or on serological examination of paired serum samples from affected and contact animals.

It is now well recognised that lung changes induced by some microbiological agents may be quite specific (68), and providing tissues are obtained from early uncomplicated cases of the disease and adequately fixed (in 10% formalin or other suitable tissue fixative), histopathology may provide an accurate method of identifying the causative agent in some cases. PI-3, adenovirus and IBR infected tissues, for example, can usually be identified microscopically. However, since tissues submitted for histological examinations are usually obtained from animals in the late stages of the disease, the specific changes caused by the primary pathogens may have disappeared or may have been masked by changes induced by secondary agents. It is possible that the fluorescent antibody technique may eventually be applied to routine diagnosis of pneumonia in calves, and development in this field is awaited with interest. This technique would allow rapid and accurate location of specific antigens in lung tissue or respiratory exudates, and has been satisfactorily applied to lung tissues from experimental calves (Ide, Phillip & Darbyshire, unpublished observations).

Similar comments are applicable to diagnostic techniques based on isolation of the causative agent(s) from infected lung tissue for, if lesions are of long standing or if secondary infection is present, the chances of isolating a specific causative agent are greatly reduced. If possible, representative samples (approx. 1 or 2 cm^3) of tissues for virological examination should be obtained from early cases immediately after death, immediately chilled and shipped in cracked ice (about 4°C) to the diagnostic laboratory. A satisfactory technique for most viruses, which allows maintenance of neutral pH (some viruses are very sensitive to pH changes) and also controls bacterial proliferation, is to place the tissue in sterile glycerol-saline (50% neutral high quality glycerol is isotonic saline buffered to pH 7.4) prior to chilling. If samples can not be stored at 4°C and taken to the diagnostic laboratory within one day after collection they should be frozen at about –20°C or lower, and samples in glycerol can also be stored at this temperature. It is important to remember that some viruses are sensitive to heat (even body temperature), drying, sunlight or the products of tissue degeneration, and samples for virological examination must be handled properly if any benefit is to be derived from their submission to a laboratory. Nasal or

fecal swabs should also be immersed in a suitable fluid environment to prevent drying (a suitable "transport medium" will usually be supplied by the diagnostic laboratory). Samples for bacteriological examination should be chilled in the absence of glycerol. Samples for fluorescent microscopy should not be submitted in glycerol, but should merely be frozen (57).

Probably the most rewarding diagnostic tool for the investigation of bovine respiratory disease at present is that of serology, but it is important to remember that a single serum sample offers no indication of the progress of an antibody response and is virtually useless as a diagnostic aid. For this reason serum samples must be obtained at the acute (when the outbreak is first noted) and convalescent (about two weeks later) stages of the disease and should preferably be collected from both affected and in-contact animals. Serum should not be contaminated, and should not contain preservatives (as some preservatives are virucidal) (57) unless specified by the laboratory. A fourfold or greater increase in antibody titre to a specific agent is usually taken as being indicative of active immunity and provides evidence that the agent was replicating in a manner sufficient to stimulate an antibody response. While it does not prove that the agent caused the disease, such a finding suggests that it played a part in the syndrome. Serological data are only circumstantial evidence of etiology, but they have been useful in the study of the epizootiology of bovine respiratory disease (28).

Factors Influencing Susceptibility of the Host

Although uncomplicated virus pneumonia would be unaffected by antibiotic therapy the fact that bacteria may play a role in the initiation or propagation of some cases of enzootic pneumonia justifies the use of such therapy in dealing with the disease. Clinical experience suggests that a proportion of cases respond favourably to treatment with antibiotics.

The role of stress in the production of cattle respiratory disease is a recognised (70, 86) but as yet ill-defined factor. It is accepted that husbandry plays an important role in respiratory infections. A respiratory pathogen exhaled in droplet form by an infected animal stands a better chance of being inhaled by susceptible animals if sick cattle are not isolated or if cattle are overcrowded or housed in poorly ventilated unhygienic areas, because it will be present in greater concentration in the environment (60). It is also known that the ability of the mammalian respiratory tract to clear itself of invading microbiological agents may be reduced by various factors including acute starvation for 24 hours, hypoxia, acidosis, drying of mucous membranes, and chemicals such as ethyl alcohol, ammonia or sulfur dioxide (52, 91). It has also been shown that chilling may predispose laboratory animals to infection (87) and that cold may alter air flow in the upper respiratory tract so as to predispose to local drying of the mucosa (91).

The role of colostrum in the control of respiratory disease is not often considered because calves which develop the disease are usually two or more months of age, by which time circulating antibodies derived from the colostrum have fallen to a non-protective level. Dawson's (30) results indicated that colostrum-derived antibody to PI-3 virus retained serum levels in the calf which would, at least theoretically, protect the animal against PI-3 infection for an average of 10 weeks after birth. The sound management practice of feeding colostrum probably contributes to the control of respiratory disease, and the importance of sound husbandry methods cannot be overemphasised.

The ultimate practical aim of research into respiratory pathogens of cattle is the production of appropriate vaccines for prophylactic use. This subject was discussed at length in a recent symposium (81), to which the reader is referred for details, but it would appear that it is too early yet to assess the efficacy of available vaccines in the control of naturally-occurring enzootic pneumonia in calves.

Summary

Enzootic pneumonia of calves is not a specific disease attributable to a single etiological agent and the variety of microbiological agents that may be associated with the condition are reviewed. Problems associated with the determination of the cause of a specific outbreak are discussed, and recommendations are made to the practitioner regarding the collection and shipment of samples for diagnostic examination. The significance of accurate etiological information in the development of effective vaccines is stressed.

Résumé

La pneumonie enzootique des veaux n'est pas une maladie spécifique attribuable à un agent isolé; l'auteur passe en revue les différents facteurs microbiens susceptibles de jouer un rôle dans cette affection. Il traite des problèmes rattachés à l'identification de la cause d'une éclosion donnée de la maladie et il sou-

met des recommandations aux practiciens pour la récolte et l'expédition des échantillons en vue de ce diagnostic. Il souligne l'importance de la précision du diagnostic étiologique pour être en mesure de produire des vaccins efficaces.

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