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SEROLOGIC STUDY OF PHOCINE DISTEMPER IN A POPULATION OF HARBOR SEALS IN SCOTLAND

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ABSTRACT: A serologic survey of the prevalence of morbillivirus antibodies was conducted in a population of harbor seals (*Phoca vitulina*) from northeastern Scotland, where mortality was comparatively low during the 1988 phocine distemper virus outbreak. None of the 12 seals sampled before the epizootic were seropositive. Thirty-five (52%) of 68 seals sampled after the beginning of the epizootic were seropositive, although there were significant age-related differences in both the number of seropositive individuals and in antibody levels. Marking studies showed that most seropositive seals caught during the peak of the epizootic survived for several months. Thus, the low mortality observed in this population did not appear to result from a lack of contact with the virus.

Key words: Harbor seal, *Phoca vitulina*, Phocine distemper virus, morbillivirus, epizootic, serologic survey, field study.

INTRODUCTION

During 1988, a major epizootic occurred among harbor seal (*Phoca vitulina* L.) populations in the North Sea, killing an estimated 17,000 individuals (Dietz et al., 1989b; Harwood et al., 1989). Harbor seals in all areas were affected to some extent, but mortality rates differed considerably between populations, varying from only 10 to 20% in northeastern Scotland (Thompson and Miller, 1991) to 60% in some Scandinavian waters (Dietz et al., 1989b).

Although seals generally died of secondary infections such as acute bacterial pneumonia, the primary cause of the epizootic was a previously undescribed morbillivirus, phocine distemper virus (PDV) Kennedy et al., 1988; Osterhaus and Vedder, 1988). Virus-neutralization tests developed for the closely related canine distemper virus (CDV) confirmed that both harbor and gray seals (*Halichoerus grypus*) from the North Sea had been exposed to PDV during 1988 (Osterhaus and Vedder, 1988; Harwood et al., 1989). In addition, morbillivirus antibodies were detected from other regions, in samples from Baikal seals (*Phoca sibirica*) (Likhoshway et al., 1989), and harp seals (*Phoca groenlandica*) and ringed seals (*Phoca hispida*) from Greenland (Dietz et al., 1989a). However, only limited data are available on the prevalence of morbillivirus antibodies in North Sea seal populations before and during the epizootic. In the Netherlands, Osterhaus et al. (1989) found antibodies to an antigenically different morbillivirus in serum samples from seals brought in to a rehabilitation center between 1984 and 1987. On the other hand, in the only samples from UK waters, Harwood et al. (1989) found no evidence of morbillivirus antibodies in serum samples taken from gray seals in the United Kingdom between 1977 and 1987.

If the epidemiology of the 1988 epizootic is to be understood in detail, data are required on the proportion of the population which came into contact with the virus and on the fate of those individuals exposed to it. Such information can be used to assess whether observed differences in mortality resulted from variations in rate of contact with the virus, or of variations in resistance to the virus.

This paper presents data from a serologic survey of a population of harbor seals

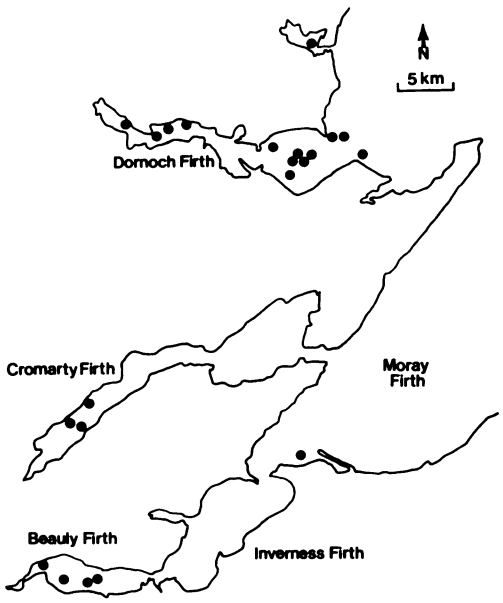

FIGURE 1. Location of the study area and position of harbor seal haul out sites (●).

in the Moray Firth, northeastern Scotland (57°35'N; 4°0'W). Mortality in this population was low (10 to 20%), with most deaths occurring during August, September and October 1988 (Thompson and Miller, 1991). A capture and marking program was started early in 1988 and blood was collected routinely from all captured seals. Sera were analyzed to assess changes in prevalence of morbillivirus antibodies in seals caught before, during, and after the epizootic. In addition, observations were made on seals marked during the epizootic to assess the fate of individuals with known antibody levels.

METHODS

Study area and capture techniques

The Moray Firth contains a population of at least 1,100 harbor seals which haul-out regularly on intertidal sand and mudbanks in the inner part of the firth (Fig. 1). Seals were captured by rushing at haul out groups with an inflatable boat or fourwheel drive vehicle and catching animals individually using hoop nets. They were then transfered to a restraining board and, where necessary, sedated lightly with either ketamine hydrochloride (Vetalar, Parke Davis/Warner Lambert, Pontypool NP4 8YA, United Kingdom) and diazepam (Valium, Roche Products Ltd., Wellwyn Garden City, AL7 3AY, United Kingdom) (Baker et al., 1988) or tiletamine hydrochloride and zolazepam (Zoletil, Reading, Z.A.C., 17 rue des Marronniers, 94240 L'Hayles-roses, France) (Stirling and Sjare, 1988). Standard length and girth measurements were taken while seals were on the restraining board. All seals less than 1.1 m standard length were assumed to be juveniles in their first or second year (Harwood et al., 1989). It was not possible to determine the precise age of older animals; seals longer than 1.1 m were therefore classed as adult.

Blood sampling and marking techniques

Blood samples were taken from the epidural vein of restrained seals using either plain or heparinized (heparin hydrochloride) blood vacuum tubes (Vacutainers, Becton Dickinson, UK Ltd., Between Towns Road, Cowley, Oxford, OX4 3LY, United Kingdom). Serum or plasma was removed within 24 hr and frozen at −20 C for ≤12 mo. Between April 1988 and June 1989, blood samples were obtained from 79 individual harbor seals, with samples being taken before, during and after the period when most deaths occurred (Table 1). Repeat blood samples were also obtained from two individuals in different seasons.

During August and September 1988, 28 harbor seals (14 adults, 14 juveniles) were caught at haul out sites in the Inverness and Dornoch Firths. All seals were marked with a numbered tag in each hind flipper and twenty four individuals also were marked with a large colored number or letter (approximately 20 cm high) on their back (Thompson, 1989). Very high frequency (VHF) radio-transmitters were glued (Fedak et al., 1983) to the hair on the heads of six adult seals from the Inverness Firth.

Throughout 1988 and 1989, observations were made at known haul out sites at least twice each month. Haul out groups were counted using a 30 × 70 telescope and the presence of any color-marked individuals was noted. Flipper tags could not be read using a telescope but were used for the identification of carcasses or individuals that were recaptured after the color-marks had been lost in the moult. Radio-tagged individuals were located daily by triangulation during November 1988 and January 1989, and their presence and activity around haul out sites was monitored using automatic recording equipment (Thompson and Miller, 1990).

Virus neutralization test

Neutralization tests were carried out in microtitration plates by a method based on that described by Appel and Robson (1973). Four-

TABLE 1. Age and sex of harbor seals caught and bled in the Moray Firth 1988–1989, before, during and after a phocine distemper epizootic.

Sampling period	Juvenile			Adult	
	Male	Female	Unknown	Male	Female
Pre-epizootic April 1988	2	1	—	5	4
During epizootic Aug–Sept 1988	9	4	1	12	2
Post-epizootic Feb–May 1989	10	15	—	6	10
Total	21	20	1	23	16

fold dilutions of test serum, from 1:16 to 1:16,384, were made up with graduated pipettes. The Onderstepoort-Bussell Strain (A. E. Churchhill, formerly of Intervet, UK Ltd., Science Park, Cambridge CB4 4FP, United Kingdom) of canine distemper virus (CDV) was diluted to 80–320 tissue culture infectious doses 50% (TCID50) per ml of growth medium with 5% fetal bovine serum (FBS) and 1 ml of this added to an equal volume of each serum dilution. The serum-virus mixtures were left in the dark at room temperature (18–20 C) for 1 hr and at 4 C for a further 1.25 hr to allow neutralization to proceed. Each mixture (0.2 ml/well) was then inoculated into four wells of Vero cell monolayers in a 96 well microtiter plate (Nunclon, Gibco Ltd., P.O. Box 35, Trident House, Paisley, PA3 4EF, Scotland). These cultures had been prepared the previous day by seeding the wells with a suspension containing 200,000 cells per ml, 0.2 ml per well. Growth medium consisted of medium 199 with 25 mM Hepes buffer and L-Glutamine and 10% FBS.

Following inoculation, plates were incubated at 37 C for 3 days in a humidified atmosphere containing 3% CO_2. Each well was then examined microscopically and the proportion of wells showing the characteristic cytopathogenic effect was recorded for each serum dilution. Titers were expressed as the reciprocal of the serum dilution which reduced the proportion of wells infected from 100% to 50%. All samples with titers ≥90 were assumed to be seropositive. Serum and virus controls were included on each occasion that the test was carried out.

RESULTS

Changes in the prevalence and level of antibodies

Antibody titers before the epizootic were all low, ranging from ≤8 to 32. Blood samples taken after August 1988, when the first deaths due to PDV were seen, frequently had much higher levels of antibody (Fig. 2).

Thirty-five (52%) of 68 seals caught after 1 August 1988, were seropositive. Significantly more adults were seropositive than juveniles ($\chi^2 = 5.25$, 1 df, $P < 0.05$) in both autumn 1988 and spring 1989 samples (Fig. 3). Furthermore, the $\log_{10}$ titers of seropositive adults were significantly higher than those of seropositive juveniles ($\bar{x}$ adult titer = 2.83, $\bar{x}$ juvenile titer = 2.41, $t = 2.69$, 33 df, $P < 0.05$). There were no significant differences in $\log_{10}$ titers between samples taken in autumn 1988 and spring 1989 (t-test, $P > 0.5$).

Survival of marked seals with known antibody titers

Eleven color-marked seals were seronegative. Three of these individuals were found dead 4 to 6 wk after capture and another three were seen alive and apparently healthy after 21 to 30 wk (Table 2).

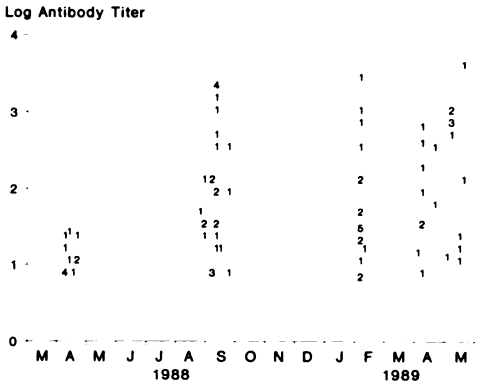

FIGURE 2. Serum morbillivirus antibody titers in free-living harbor seals from before the phocine distemper epizootic (April 1988), during the epizootic (August to September 1988), and after the epizootic (February to May 1989).

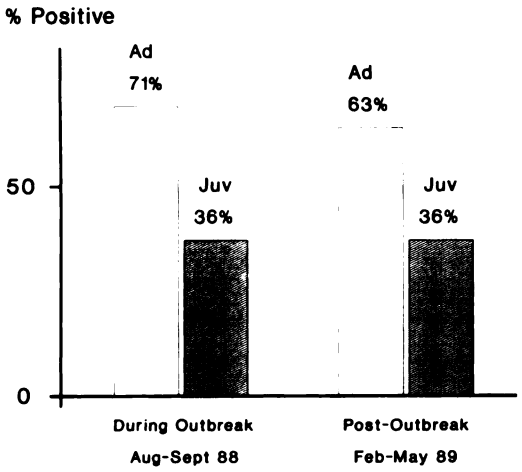

FIGURE 3. Age differences in the proportion of seropositive seals caught during and after the 1988 epizootic. Sample sizes are presented in Table 1.

One juvenile seal (32038) was found sick after 3 wk. It displayed none of the classic signs of PDV infection (see Dietz et al., 1989b) and its titer remained at ≤8. After treatment with antibiotics it recovered and was released to the wild. The remaining seals were either not subsequently seen or were seen alive only soon after marking.

Two of thirteen color-marked seropositive seals were recovered dead, 1 and 3 wk after marking. An adult female which had been radio-tagged (32041) probably also died because her radio-signal disappeared after less than 1 wk. This disappearance was preceded by several days of unusual behavior when she spent almost all her time either on the shore or at the surface. A further six individuals were observed alive between 13 and 44 wk, well beyond the period when deaths due to PDV were

TABLE 2. Fate of seals with and without morbillivirus antibodies marked during August and September 1988 when most seal deaths were seen in the Moray Firth.

Seal number	Age	Sex	Antibody titer to morbillivirus	Date caught	Number of weeks to last sighting	Status at last sighting
Seals with no antibodies against morbillivirus						
32030	A*	M	32	8.29.88	30	Alive
32032	A	M	32	9.2.88	6	Dead
32035	J	M	16	9.10.88	3	Alive
32036	J	?	<8	9.10.88	4	Dead
32038	J	M	<8	9.12.88	3	Sick
32039	J	M	<8	9.12.88	24	Alive
32040	J	M	24	9.12.88	—	Not sighted
32025	A	M	32	9.13.88	3	Alive
32047	J	F	24	9.15.88	—	Not sighted
32051	J	M	32	9.15.88	21	Alive
32055	J	F	<8	9.26.88	6	Dead
Seals with antibodies against morbillivirus						
32031	A	F	128	8.31.88	34	Alive
32041	A	F	90	9.13.88	<1	Unclear
32042	J	M	90	9.13.88	6	Sick
32043	A	M	512	9.14.88	44	Alive
32044	A	M	2048	9.14.88	—	Not sighted
32045	A	M	2048	9.14.88	3	Dead
32046	A	M	367	9.15.88	<1	Alive
32048	J	F	1024	9.15.88	—	Not sighted
32049	A	M	2048	9.15.88	39	Alive
32050	A	M	2048	9.15.88	13	Alive
32052	J	F	1024	9.15.88	43	Alive
32053	A	M	367	9.26.88	1	Dead
32054	J	M	90	9.26.88	39	Alive

* A = adult; J = juvenile.

reported from this area. Three additional seals were either never seen or seen alive only a few days after marking. Finally, a juvenile male (32042) was found alive but in poor condition 6 wk after marking. It also displayed no signs of PDV infection and after treatment it recovered and was released.

DISCUSSION

One or two samples collected before the 1988 epizootic were in the range which has been considered seropositive in other studies (Osterhaus and Vedder, 1988; Osterhaus et al., 1989). However, because the CDV neutralization test measures heterotypic antibody, and there could be group cross-reactions, we suggest that these low titers do not necessarily indicate the presence of PDV antibodies. Furthermore, low dilutions of seal sera sometimes appeared to adversely effect the Vero cell cultures, and this could have masked any specific cytopathogenic effects. Consequently, we only accepted titers of ≥ 90 as proof of morbillivirus infection. Conversely, we cannot be sure that these early samples were seronegative for morbillivirus antibodies. Seals could have previously been exposed to a different morbillivirus infection which resulted in a weaker immune response than PDV, or antibodies to other morbilliviruses could have been detected less effectively by our CDV antibody test. Harbor seals may live for up to 30 years (Boulva and McLaren, 1979). Thus, if seal populations are exposed to morbilliviruses only infrequently, seropositive individuals could be rare. Lack of a precise diagnostic test for PDV, together with the small sample sizes involved, therefore prevents a reliable assessment of whether these populations have been exposed to PDV or other morbilliviruses.

Comparison of samples taken in April 1988 and August/September of 1988 showed that most of the later samples were positive (Fig. 2). Thus, a high proportion of the Moray Firth seals which survived the 1988 epizootic, particularly adults, are likely to be immune to further outbreaks of PDV. Phocine distemper virus may have arrived in the Moray Firth during the period April to August 1988, but it is generally believed that it arrived in Scotland during late July or August (Dietz et al., 1989b). If so, the high proportion of seropositive animals in our sample from August and September 1988 (Fig. 3) suggests the virus spread quickly through this population. This is highlighted by the similarity, in both the prevalence of seropositive individuals (Fig. 3) and antibody levels, between samples taken during the peak of the epizootic and those taken the following year. However, it is also possible that the virus arrived in the Moray Firth earlier in the year and that the mortalities seen from August to October were precipitated by other factors. For example, harbor seals are generally in poor body condition in late summer after the breeding season and moult (Drescher, 1979; Pitcher 1986) and seals may have been more vulnerable to secondary pathogens at this time. Unfortunately, we know of no blood samples being taken from United Kingdom seals during the summer of 1988 which could be used to identify the time of arrival of PDV more precisely.

The observed age-difference in the proportion of seropositive individuals (Fig. 3) was also seen in a sample of seals from throughout the United Kingdom (Harwood et al., 1989), which included some of the samples reported in our study. Harwood et al. (1989) presented data from seals caught during the spring of 1989, and suggested that many of the young seals had not been exposed to the virus at haul out sites due to social factors. For example, juvenile harbor seals sometimes form fairly discrete haul-out groups (Drescher, 1979) and there is some evidence that pups haul-out only infrequently in the period immediately following lactation (Thompson and Harwood, 1990). The observed differences could also result from juveniles incurring greater mortality than adults. However, studies of carcasses found no ev-

idence for higher mortality amongst young seals (Hall et al., 1991; Thompson and Miller, 1991). An alternative explanation is that age-related differences in immune response resulted in there being false negatives in the juvenile sample. Two lines of evidence appear to support this latter hypothesis. First, we found the same age-differences in the prevalence of seropositives in samples collected during the peak of the epizootic and in those collected in 1989 (Fig. 3). During the epizootic, juveniles were caught from groups containing all age-classes of seals, including some adults which displayed signs of PDV and related secondary infections. Secondly, we found that seropositive juveniles had significantly lower titers than seropositive adults, which may indicate that the immune system of juvenile harbor seals is less well developed. Carter et al. (1990) have shown that gray seal pups have much lower serum immunoglobulin levels than adults, and suggest that the immune system of young seals may be compromized. Alternatively, the morbillivirus antibody levels that we found in juvenile harbor seals may result from a normal response to a primary infection, and older animals may have elevated levels due to anamnestic response from previous morbillivirus infection (Norrby and Appel, 1980).

Resighting data from seals marked during the peak of the epizootic confirm that most seropositive individuals survived the period of the epizootic (Table 2). A few seropositive animals were known to have died but the cause of death could not be confirmed. The varied fates of seals from both seropositive and seronegative groups suggest that serum antibody levels are of limited use as a prognostic tool during a disease outbreak of this kind. However, further studies of these individuals, and collection of serial serum samples, should allow an assessment of whether morbillivirus antibodies remain detectable in future years.

In conclusion, although we cannot confirm whether or not harbor seals from the Moray Firth had previously been exposed to morbilliviruses, these data show that a high proportion of adults came into contact with the virus during 1988. The cause behind the lower proportion of seropositive samples from juveniles remains unclear, but highlights the need for further work on the immune response in this species. Despite these uncertainties, these data show that the low mortality observed in this population was not the result of seals failing to come into contact with PDV.

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