Prevalence of morbillivirus antibodies in Scottish harbour seals

P. M. THOMPSON, H. THOMPSON, A. J. HALL

SINCE May 2002, a new outbreak of phocine distemper virus (PDV) in harbour seals (*Phoca vitulina*) has been spreading through the North Sea (Harding and others 2002, Jensen and others 2002). By late September 2002, over 17,200 dead harbour seals had been reported dead in European waters (Reineking 2002), approaching the 18,000 deaths resulting from the PDV outbreak in 1988 (Heide-Jørgensen and others 1992b). In the UK, the first cases of PDV were reported in mid-August, and over 1800 seals had been found on the English coast by late September 2002. However, most of the UK's 36,000 plus harbour seals are found around the Scottish coast, where PDV was only confirmed to be present in mid-September. Serological studies following the 1988 outbreak of PDV indicated that a high proportion of surviving seals came into contact with the virus (Heide-Jørgensen and others 1992b). The spread and impact of the current outbreak depends critically on levels of resistance in these different North Sea populations, but few serological data exist to help evaluate this threat. This short communication describes a study undertaken to determine how the prevalence of morbillivirus antibodies has changed in the Scottish populations since 1988, and to assess the current vulnerability of these animals to the spread of PDV.

Blood was collected from free-living harbour seals in the Moray Firth between 1988 and 1996, and in the Tay estuary between 1998 and 2000. Over 300 seals were captured at haul-out sites throughout the year, but predominantly in the spring and autumn (Thompson and others 1992). As harbour seal pups are born in June and early July, data were pooled into year classes based on the period June 1 to May 30.

Measurements of pelage hairs (Corpe and others 1998) confirmed that 108 of the captured seals were aged between two and 14 months. These seals ranged in size from 83 to 113 cm and 13·6 to 37 kg, and it was assumed that all seals longer than 115 cm or weighing more than 40 kg were more than one year of age. Samples from pups (less than 100 cm long or less than 25 kg in weight) captured before September were excluded to avoid any possibility of detecting maternally derived antibodies (Cornwell and others 1992).

Blood samples were taken using heparinised or plain vacutainers, and the plasma or serum was frozen and stored at –20°, before being sent overnight on ice to Glasgow University Veterinary School. Virus neutralisation tests were carried out in microtitration plates as described by Cornwell and others (1992). Titres were expressed as the reciprocal of the serum dilution that reduced the proportion of wells infected from 100 per cent to 50 per cent. Antibody titres from seals captured before the 1988 epizootic were all up to 32 (Thompson and others 1992). Based upon these data, and inspection of frequency distributions of titres from this study, it was assumed that all samples with titres of 68 or more were seropositive. The sensitivity of the present results to this assumption was investigated by using logistic regression to determine how the prevalence changed under different threshold titres.

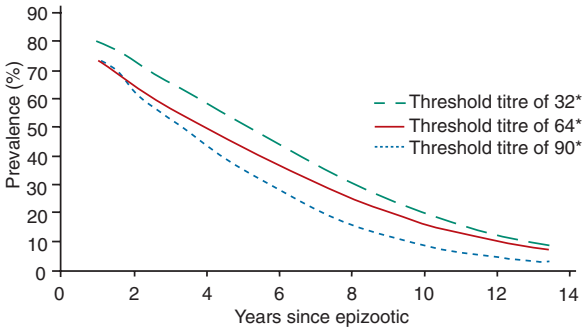

FIG 1: Changes in the prevalence of morbillivirus antibodies in Scottish harbour seals since the 1988 phocine distemper virus outbreak. The trend was estimated using logistic regression, with each fitted curve representing a different threshold level for seroprevalence. *P<0·001

Of the 300 plus seals which were captured and sampled, 81 were confirmed to be in their first year and born after 1990. None of these seals was seropositive, even using a threshold titre of 32. Since the 1988 epidemic, there had been a significant decline in the prevalence of antibodies among seals over one year of age (Fig 1). These data suggest that between 2·7 per cent and 8·6 per cent of the current population were exposed to PDV during 1988. Mean $\log_{10}$ titres of seropositive seals also showed a significant decline over this period (r^2=0·59, P<0·05) (Table 1).

These data represent the most extensive serological survey of healthy, free-living seals in any of the areas affected by the PDV outbreak in 1988. Analyses of samples from seals born since 1990 provided no evidence for the continued circulation of PDV on the east coast of Scotland. The decline in both prevalence (Fig 1) and mean titres (Table 1) further indicate that there was no exposure to the virus after 1988, in line with epidemiological models which suggest that PDV should not persist in populations affected in 1988 (Grenfell and others 1992, Swinton and others 1998). Most other serological studies have been based on samples from orphaned pups around North Sea coasts. These, too, generally found no evidence of circulating PDV beyond 1989 (Hughes and others 1992, Harder and others 1993), and the only exception to this pattern (Visser and others 1993) is now recognised to result from the detection of maternally derived antibodies (Jensen and others 2002).

Accounting for uncertainty over the most appropriate threshold titre, the present data indicate that only 3 to 9 per cent of animals from current Scottish populations have previously been exposed to PDV. The underlying pattern of decline follows a trend which would be predicted from population turnover and changing age structure (Heide-Jørgensen and others 1992a, Harding and others 2002), and suggests that a PDV epidemic could develop rapidly now that infective animals have arrived in Scottish waters.

TABLE I: Number of seropositive seals more than one year of age recorded in each year, with mean $\log_{10}$ titres for the seropositives (titres of 64 or more)

	Year								
	1988	1990	1991	1992	1993	1994	1998	1999	2000
Number of samples	36	24	36	28	22	20	19	31	7
Number seropositive	26	16	28	11	8	5	2	9	0
Mean $\log_{10}$ titre	2·83	2·32	2·33	2·19	2·16	1·99	2·18	1·96	–

ADDENDUM

By the end of October 2002, the total number of dead seals recorded had risen to almost 21,000, with just under 3000 reported from UK waters (Reineking 2002).

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Detection of PrP^{CWD} in mule deer by immunohistochemistry of lymphoid tissues

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CHRONIC wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) of native North American deer and elk (Williams and Young 1980, 1982). The neuropathology of clinical CWD is well described (Williams and Young 1980, 1982, 1992, 1993, Spraker and others 1997), but studies of CWD-infected cervids employing immunohistochemistry have been undertaken only recently. These studies have demonstrated that brain and various lymphoid tissues from clinically affected deer and elk stain intensively with anti-prion protein (PrP) immunostaining (Williams and Young 1992, Spraker and others 1997, O'Rourke and others 1998a). Positive staining of lymphoid and brain tissue can also occur in the absence of spongiform lesions (Sigurdson and others 1999, Miller and others 2000).

Accumulation of PrP^{Sc} in peripheral lymph nodes and spleen has been shown to precede the development of detectable neurological lesions and clinical signs of scrapie in sheep (Schreuder and others 1996, 1998, van Keulen and others 1996, Andréoletti and others 2000), but not of bovine spongiform encephalopathy in cattle (Wells and others 1998). The presence of PrP^{Sc} in tonsillar and nictitating membrane lymphoid tissues has led to the acceptance of lymphoid immunohistochemistry as a reliable diagnostic test for scrapie in sheep (Miller and others 1993, Schreuder and others 1996, 1998, van Keulen and others 1996, O'Rourke and others 1998a, 1998b, 2000, Andréoletti and others 2000). Positive staining of lymphoid tissues (including tonsil and retropharyngeal lymph node) in the absence of either lesions or staining in brain tissue also has been observed in mule deer (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginianus*) exposed to CWD (Sigurdson and others 1999, Miller and others 2000, E. S. Williams, unpublished observations), and seems to be an early indicator of CWD infection in these species. This short communication presents data that demonstrate the use and reliability of tonsil and retropharyngeal lymph node tissue immunohistochemistry in detecting PrP^{CWD} and estimating the prevalence of CWD in free-ranging mule deer populations.

Tissue samples from 1372 mule deer, collected between October 2000 and April 2001, were examined to compare the sensitivity, specificity and agreement of tonsil and retropharyngeal lymph node immunohistochemistry with medulla oblongata (obex) immunohistochemistry in detecting PrP^{CWD}. All samples were from randomly harvested or culled, apparently healthy, free-ranging mule deer, with the exception of three captive, clinically affected individuals from the Colorado Division of Wildlife's Foothills Wildlife Research Facility. Of the samples collected, 290 were from populations where CWD had never been diagnosed and 1079 were from populations in which CWD was known to be endemic (Miller and others 2000), and in which its prevalence had previously been estimated at about 5 per cent (Miller and others 2000). Surveillance and sampling methods were generally as described by Miller and others (2000).

Representative samples of medulla oblongata (sectioned at the obex), tonsil and retropharyngeal lymph node tissues were collected from each animal, and the tissues were preserved in 10 per cent neutral buffered formalin. Subsamples of all three tissues from each animal were cut into sections approximately 2 to 3 mm thick, immersed in 98 per cent formic acid for one hour, and rinsed under flowing tap water for at least four hours. These tissues were embedded in a common paraffin block and sectioned at 5 to 6 μm for staining, thereby minimising opportunities for method-associated staining variation among tissues from an individual animal. The tissues were examined by immunohistochemistry using monoclonal antibody (MAb) F99/97.6.1 (Cell line F99/97.6.1; VRMD) and staining techniques described by O'Rourke and others (2000) and Spraker and others (2002). Briefly, sections were mounted on positively charged glass slides, deparaffinised and hydrated. Pretreatments to enhance epitope exposure included immersion in 98 per cent formic acid solution for 20 minutes, followed by a rinse in water and autoclaving for 20 minutes at 121°C in acid retrieval solution (DAKO Target Retrieval Solution; DAKO) with cooling for 15 minutes in the retrieval solution, followed by 15 minutes in buffer solution (APK Wash, pH 7·4; Ventana Medical

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