



Observations on the helminths of harbour porpoises (*Phocoena phocoena*) and common guillemots (*Uria aalge*) from the Belgian and German coasts

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Veterinary Record 1996 139: 254-257

doi: 10.1136/vr.139.11.254

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The liver is of major importance in certain haemorrhagic diseases because most coagulation factors are synthesised there (Oldstone and others 1982). The hepatic lesions, including the destruction of hepatocytes, reported in this study after seven and 12 days, correspond with the histological (Sierra and others 1991), immunohistological (Fernández and others 1992a, Pérez and others 1994) and ultrastructural (Sierra and others 1991) findings of acute African swine fever. Although such lesions may account for the decreased synthesis of coagulation factors during the most critical phases of the disease, physiopathological results indicate that the destruction of hepatocytes, although certainly involved, does not determine the final outcome of this haemorrhagic disease (Villeda and others 1993).

The results of this study suggest that African swine fever virus isolate E75 acts directly and indirectly on various cell types actively involved in haemostasis. Morphopathologically, the mechanisms related to haemorrhage after the infection consisted of the activation and extensive destruction of monocytes/macrophages, disseminated intravascular coagulation, and infection and necrosis of megakaryocytes. These findings resemble the pathogenesis of haemorrhagic fever in human beings by other viruses which act through direct effects on cell function and/or through the activation of immune and inflammatory pathways (Halstead and O'Rourke 1977, Cosgriff 1989).

Acknowledgements. – The authors thank Dr F. Gonzalvo and Dr J. Dominguez, at the Institut Nacional de Investigaciones Agrarias de Madrid, for determining the African swine fever antibody titres.

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Observations on the helminths of harbour porpoises (*Phocoena phocoena*) and common guillemots (*Uria aalge*) from the Belgian and German coasts

L. Brosens, T. Jauniaux, U. Siebert, H. Benke, F. Coignoul

Veterinary Record (1996) **139**, 254-257

Between February 1990 and July 1991, 18 harbour porpoises (*Phocoena phocoena*) and 248 common guillemots (*Uria aalge*), found dead along the Belgian and German coasts, were examined for their burden of helminths. A total of three species were found in the guillemots (one cestode, one nematode and one pentastomid), and six species in the porpoises (one trematode, one cestode and four nematodes). Among the guillemots the burden of helminths was not statistically different between juvenile and adult birds. The deaths of the birds were apparently not related to the parasite infections. In contrast, the adult porpoises were more heavily parasitised than the juveniles, except for one young porpoise stranded on the Belgian coast. In the porpoises, four species of parasites had a

pathological effect and *Torynurus convolutus* was responsible for the death of one animal from the Belgian coast and three from the German coast.

IN the past few years, increased numbers of dead marine mammals and seabirds have been reported along the shores of the North Sea (Camphuysen 1989). Various causes have been suggested, including infectious agents, toxic chemicals, parasites, oil pollution, the gradual exhaustion of food supplies and accidental catches by fishermen (Kinze and others 1988). A better understanding of the physiology, ecology, ethology and pathology of these species is a prerequisite to understanding the complex phenomenon of their decline, and a multidisciplinary approach will be necessary.

In the context of studies carried out in Belgium (Jauniaux and Coignoul 1992) and Germany (Siebert and Frese 1993), the authors have been interested in the parasitological infestation of two species: a marine mammal, the harbour porpoise (*Phocoena phocoena*); and a sea bird, the common guillemot (*Uria aalge*). Parasites can be responsible for or involved in various diseases, and can be a cause of the stranding of solitary cetaceans (Geraci and Lounsbury 1993). Moreover, parasites can be biological indicators of the behaviour and the distribution of their hosts (Raga and Balbuena 1992). Their presence in large numbers could also

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TABLE 1: Helminths recorded in 248 common guillemots found dead on the Belgian coast

Helminth species	Birds infected /examined	Parasitised birds (%)	Location
Cestodes			
Unidentified	1/248	0.4	Intestine
Nematodes			
<i>Contracaecum spiculigerum</i>	64/248	25.8	Gizzard, oesophagus
Pentastomids			
<i>Reighardia sterna</i>	7/248	2.8	Air sacs

indicate changes in the marine ecosystem as a result of an imbalance between the parasites and their hosts (Borrens and Coignoul 1992).

Materials and methods

Necropsies and sampling

Eighteen harbour porpoises and 248 common guillemots were examined between February 1990 and July 1991. The porpoises had been found stranded or were bycaught, two along the Belgian coast and 16 along the German coast (eight of them being bycaught). The guillemots were found on the Belgian coast.

The necropsies were carried out according to standard protocols specific for sea birds (Pionneau 1987, Dorrenstein and Van Der Hage 1993) and marine mammals (Siebert and Frese 1993). The specimens for helminth evaluation were collected and rinsed in 0.9 per cent normal saline to remove mucus and debris, and then fixed in a large volume of fixative. The parasites were identified largely on the basis of the following tables and determination keys: Price (1932), Yamaguti (1958-1961), Self (1969), Arnold and Gaskin (1975), Berland (1982), Hong-Kean and Masashi (1982), Abril and others (1984) and Andersen (1987). Additional investigations including histology, bacteriology, virology and toxicology were also made.

Identification of trematodes and cestodes

Trematodes and cestodes were fixed in cold 4 per cent buffered formaldehyde, and extended in xylene between two slides. They were stained by immersion in boracic carmine (Langeron 1949), cleared in hydrochloric acid diluted in ethanol, and neutralised in 70 per cent ethanol saturated with sodium bicarbonate. The samples were dehydrated by immersion in graded alcohols and mounted in a synthetic medium. For the cestodes, the integument and underlying layers of muscle were stripped from the ventral surface of the strobilae to provide better details of the ducts (Berland 1982, Jones 1990). The best results were obtained by initial immersion in xylene before the stripping procedure.

In the porpoises, only a macroscopic examination of the stomach was made.

Identification of nematodes

The samples were fixed in cold 70 per cent alcohol and 2 per cent glycerine to prevent them from becoming dehydrated. The nematodes were examined as wetmounts in clearing agents, using glycerine for small worms and lactophenol for large worms.

Determination of age and maturity

The maturity of the guillemots was determined by an examination of the genital organs following the technique by Camphuysen and van Franeker (1992). Immature birds were considered to be

TABLE 2: Prevalence of infestation of 248 common guillemots with the helminth *Contracaecum spiculigerum* and the mean burden of worms in relation to the sex and age of the birds

Categories	Number	Prevalence	Mean burden
Males	162	28.4	5.2
Females	86	20.9	4.8
< 1 year old	23	17.4	4.3
> 1 year old	225	26.7	5.1

less than one year old and assigned to group 1, and mature birds were considered to be at least one year old and assigned to group 2. The age of the porpoises was determined by an examination of their teeth, using the methods described by Kremer (1987). The animals were separated into two age groups, those less than one year old (group 1) and older animals (group 2).

Statistical analyses

The relationships between the number of parasite species and the age or sex of the hosts were evaluated by the Kruskal-Wallis and Mann-Whitney tests.

The relationship between the ratio of parasitised and non-parasitised animals (the prevalence) was determined by the χ^2 test for the guillemots and by the exact test for the porpoises, because of the small number of animals.

Parasite species occurring in at least 10 per cent of the animals were listed as common for that host, and only these species have been included in the statistical analyses.

Differences with $P < 0.05$ were accepted as statistically significant.

Results

Helminths belonging to three genera were collected from the 248 guillemots. Seventy-two guillemots were infected, the most common helminth being a nematode found in the gizzard of 64 birds.

A total of six helminth species were identified in the harbour porpoises, 16 (89 per cent) of which were infested by these parasites.

Parasitic burdens of the guillemots

Pentastomids of the species *Reighardia sterna* (Self 1969) occurred in the air sac. This species is generally not regarded as a primary cause of death in marine birds.

The nematode found in the gizzard and the oesophagus was identified as *Contracaecum spiculigerum*.

A chain of immature, unidentified proglottids was found in one bird.

Among the three species identified, only *C spiculigerum* occurred in more than 10 per cent of the hosts (Table 1).

A χ^2 analysis of the numbers of immature and adult birds infected with *C spiculigerum* did not indicate a statistically significant difference in the number of parasites between the two age groups, the mean burden (mean number of worms/birds) being light in both groups (Table 2).

There was no significant difference between the number of male and female birds which were infected.

Parasitic burdens of the porpoises

One species of trematode, one cestode, and four nematodes were found in the porpoises. All the species except *Diphyllobothrium stemmacephalum* had a prevalence over 10 per cent (Table 3).



TABLE 3: Helminths recorded in 18 harbour porpoises found dead on the Belgian and German coasts

Helminth species	Porpoises infected /examined	Parasitised porpoises (%)	Location
Trematodes			
<i>Campula oblonga</i>	5/18	28	Liver
Cestodes			
<i>Diphyllobothrium stemmacephalum</i>	2/18	11	Intestine
Nematodes			
<i>Pseudalius inflexus</i>	16/18	89	Bronchus, artery, heart
<i>Torynurus convolutus</i>	8/18	44	Bronchus, pharynx
<i>Stenurus minor</i>	5/18	28	Middle ear
<i>Anisakis simplex</i>	6/18	33	Stomach

Trematodes of the species *Campula oblonga* (Campulidae) occurred in the bile and pancreatic ducts (Table 3). This species was associated with chronic fibrotic cholangitis and hyperplasia of the bile ducts in all the animals infested. *C. oblonga* has previously been reported in the liver and in the bile and pancreatic ducts of harbour porpoises along the coasts of Britain, Denmark and France (Clausen and Andersen 1985, Balbuena and others 1987, Baker and Martin 1992) and was associated with massive hyperplasia of the musosa. These histological observations are comparable with those observed during this survey.

One cestode of the species *D. stemmacephalum* (Diphyllobothriidae) was found in the intestine of two porpoises (Table 3).

The nematode, *Anisakis simplex* (Ascaridoidea: Anisakidae) was recovered from the stomach (Table 3). This worm was associated with ulcers in three of the porpoises, and the prevalence of the ulceration was comparable with that observed by Baker and Martin (1992).

The last three species of nematodes identified, namely *Pseudalius inflexus*, *Torynurus convolutus* and *Stenurus minor*, belong to the Pseudaliidae family, which is composed almost exclusively of parasites of odontocetes (Table 3). *P. inflexus* was recovered from the bronchi and, in five cases, from the heart and pulmonary arteries. *T. convolutus* was found in the bronchi. In seven cases these worms were present in such large numbers that they occluded the bronchi almost completely or completely. They were associated with a chronic interstitial pneumonia. Clusters of macrophages and neutrophils were observed around the parasite larvae in the pulmonary tissue. The deaths of one porpoise from the Belgian coast and three from the German coast were attributed to this parasite. *S. minor* was recovered from the middle ear, but no pathological changes were observed in association with these worms.

The prevalence of the parasites increased with the age of the porpoises except for *S. minor* (Table 4). In contrast, Clausen and Andersen (1985) observed an increase in the prevalence of *S. minor* with the age of porpoises in Danish waters.

The exact test showed a significant increase in the prevalence of parasites with age, but no difference between the sexes.

The Mann-Whitney test indicated that there was no significant difference between the number of parasite species in the male and female porpoises.

Discussion

The environment of the host is an important factor in relation to the presence of helminth species. When compared with guillemots from Newfoundland (Threlfall 1971), those collected along the Belgian coast were not heavily parasitised in terms of either the number of species or the parasitic burden. Twelve genera of helminths were found in the birds from Newfoundland compared with three genera in the birds collected on the Belgian coast. The low incidence of worms in the Belgian guillemots may be the result of environmental conditions in the area where the birds feed, these conditions possibly being unsuitable for the development of the larvae.

TABLE 4: Numbers of harbour porpoises infested with five common helminths in relation to the sex and age of the animals

Categories	Number	Pi	Tc	Sm	As	Co
Males	9	6	4	2	2	1
Females	9	9	4	3	4	4
< 1 year	8	6	3	3	2	1
> 1 year	10	9	5	1	4	4

Pi *Pseudalius inflexus*, Tc *Torynurus convolutus*, Sm *Stenurus minor*, As *Anisakis simplex*, Co *Campula oblonga*

Oiling of birds could also be a factor in explaining the weak infestation of the guillemots from the Belgian coast, the oiled birds having lost their appetite and therefore having not picked up an infection (Borgsteede 1991). Another possibility is that crude oil can act as an antiparasitic substance; substances derived from crude oil, such as carbon tetrachloride and benzene derivatives, have been used as antiparasitic drugs.

The sex of the guillemots had virtually no influence on the structure of the helminth population. This was not surprising since there is no documented evidence that male and female guillemots display different behaviours in breeding or wintering. In contrast, in herring gulls (*Larus argentatus*), a species in which the male and female birds live in different habitats for most of the year, a difference in the helminth population of the sexes has been reported (Threlfall 1968).

The age of the host might be expected to be an important determinant of the composition of the burden of helminths. Very young birds have few parasites and are, therefore, not representative of the whole population (Bush 1989). However, the helminths present in the juvenile birds were very similar to those present in the older birds, probably because all the juvenile birds were more than three months old. These observations are comparable to those in communities of willets (*Catoptrophorus semipalmatus*), a species in which the immature birds live in similar conditions and feed like the adults from a very young age (Bush 1989).

To the best of the authors' knowledge, the life cycle of *C. spiculigerum*, the only common parasite found in the guillemots from the Belgian coast, is still unknown. It is likely, however, that its intermediate hosts are planktonic crustaceans and fish (Lick 1990).

The helminth fauna of the porpoises from Belgian and German waters did not seem to differ substantially from that of porpoises from the rest of the European Atlantic, although no gastric trematodes were found. However, the lack of trematodes may have been due to the fact that the stomach of the porpoises was not examined thoroughly.

In the porpoises, the sex of the host seemed, as in the guillemots, to have no influence on the structure of the helminth fauna. The increase in the prevalence of parasites with age for certain species was different from observations in domestic animals (Kinze and others 1988), in which immature individuals are more sensitive to parasites. In adults, resistance develops to infestation by additional parasitic worms and recovery is prompted by the development of an immune response. However, when defence mechanisms are deficient, as a result of disease or debilitation, an increase in the number of parasites is observed.

The life cycle of *A. simplex* is better known than that of the other parasitic species found in the porpoises, because its larvae are present in edible teleosts and can cause gastric and other lesions in human beings eating raw fish. Some intermediate hosts of *C. oblonga* are still unknown. Apart from molluscs, other hosts most probably play a part in the cycle before the flukes infest cetaceans (Raga and Balbuena 1992). The life cycle of the Pseudaliidae is completely unknown.

T. convolutus occurred in association with *P. inflexus* in the same animals. A similar case was reported in porpoises from French Atlantic waters (Balbuena and others 1987). The massive presence of two species in the respiratory system is a heavy burden on



diving mammals such as the harbour porpoise, and parasitic bronchopneumonia was the cause of death of four (22 per cent) of the porpoises. The guillemots from the Belgian coast were, in general, weakly parasitised. This observation could indicate either that the birds were particularly healthy, or that the environmental conditions for the complex life cycle of the parasites were unfavourable, or that the diet of the birds lacked variety. In any case, it seems probable that parasitism was not responsible for the mortalities observed among common guillemots in the North Sea, the major cause of which is oil pollution (Camphuysen and van Franeker 1992).

The harbour porpoises all carried a large number of helminths of an appreciable range of species. These helminths, particularly those that infested the respiratory tract, had a significant impact on the health of the porpoises and were responsible for the death of four of them. The immature male found dead along the Belgian coast was massively infested with pulmonary, hepatic, intestinal and vascular parasites. However, the reasons for such a severe infestation in an immature animal were not clear.

Acknowledgements. – This work was partly subsidised by the EEC (NORSPA N° 90-1/B/002-BA 3030/92/004295) and the Belgian Ministry of Public Health and Environment. The authors thank the rehabilitation centres of the Belgian coast (De Zwin, Oostende and Nieuwpoort) for collecting most of the guillemots, and the 'Institut Royal des Sciences Naturelles' for the identification of the birds. The first author expresses her gratitude to Professor Raga for his help in the identification of the parasites. Thanks are also due to Professor J.-M. Bouquegneau (Department of Oceanology of the University of Liège) who made this work possible.

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Abstracts

Bone growth and 1,25(OH)₂-vitamin D₃ in chickens

THE incidence of defects of endochondral ossification was different in two distinct strains of commercial broilers which were both fed the same nutritionally adequate diet. Between one and 21 days of age the incidence of tibial dyschondroplasia in one strain varied from 10 to 20 per cent, whereas in the other strain it varied from 10 to 70 per cent and 40 per cent of the bone samples collected at 14 days of age had lesions of calcium deficiency rickets. The serum concentration of 1,25-dihydroxycholecalciferol was significantly lower in the second than in the first strain, except in the day-old chicks. These results suggest that tibial dyschondroplasia in some strains of broiler is related to an inherent predisposition to rickets and to lower serum concentrations of 1,25-dihydroxycholecalciferol.

PARKINSON, G., THORP, B. H., AZUOLAS, J. & VAIANO, S. (1996) *Research in Veterinary Science* **60**, 173

Interaction of nutrition and genotype on haemonchosis in sheep

PROTEIN supplementation has been shown to improve the resistance of some breeds of sheep to haemonchosis. Blackface sheep are more resistant to the disease than other breeds and this paper describes experiments to test whether protein supplementation would be beneficial to this resistant breed. Blackface lambs were fed either a basal diet or the same diet supplemented with soya-bean meal to give an additional 80 g crude protein/kg dry matter; some of them were then given an initial dose of *Haemonchus contortus* followed by a trickle infection for 10 weeks. The weight gains of the supplemented lambs were greater and their carcasses were leaner, irrespective of their infection status. The infected animals on the basal diet were more anaemic and hypoalbuminaemic than those on the supplemented diet, although there were no significant differences between them in worm burdens or faecal egg counts.

WALLACE, D. S., BAIRDEN, K., DUNCAN, J. L., FISHWICK, G., GILL, M., HOLMES, P. H., MCKELLAR, Q. A., MURRAY, M., PARKINS, J. J. & STEAR, M. (1996) *Research in Veterinary Science* **60**, 138

