Taxon 20 (Fam. Pasteurellaceae) Infections in European Brown Hares (Lepus europaeus)

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Taxon 20 (Fam. Pasteurellaceae) Infections in European Brown Hares (*Lepus europaeus*)

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ABSTRACT: Hemolytic bacteria, phenotypically related to organisms previously identified as *Pasteurella haemolytica* and tentatively named Taxon 20, were isolated from cases of purulent bronchopneumonia and from conjunctivitis in European brown hares (*Lepus europaeus*). The bronchopneumonia, sometimes accompanied by lesions in other organs, occurred without other concomitant disease. The conjunctivitis was found mainly in animals suffering from the European brown hare syndrome.

Key words: European brown hare, *Lepus europaeus*, Pneumonia, *Pasteurellaceae, P. haemolytica*, Taxon 20.

Bacteria identified as *Pasteurella haemolytica* were isolated from cases of respiratory disease in captive European brown hares (*Lepus europaeus*) by Louzis (1984). They were more frequently associated with respiratory infection in this animal species than *Bordetella bronchiseptica* and *Pasteurella multocida*. Similar organisms were also isolated, but more rarely, from purulent mammary and uterine infections, from asymptomatic carriers and from wild hares (Louzis et al., 1988).

The present communication describes infections caused by this type of bacteria in hares in Belgium which were found in post mortem examinations of 42 farmed and 44 wild hares.

The farmed hares all originated from two farms (51°10′N, 4°16′E and 51°21′N, 4°42′E) and their ages ranged from 2 to 11 mo. The wild hares were shot or found dead in different locations (area located 51°03′ to 51°30′N, 2°50′ to 3°45′E) in 1989. After primary culture of macroscopically visible lesions on Columbia ox blood agar (Lab M, Bury, Lancashire, United Kingdom BL9 6AU), subcultures of haemolytic colonies of Gram-negative glucose fermenting bacteria were characterized and identified as described by Bisgaard and Mutters (1986); carbohydrate fermentation tests were carried out in Phenol Red Broth Base (Difco, Detroit, Michigan 48201 USA) and glycosidase tests using the test tablets of Rosco, 2630 Taastrup, Denmark. Controls using Strain NCTC 9380–T were obtained from The National Collection of Type Cultures, London, United Kingdom NW9 5HT. Histological examinations of organs with macroscopically visible lesions, usually the lungs, were performed using Haematoxylin and Eosin staining methods.

Infection with similar haemolytic bacteria was diagnosed in eight captive hares and in four wild animals. Four cases of purulent conjunctivitis were found, one in a farmed hare and three in wild hares. In the remaining eight cases pneumonic lesions predominated. Cases involving the respiratory system only were diagnosed in four farmed animals and in one wild hare. In one of these cases a large abscess, approximately 10 cm in diameter, was the only significant lesion; in another one purulent bronchopneumonia was evident; in the three other cases mucopurulent tracheo-bronchitis and bilateral catarrhal or purulent pneumonia, mainly involving the apical lobes, was noted. In three other captive hares the lesions were mainly respiratory (purulent tracheobronchitis and bronchopneumonia with abscess formation) but the bacteria were also isolated from other important lesions causing peritonitis (one), endocarditis and enlargement of the spleen (two) and liver (one).

The respiratory tract lesions were fairly characteristic. They can be defined as a purulent tracheo-bronchitis extending to

bronchiolitis, in some cases involving part of the lung parenchyma, eventually leading to abscess formation. Histological examination of the affected lungs revealed mainly accumulation of mucopurulent exudate in bronchi and broncheoli. The epithelial lining of the airways showed degenerative changes, characterized by cytoplasmic vacuolation, loss of cilia and desquamation of individual cells. In some areas accumulations of numerous bacteria were seen, surrounded by neutrophilic granulocytes in the lung parenchyma.

Bacteriological cultures performed on the lesions of each of the 12 cases described above revealed large numbers of haemolytic Gram-negative organisms. The colonies were easily recognized on the primary isolation plates by their narrow haemolysis zones, their small size, 0.5 to 1.5 mm in diameter, and their smooth, regular, grayish and low convex aspect. Identical phenotypic characters were obtained with all isolates investigated. They were catalase- and phosphatase-positive and indol- and urease-negative. Differences in haemolysis (calf blood), glycerol, L(−)fucose, cellobiose, beta-glucosidase, lactose, aesculin, arbutin, gentiobiose, salicin, alpha-fucosidase and beta-glucuronidase separate the hare isolates from strain NCTC 9380–T, the type strain of the species *"Pasteurella" haemolytica*, as described by Bisgaard and Mutters (1986).

In one animal the haemolytic bacteria were isolated in mixed culture with *Staphylococcus aureus* from the lung and spleen, but in all others they were isolated in pure culture. In one of the conjunctivitis cases the bacteria were isolated in pure culture from a wild hare which had also subcutaneous abscess in the mandibular region which yielded *Yersinia enterocolitica* biotype 5 (hare biotype) upon culture. The three other conjunctivitis cases involved two wild hares and one farmed hare in which European brown hare syndrome, a viral infection causing a necrotizing hepatitis (Uyttebroek et al., 1990) was diagnosed as the cause of death.

The observations described here entirely correspond to the descriptions given by Louzis (1984) and Louzis et al. (1988). The confusion in bacterial nomenclature is caused by the very complex and to date not yet resolved taxonomy of the so-called *"Pasteurella" haemolytica* complex. Evidence has been obtained which indicates that bovine and ovine strains of *"P." haemolytica* constitute a new genus within the family *Pasteurellaceae* containing several species and that porcine and avian strains represent separate groups within the same family (Mutters et al., 1986). The taxonomic significance of the phenotypic differences observed between the organisms isolated from farmed and wild hares in Belgium and *"P." haemolytica* NCTC 9380–T is unknown, but under investigation. Results obtained so far (M. Bisgaard, unpubl. data) indicate that similar organisms have not been described previously. It is consequently suggested that similar organisms in the future are referred to as Taxon 20 in the provisional classification system of *Pasteurellaceae* established by one of us (M.B.), until their final classification has been published.

Taxon 20 was associated mainly with purulent bronchopneumonia in farmed hares. The pure and profuse nature of the cultures which were obtained upon primary isolation and their constant association with the characteristically purulent respiratory lesions described above suggest that the organisms belonging to this taxon can act as primary pathogens. Final proof of this must be obtained by experimental infection.

The etiological significance of Taxon 20 bacteria in conjunctivitis, however, is less likely. These cases were all associated with other serious conditions, especially the European brown hare syndrome.

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