

The Seal Death in Danish Waters 1988

1. Pathological and Bacteriological Studies

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Heje, N.-I., P. Henriksen and B. Aalbæk: The seal death in Danish waters 1988. 1. Pathological and bacteriological studies. Acta vet. scand. 1991, 32, 205–210. – During the seal epizootic in Danish waters in 1988 a total of 81 adult seals were necropsied. The cause of death was suppurative bronchopneumonia complicated by pleurisy. Histologically, an interstitial pneumonia with cytoplasmatic inclusion bodies typical of canine distemper was identified in many of the seals. The condition was in many cases complicated with a secondary infection with *Bordetella bronchiseptica*.

pathology; pneumonia; epizootic.

Introduction

In the spring and summer 1988 an epizootic occurred in the Danish seal population (Heide-Jørgensen *et al.* 1988, Dietz *et al.* 1989). The epizootic started in the Kattegat and spread to other parts of the Danish waters during the summer and autumn 1988 (Heide-Jørgensen & Härkönen 1990). The seals obviously suffered from respiratory distress. Sick seals usually lived 3 to 4 days before they died. When left undisturbed, they headed for the beach, where some of them died while others expired in the water. In connection with the epizootic a number of seals of varying ages were subjected to post mortem examination. The necropsies were performed at the Royal Veterinary and Agricultural University, Copenhagen and the National Veterinary Laboratory, Aarhus.

Material and methods

A total of 81 adult and 27 neonatal seals were examined. Apart from 2 seals euthanized in the agonal stage, the animals had pre-

sumably died 1 to 3 days prior to the necropsy. On the basis of the post mortem findings, material from 29 seals was taken for histopathological examination and fixed in 4 % neutral buffered formaldehyde solution. The material included tissue samples of trachea, lung, tracheobronchial lymph nodes, liver, pancreas, kidneys, adrenal glands, jejunum, mesenteric lymph nodes and in certain cases also samples of brain, uterus and testicles. The fixed tissue was embedded in paraffin, cut in 6–7 µm sections, and stained with haematoxylin and eosin. According to circumstances, Gram stain, Shorr's S-3 stain for cytoplasmatic inclusion bodies (Ernø 1964), Pollack's trichrome stain ad modum *Westerfield & Dimmock* (1946) and *Masson's* trichrome stain (1929) were applied. Smears from trachea, urinary bladder and lung of 25 seals were stained with Shorr's S-3 stain for inclusion bodies.

Bacteriological examination was performed on 81 animals. Material from lung, spleen,

liver and exudate from the pleural cavity was inoculated on 2 blood agar plates (tryptose blood agar base (Difco) supplied with 5 % sterile bovine blood). One plate was incubated aerobically, another anaerobically (BTL anaerobic jars supplied with palladium catalysts and a 10 % CO₂/90 % H₂ gas mixture at 37°C for 48 h). Isolates were identified according to *Cowan & Steel* (1970). Strains of *Bordetella bronchiseptica* were identified serologically by agglutination tests.

Material from 41 adult seals was sent for virological examination at the State Veterinary Institute for Virus Research, Lindholm. Three seals were examined for rabies by immunofluorescence technique at the National Veterinary Laboratory, Copenhagen.

Results

Post mortem findings

The 27 neonatal seal pups weighing from 4.5 to 7.5 kg revealed no pathological changes. They had a fetal hair-coat and the teeth had not erupted. Macroscopically and microscopically, it was established that the pups had respired after birth since the lung tissue was completely or partly insufflated.

The age of the 81 adult seals ranged from about 1 year old to old animals. They had worn-down incisors, lacked canines and the greater part of the molars. The thickness of their subcutaneous fat was below average. The post mortem findings were rather similar in all the examined seals: The respiratory tract was affected by fibrino-purulent tracheitis with small necrotic foci and an extensive bronchopneumonia with bronchiolar necrosis. Two seals revealed a severe lobar pneumonia. Nineteen seals had also pleurisy with considerable amounts of exudate. Two seals had extensive cardiac dilatation with thrombopurulent valvular endocarditis with several nematodes attached to the valves. Nearly

all the seals had nematodes in the bronchi, stomach and intestine. Twenty-nine of the seals revealed purulent conjunctivitis and rhinitis. The liver and spleen were severely congested in most of the seals.

Microscopically, the trachea showed multifocal epithelial necrosis with an extensive fibrinous exudate and neutrophilic infiltration. In 1 seal a few tracheal epithelial cells revealed slightly eosinophilic intranuclear inclusion bodies with the haematoxylin and eosin stain. The Shorr's S-3 stain revealed 1 to several red cytoplasmic inclusion bodies in tracheal and pulmonary smears from the majority of the examined seals (Table 1).

In the majority of the seals, the lung showed extensive suppurative inflammation in the bronchi with varying desquamation of epithelium and large amounts of exudate in bronchiolar lumina, which were obturated in some cases. The inflammation spread bronchiogenically with varying degrees of inflammation in the alveoli, from slightly suppurative conditions in the centrolobular alveoli to serous inflammation intermedially, and atelectasis in the paraseptal part of the lobuli according to the special anatomical structure of the seal lung. In some cases the suppurative conditions were diffusely spread in the lobuli and there could be more or less coalescence of the alveolar structures with formation of abscesses, or the lobuli were the seat of croupous or necrotizing inflammation. In the latter type of inflammation, considerable colonies of Gram-negative rods were present in the necrotic foci. Nematodes were found in many of the bronchioli and alveoli with or without concurrent inflammation. The nematodes were classified as *Parafilaroides gymnurus* according to *van der Kamp* (1987).

In 18 seals bronchiogenic suppurative inflammation was found as well as interstitial inflammation with proliferation of alveolar

Table 1. Demonstration of inclusion bodies in smears and tissue sections of various hollow organs and tissues.

Case no.	Smears from			Tissue sections					
	Trachea	Bladder	Lung	Lung			Lymph node		
	1	1	1	1	2	3	1	2	3
V 115/88	+	-	+	-			0		
V 121/88	-	-	+	+			0		
V 134/88	+	+	+	+			0		
V 140/88	-	0	+	-			0		
V 141/88	+	-	+	+			0		
V 142/88	+	-	-	-			0		
V 143/88	-	0	0	-			0		
V 144/88	+	0	0	-			0		
V 145/88	+	+	+	+			0		
V 146/88	-	-	-	-			0		
V 149/88	+	-	-	-			0		
V 151/88	-	-	-	-			0		
V 152/88	+	+	+	+			0		
V 153/88	+	-	+	+			0		
V 158/88	0	0	0	-			0		
V 159/88	+	+	+	+			0		
V 160/88	+	-	+	+			0		
V 184/88	+	-	+	+			0		
V 185/88	+	0	0	0			0		
V 186/88	+	0	0	0			0		
V 187/88	+	0	0	0			0		
V 204/88	-	0	+	0			0		
V 249/88	+	0	0	0			0		
V 250/88	+	0	0	0			0		
V 252/88	+	0	0	0			0		
14288/88				+	+	+	0	0	0
14289/88				+	+	+	0	0	0
14291/88				+	+	+	0	0	0
14293/88				+	+	+	+	+	-
14297/88				+	+	-			
14516/88				+	+	+			

1) Haematoxylin - S-3.

2) Pollack's trichome.

3) Masson's trichome.

+: positive, -: negative, 0: not examined.

type II cells, free alveolar macrophages and infiltration with lymphocytes and plasma-cells in the alveolar septa. Eosinophilic cytoplasmatic inclusion bodies were observed in a few macrophages in 16 seals (Fig. 1

and Table 1). Additionally, intranuclear inclusion bodies were found in alveolar macrophages in 2 seals.

The tracheobronchial and mesenteric lymph nodes revealed atrophy of the paracortical

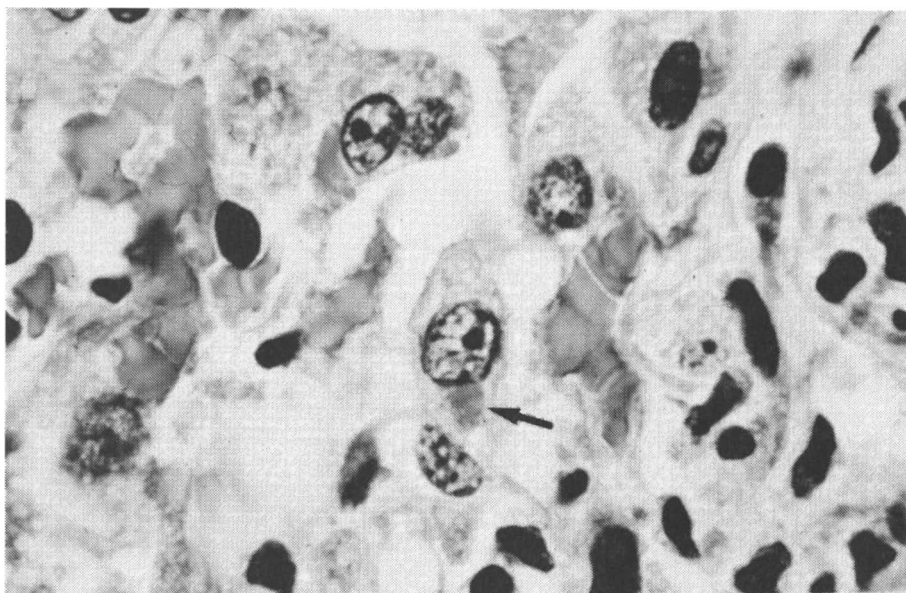


Figure 1. Seal lung. Macrophage containing cytoplasmic inclusion bodies (arrow). Objective 100 \times .

areas with lack of germinal centers and occurrence of cytoplasmic inclusion bodies.

The brain of 1 seal showed small foci of demyelination in the pons and gemistocytic astrocytes with a few intranuclear inclusion bodies.

No significant lesions were identified in other organs.

Rabies was not demonstrated in 3 seals examined.

Bacteriological findings

The bacteriological findings are shown in Table 2. *Bordetella bronchiseptica* was isolated from the lung of 37 of the 81 examined seals, hence being the organism most frequently demonstrated. No growth was achieved in material from 11 of the examined seals.

Table 2. Bacteriological findings in 81 seals with pneumonia.

Bacteria isolated from the lung	Number of seals
<i>Bordetella bronchiseptica</i>	37
Haemolytic streptococci	18
Staphylococci (<i>S. aureus</i> and <i>S. intermedius</i>)	8
<i>Pasteurella multocida</i>	6
<i>Listeria monocytogenes</i>	1
"Sterile"	11

Discussion

The disease affected both males and females regardless of age. A considerable number of abortions in the seal population have been reported by fishermen. The neonatal seal pups were considered to be aborted fetuses,

because the fetal hair-coat had not been exfoliated and the teeth had not erupted. The cause of abortion was not established.

The adult seals were considered to have died from bronchopneumonia which, in many cases, was complicated by exudative pleurisy.

The observed interstitial pneumonia characterized by septal mononuclear infiltration, proliferation of alveolar type II cells and free alveolar macrophages is frequently found in canine distemper (Jubb *et al.* 1985, Thomson 1988). The cytoplasmatic and occasionally intranuclear inclusion bodies are similar to those described in canine distemper (Ernø 1964, Jubb *et al.* 1985). Moreover, the demyelinating encephalitis demonstrated in 1 seal is often observed in canine distemper (Jubb *et al.* 1985). Demyelinating encephalitis was also found in seals from the epizootic in the German Wadden Sea (Hofmeister *et al.* 1988).

These findings agree with the demonstration of a distemper-like virus in the seals (Osterhaus *et al.* 1988, Have *et al.* 1991). Additionally, Have *et al.* (1991) isolated a herpes virus from some of the seals and the morphology of the demonstrated intranuclear inclusion bodies in pulmonary epithelial cells of a few seals is consistent with a herpes virus infection (Jubb *et al.* 1985).

The extensive suppurative and occasionally necrotizing bronchopneumonia could probably be correlated to secondary infection with *Bordetella bronchiseptica* and other bacterial species. *Bordetella bronchiseptica* is often isolated from pneumonic tissue in canine distemper (Jubb *et al.* 1985).

Most probably the nematodes found were without aetiological significance in the epizootic. Lungnematodes are very common in seals from the Wadden Sea and in most cases without significant pulmonary lesions (van der Kamp 1987).

Conclusion

The cause of the death in the seal epizootic in Danish waters was, in the majority of cases, bronchopneumonia complicated by suppurative pleurisy. The type of pathological alterations and inclusion bodies were similar to those observed in canine distemper, and the epizootic was probably caused by the introduction of a canine distemper-like virus into a highly susceptible seal population in the Danish waters.

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Sammendrag

Sældøden i danske farvande i 1988. 1.

Patologiske og bakteriologiske undersøgelser.

I forbindelse med epizootien blandt sæler i danske farvande i 1988 blev 81 voksne sæler obduceret. Dødsårsagen var suppurativ bronchopneumoni. Ved histopatologisk undersøgelse fandtes der hos de fleste voksne sæler interstitiel pneumoni med cytoplasmatiske inklusionslegemer typisk for hundesyge. Sekundær infektion med *Bordetella bronchiseptica* i lungerne og lungeormangreb var almindelige komplikationer.

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