PARAMYXOVIRUS ASSOCIATED WITH PNEUMONIA IN A DWARF RABBIT

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SUMMARY : Respiratory diseases in rabbits usually are associated with *Pasteurella multocida* infections. With the exception of myxomatosis and Rabbit Haemorrhagic disease, naturally occurring viral respiratory diseases have not been described in rabbits to our knowledge. In this report a natural outbreak of acute fatal pneumonia associated with the presence of paramyxovirus-like particles is described. The lung lesion was characterized by giant cell alveolitis. The results of this study should encourage virus isolation attempts in cases of acute respiratory disease in rabbits, in order to gain further insight in the possible role of viruses in rabbit pneumonia.

RÉSUMÉ : Paramyxovirus associé à la pneumonie chez le lapin nain.
Les maladies respiratoires chez le lapin sont le plus souvent associées avec les infections à Pasteurella multocida. *En dehors de la Myxomatose et de la Maladie Hémorragique virale du lapin, aucune autre maladie respiratoire spontanée d'origine virale n'a été décrite chez le lapin à notre connaissance. Dans le présent article nous rapportons un cas de pneumonie aigüe et létale, associée à la présence de particules virales de type paramyxovirus. La lésion dans les poumons était caractérisée par une alvéolite à cellules géantes. Les résultats de cette étude devraient encourager les efforts en vue de l'isolement de virus à partir de cas de maladies respiratoires aigües chez le lapin, afin d'éclaircir le rôle éventuel des virus dans la pneumonie chez cette espèce.*

INTRODUCTION

Respiratory diseases represent a significant draw-back to the industrial rabbit production. Also in rabbits kept as pets respiratory diseases are common. Clinically these diseases are referred to as snuffles and as pneumonia (CHEEKE *et al.*, 1987). Snuffles comprises all upper respiratory tract infections, whilst "pneumonia" covers all lower respiratory tract infections.

Both snuffles and pneumonia usually are attributed to *Pasteurella multocida* infections (CHEEKE *et al.*, 1987 ; JONES, 1988 ; OKERMAN, 1994). Several other bacterial agents have been associated occasionally with respiratory disease in rabbits (JONES, 1988 ; OKERMAN, 1994). In contrast to most other domesticated mammalian species, the rabbit seems to be remarkably free of respiratory viruses. There are a limited number of reports suggesting a possible role of myxomatosis virus in respiratory disease in large production units (ARTHUR, 1989 ; JOUBERT *et al.*, 1982). In Rabbit Haemorrhagic disease, respiratory symptoms may occasionally be present (MARCATO *et al.*, 1991). To our knowledge there are no other reports of naturally occurring respiratory disease in rabbits where a viral etiology was documented.

Hereafter a description is given of a natural case of pneumonia in a rabbit, which was associated with the presence of virus-like particles in the lungs. These virus-like particles were similar in morphology and morphogenesis to paramyxoviruses.

MATERIALS AND METHODS

From a small colony of 16 dwarf rabbits one animal was presented for autopsy. Specimens from various organs were taken for impression smear cytology using a rapid blood staining set (Haemacolor, Merck). Several samples were taken from lungs and intestine for bacteriological and histological examination, and from the lung for transmission electron microscopical examination. For bacteriological examination the lung samples were inoculated on blood agar plates and intestinal samples were inoculated on Mac Conkey agar n°3, as described for the isolation of *E. Coli* (OKERMAN, 1989). For

histological examination samples were fixed in phosphate buffered formalin, embedded in paraffin, sectioned and stained with haematoxylin and eosin. Samples for transmission electron microscopy were fixed in glutaraldehyde – paraformaldehyde, embedded in a mixture of Spurr's and araldite medium, sectioned and contrasted with uranylacetate and leadcitrate.

RESULTS

In a small colony of 16 weanling dwarf rabbits at eight weeks of age, four suddenly showed signs of anorexia and dullness. All four rapidly became very lethargic and died without excitement within 24 to 48h. There was no history of previous contact with other rabbits. They received no medication. One of these rabbits was presented for autopsy. At post mortem examination, there was distension of the heart. The liver showed greyish nodular lesions which were identified as *Eimeria stiedae* pericholangitis. The kidneys were pale and swollen. The content of the small intestine was watery. The predominant lesions however were in the lungs. These were severely congested and multiple white miliary foci were scattered throughout the lung parenchyma.

Bacteriological cultures from the lungs were negative. Also from the intestine no specific pathogenic bacteria could be cultured.

The histologic lesion in the lungs essentially was an alveolitis (Fig. 1). There was thickening of the interalveolar septa and extensive detachment of epithelial cells. Alveoli were filled with exudate containing detached epithelial cells, numerous macrophages and cellular debris. Many detached cells showed cytoplasmic vacuolization or eosinophylic necrosis. Few of these vacuolated cells contained one or more very small eosinophylic intracytoplasmic inclusion bodies (Fig 2). Also occasional multinucleated syncytia were seen (Fig. 3). These cells had an eosinophylic cytoplasm and 2 to 6 nuclei. Aggregates of lymphocytes were present in the interstitial space, mainly around small arterioles and distended capillaries. Few neutrophylic granulocytes were scattered in the interalveolar tissue. Bronchial and bronchiolar epithelium appeared normal.

Figure 1 : Histological section of the lung showing alveolitis. Alveoli are filled with detached vacuolated epithelial cells, macrophages and necrotic debris. Interalveolar septa are thickened. Haematoxylin and eosin staining, bar = 20 μm.

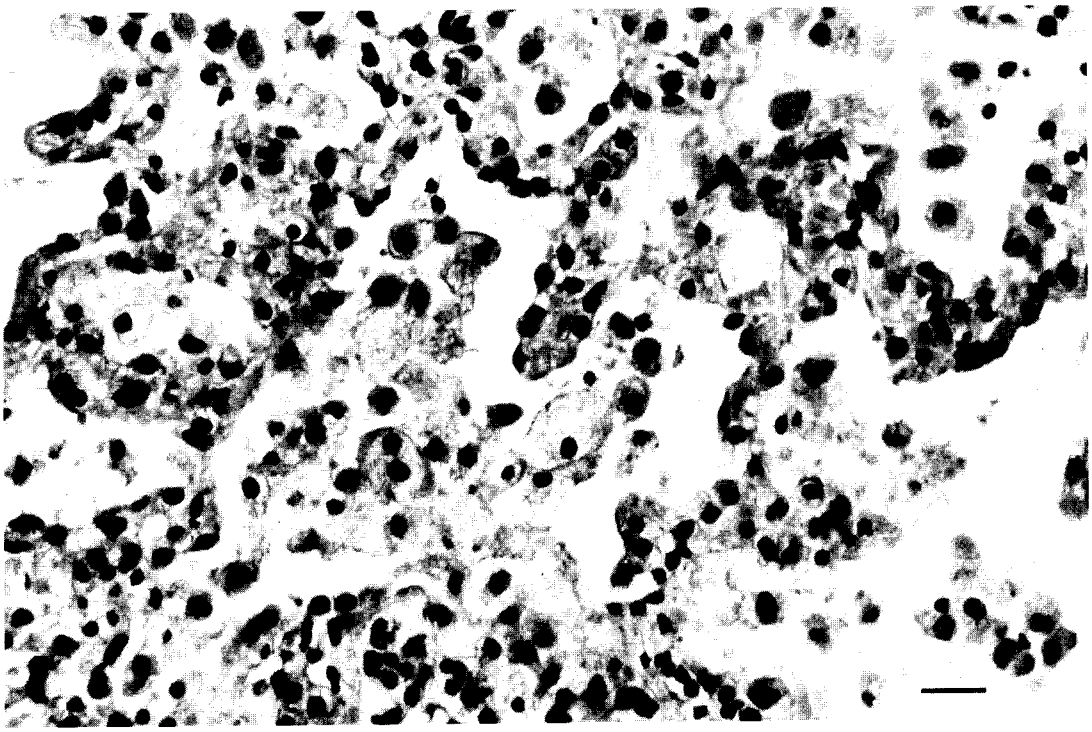


Figure 2 : Histological section of the lung showing a large vacuolated intra alveolar cell containing few small eosinophylic intracytoplasmic inclusion bodies (arrow). Haematoxylin and eosin staining, bar = 20 μm.

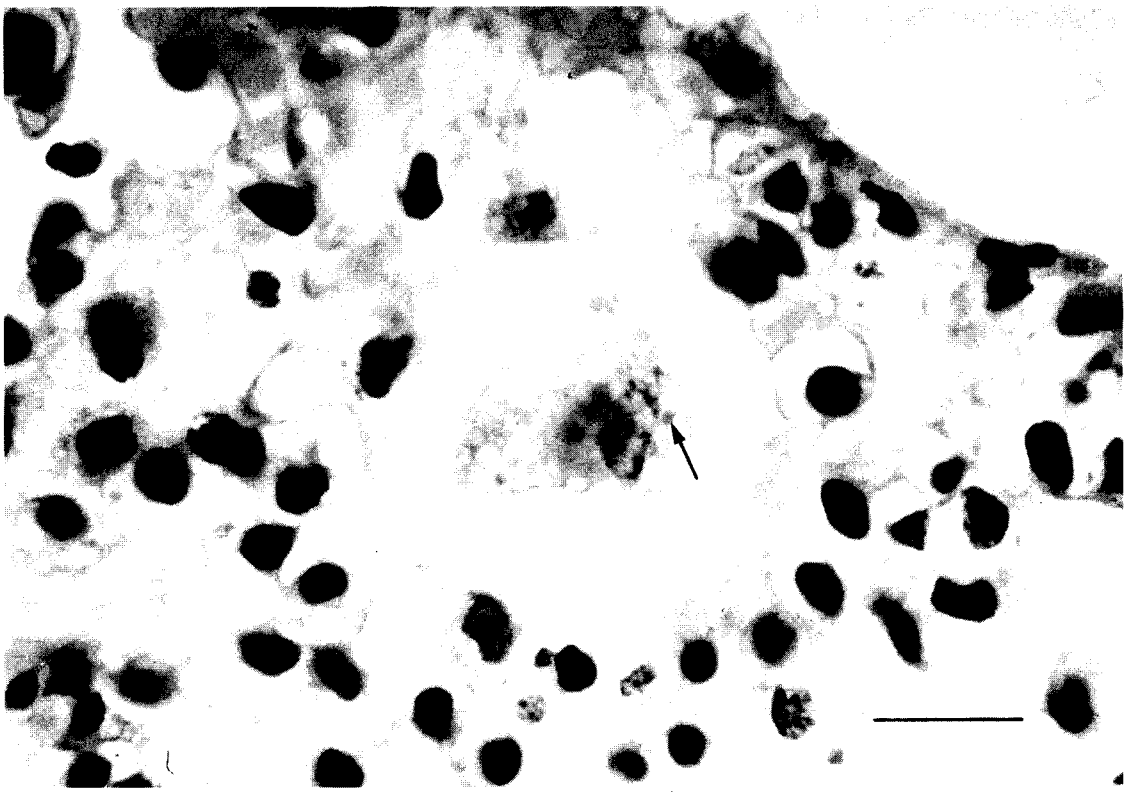
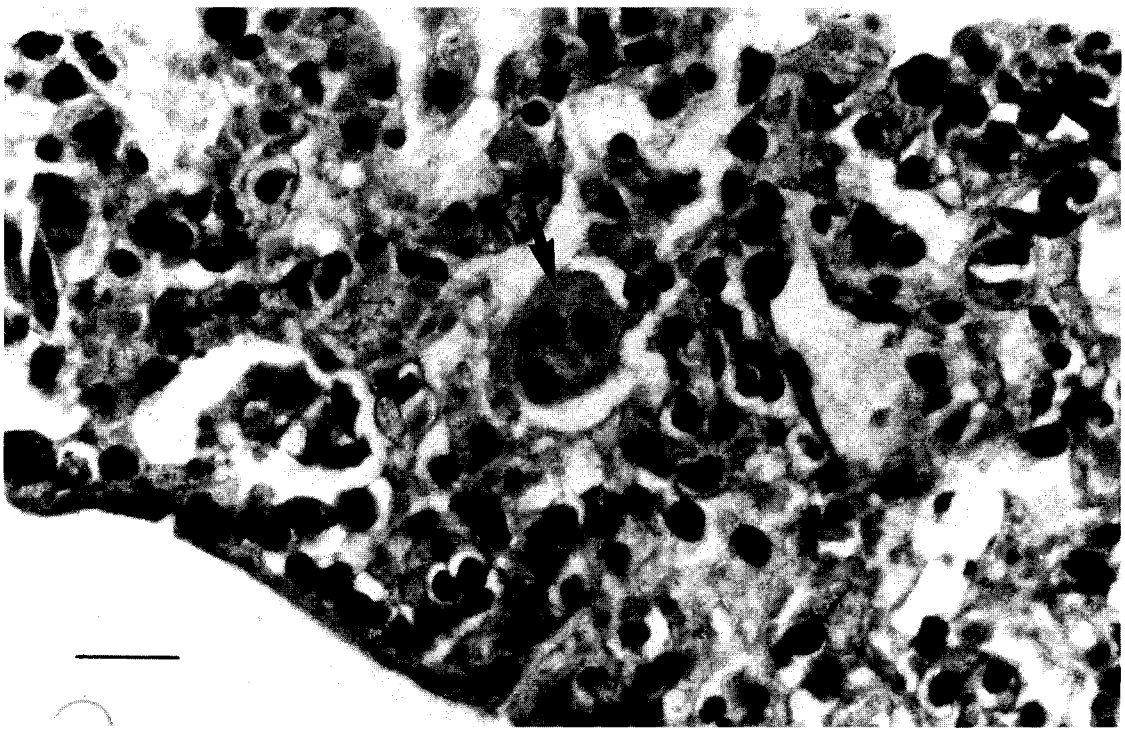


Figure 3 : Histological section of the lung showing a multinucleated cell in the alveolar lumen (arrow). Haematoxylin and eosin staining, bar = 20 μm



By transmission electron microscopy it was found that many type one alveolar epithelial cells were rounded and detached from the basal lamina. Type two alveolar epithelial cells contained a decreased number of multilamellated bodies. No bacteria were seen. The type one cells had an electron translucent cytoplasm with few organelles. The nucleus contained condensed clumps of chromatin. The plasma membrane on many occasions was found to be covered with a fuzzy continuous layer of granules. In these areas virus–like particles were seen budding–off from the plasma membrane into the interstitium (Fig. 4). The buds were characterized by a local thickening of the plasma membrane, which was lined at the intracytoplasmic side by fine granular material. Numerous pleomorphic virus–like particles were found in the interstitium in these areas (Fig. 5). These particles ranged in diameter from 150 to 180 nm. Most of them were roughly spherical, but some appeared more elongated, and occasionally filamentous particles were seen.

Figure 4 : Ultrastructure of detached cells in the alveolar lumen, showing electron-translucent cytoplasm (C) and budding of virus–like particles from the plasma membrane (arrow). bar = 320 nm.

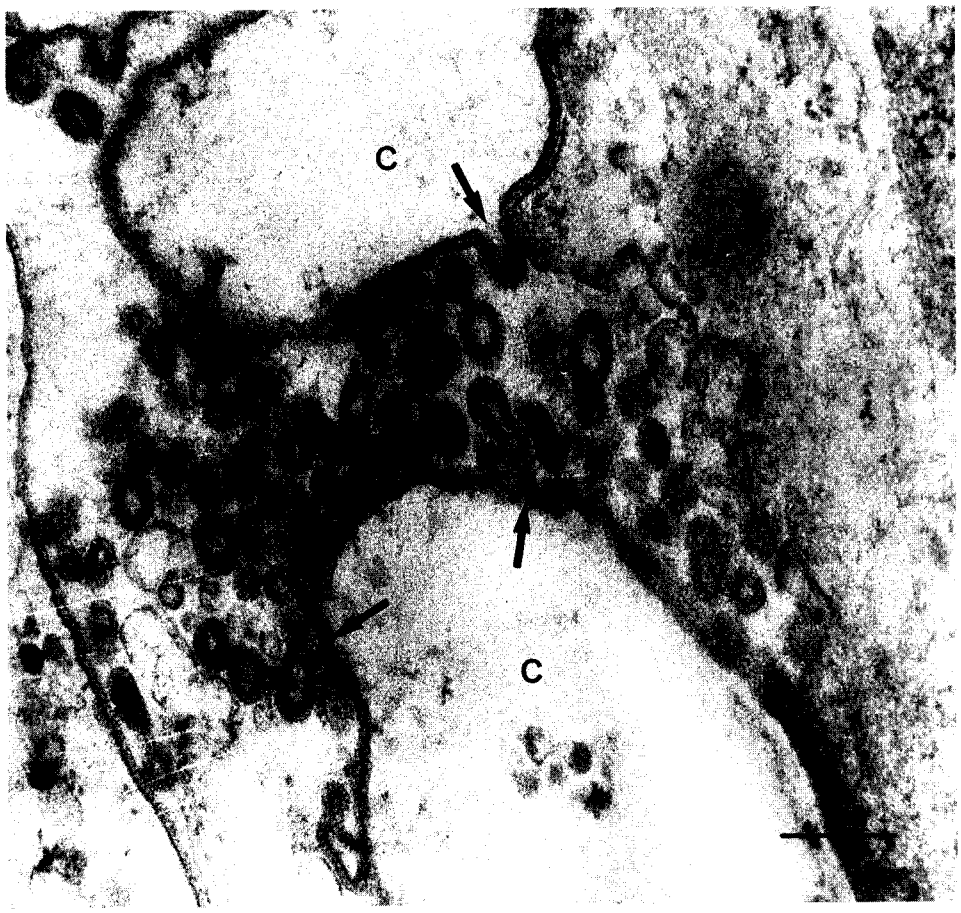
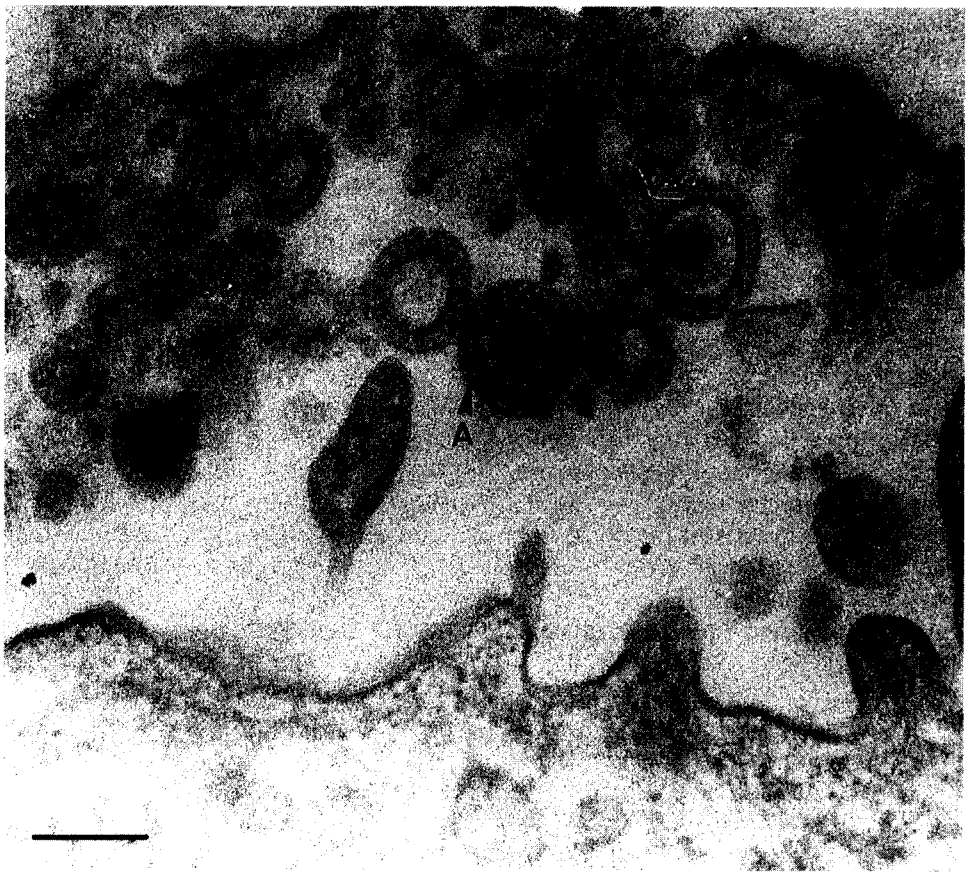


Figure 5 : Pleomorphic virus-like particles between detached cells in the alveolar lumen. Notice the dense rim of short surface projections (A). bar = 145 nm



DISCUSSION

Respiratory disease in rabbits usually is associated with infection of *Pasteurella multocida*. Different serotypes of different pathogenicity are known. The pasteurellae colonize the mucosal surfaces of the pharynges of rabbits in the carrier state or with recurrent rhinitis (GLORIOSO *et al.*, 1982). Pasteurella is considered to be of primary pathogenicity. Infections with highly pathogenic serotypes lead to acute septicaemia (OKERMAN *et al.*, 1990). Less pathogenic strains may cause enzootic pneumonia with poor environmental conditions acting as triggering or complicating factors. The important role of the environmental conditions has clearly been established, since rabbits have been shown to be particularly sensitive not only to high concentrations of ammonia, but also to high velocity–low volume ventilation (MORISSE *et al.*, 1978 ; MORISSE, 1989). These factors contribute considerably to the severity of lesions observed after *Pasteurella multocida* infection. Other bacterial agents occasionally have been associated with respiratory disease in rabbits (JONES, 1988 ; OKERMAN, 1994). Generalized *Staphylococcus aureus* infection may give rise to small abscesses in the lungs. *Pseudomonas aeruginosa* infection can on some occasions lead to coryza and pneumonia. *Bordetella bronchiseptica* very frequently can be isolated from the respiratory tract of rabbits, but the lesions associated with this infection are usually very mild. Mycoplasma infections occasionally have been recorded, but their pathogenic significance seems questionable. Also mycobacterium infections of the respiratory tract have been reported in rabbits. In the present case, bacteriological cultures from the lungs revealed negative results. No attempts were made to culture from the upper respiratory tract. However, any positive culture from the latter site would not explain the lesions observed in the lower respiratory tract. Moreover the histologic and ultrastructural lesions observed in the lungs were not at all indicative of a bacterial infection.

Only few viral agents have been described in relation to respiratory disease in rabbits. There have been suggestions towards a possible role of myxomatosis virus in respiratory disease in large production units (ARTHUR, 1989 ; JOUBERT *et al.*, 1982). Infections with the Rabbit Haemorrhagic Disease Virus (RHDV) give rise to gross lesions in the lungs which are merely multifocal haemorrhages associated with vascular thromboses and not with inflammatory lesions (MARCATO *et al.*, 1991). Strictly there is no pneumonia in RHDV infection. To our knowledge these are the only viruses associated with naturally occurring respiratory disease in rabbits. In laboratory rabbits however, coronavirus–like particles occasionally have been found in cases of pleural

effusion disease (OSTERHAUS *et al.*, 1982). Sendai virus is one of the most prevalent viruses in laboratory rodents. Nevertheless there have been no reports demonstrating natural infection of Sendai virus in rabbits, although anti-Sendai virus antibodies have been detected in 50 % of sera from conventional rabbits in one study (MACHII *et al.*, 1989).

The ultrastructure of the virus-like particles observed in the present study is in agreement with descriptions in the literature on the morphology and morphogenesis of Paramyxoviruses in ultra-thin sections (CHEVILLE, 1983 ; COMPANS *et al.*, 1967). Also the histological lesions are in agreement with the cell and tissue pathology seen in paramyxovirus induced pneumonia in other species, e.g. in the bovine (CHEVILLE, 1983). The presence of alveolitis with multinucleated syncytia and eosinophylic cytoplasmic inclusion bodies is indeed characteristic. This type of histologic lung lesion has been described previously in rabbits by MARCATO and ROSMINI (1986) as a giant cell alveolitis in enzootic pneumonia. These authors excluded Chlamydia as a causative agent, but did not demonstrate or suggest any possible alternative cause.

To our knowledge this is the first report describing pneumonia in rabbits associated with the presence of paramyxovirus-like particles. Sendai virus is the only paramyxovirus associated with rabbits as far as we know. Antibodies against Sendai virus have been detected in rabbit sera, but there are no reports of natural Sendai infection. After experimental infection of rabbits with Sendai virus no lung lesions were obtained and virus replication only was demonstrated in the nasal epithelium (MACHII *et al.*, 1989). No serological tests or attempts to isolate the virus were made in the present study. Nevertheless this study suggests that the lung pathology in rabbits possibly is not so very different from that of other mammalian species, in the sense that also in the rabbit viral agents possibly can induce a primary lung lesion after which bacteria can invade and replicate. Further investigations are needed to isolate and characterize viruses from rabbits with acute respiratory disease.

Acknowledgements : The technical assistance of M. Gheyle and C. Puttevils is gratefully acknowledged.

Received : March 24, 1994.
Accepted : May 5, 1994.

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