in air would be more suitable for commercial application. This mixture induces a rapid loss of brain function as shown by the SEPs in this study, and it also reduces the effect of the pungency associated with high concentrations of carbon dioxide in air. The time to the onset of an isoelectric EEG was also shorter with this mixture suggesting that death ensues rapidly during exposure to it.

Even though the time to loss of SEPs was longer during exposure to 90 per cent argon in air this mixture is considered to be acceptable for stunning turkeys because the induction of anaesthesia is smoother, owing to the fact that both nitrogen and argon are tasteless and odourless gases and probably do not induce any sense of pungency or breathlessness.

The susceptibility of the turkey's brain to the toxic nature of the carbon dioxide-argon mixture (hypercapnic anoxia) appears to be similar to that of hens, as it has been reported that hens lost their SEPs in 19 seconds during exposure to a carbon dioxide-argon mixture (Mohan Raj and others 1992). However, the onset of an isoelectric EEG occurred more quickly in turkeys which suggests that brain death ensues more quickly in this species during exposure to the carbon dioxide-argon mixture. The times to the loss of SEPs by turkeys during exposure to this mixture and by hens (Mohan Raj and others 1992) are shorter than the times to loss of SEPs during exposure to 90 per cent argon in air. Turkeys take longer than hens to lose their SEPs in this mixture. In the case of hens there was a mean difference of 10 seconds between the time to loss of SEPs in the carbon dioxide-argon mixture and 90 per cent argon in air (Mohan Raj and others 1992) and the present study showed that this difference was 22 seconds for turkeys. This longer time would imply that the turkey brain is relatively tolerant to anoxia induced with 90 per cent argon in air and this finding is also supported by the findings of Gregory and Wotton (1988) who reported that the visual evoked potentials were present in turkeys for more than a minute after severing both the carotid arteries. Owing to their resistance, turkeys probably require exposure to anoxia for three minutes to ensure the death of all the birds, instead of the two minutes required for chickens.

It is concluded that stunning/killing turkeys with either a mixture of 30 per cent carbon dioxide and 60 per cent argon in air, or by 90 per cent argon in air are acceptable methods, and it is considered that both would be suitable for stunning turkeys in their transport containers under commercial conditions. Separate studies have shown that exposure times of two and three minutes, respectively, are required to kill turkeys in batches of two or three birds per crate with the carbon dioxide-argon mixture and 90 per cent argon in air (unpublished data). Using 65 per cent carbon dioxide in air for stunning turkeys is considered to be unacceptable owing to the pungency of the gas at this concentration.

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Short Communications

Continued presence of phocine distemper virus in the Dutch Wadden Sea seal population

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SINCE the emergence of phocine distemper caused by phocid distemper virus-1 (PDV-1), which first affected the harbour seal (*Phoca vitulina*) and grey seal (*Halichoerus grypus*) populations of northwest Europe in 1988 (reviewed by Osterhaus and others 1990), there have been many speculations about the future persistence of this virus in these populations (Harwood and others 1989, Reijnders 1989, Simmonds 1991). In 1989 the authors documented that some of the vaccinated seals in the Seal Rehabilitation and Research Centre (SRRC) at Pieterburen, the Netherlands, became infected with PDV-1. This infection only caused mild respiratory signs and conjunctivitis in these animals (Visser and others 1992). It indicated that PDV-1 had been reintroduced into the SRRC in that year by infected animals admitted to the centre. Recently, Hughes and others (1992) documented that there has been no evidence of the continued presence of PDV-1, either clinically or from serological assays, in common or grey seals in the Wash and other British sites of the North Sea after the 1988 outbreak. The present study provides evidence for the continued presence of PDV-1 among harbour and grey seals in the Dutch Wadden Sea till the present time.

The authors have tested sera from harbour and grey seals admitted to the SRRC at Pieterburen from 1988 to 1992, in a previously described ELISA for the detection of morbillivirus specific serum antibodies (Visser and others 1989). Antibody titres ≥ 10 in this assay were considered positive. The animals were divided into three categories: babies (younger than three months), juveniles (three months to one year) and subadults (older than one year). Absence of morbillivirus specific serum antibodies in baby seals may reflect the absence or low levels of colostrum intake, or the absence or low levels of morbillivirus specific serum antibodies in the cows of grey seals and harbour seals (Carter and others 1990, 1992). Morbillivirus specific serum antibodies in baby seals may be of maternal origin, or result from active infection. Morbillivirus specific serum antibodies in juvenile seals, which have lost their maternal antibodies, should have resulted from

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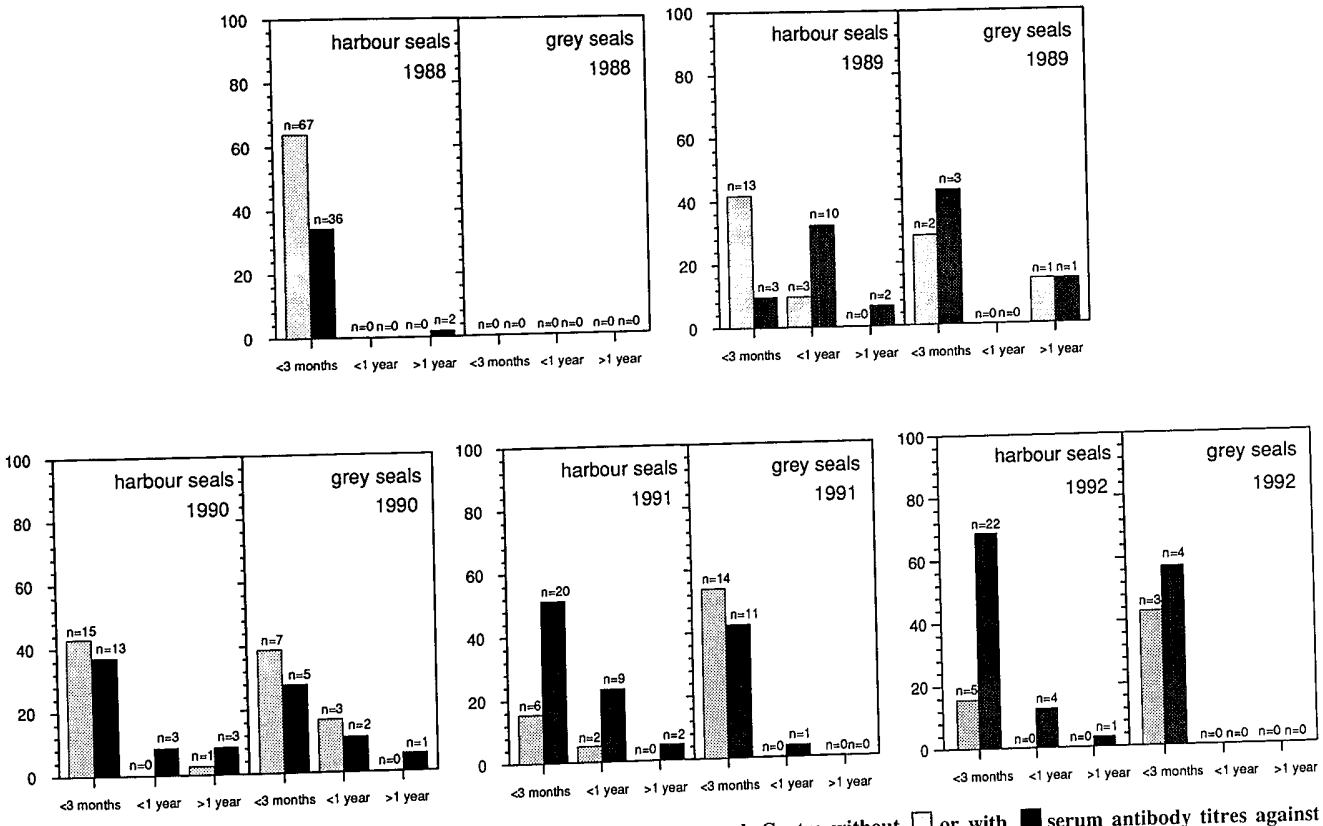

FIG 1: Percentages of seals admitted to the Seal Rehabilitation and Research Centre without □ or with ■ serum antibody titres against canine distemper virus upon admittance (n = absolute numbers)

infection during their first year of life. In subadult seals morbillivirus specific serum antibodies should also be the result of an active infection that may, however, have occurred more than one year before. Therefore, serological data from juvenile seals, may provide information about the continued presence of PDV-1 in the seal population.

Serological data obtained from the three described categories of harbour and grey seals admitted to the SRRC at Pieterburen since the outbreak of 1988, are shown in Fig 1. In 1988 about one third (n=36) of the baby harbour seals (n=103) were seropositive upon arrival. Besides baby seals, in that year only two other seals – juvenile harbour seals – admitted to the SRRC, were included in this study. They were both found to be seropositive. In 1989 only three of the 16 baby harbour seals arrived with morbillivirus specific serum antibodies. In that year the majority of the juvenile harbour seals (10 of the 13 admitted) had morbillivirus specific serum antibodies. Both of the subadult harbour seals and one of the two subadult grey seals tested, had morbillivirus specific serum antibodies upon arrival. In 1990 almost half of the baby harbour and grey seals (18 of the 40 animals admitted) had morbillivirus specific serum antibodies. All the three juvenile grey seals admitted in that year had morbillivirus specific serum antibodies, and three out of four subadult seals were seropositive.

In 1991, of the 51 admitted baby seals studied, 31 were seropositive, whereas 10 of the 12 juvenile seals had morbillivirus specific serum antibodies upon arrival. Both subadult harbour seals tested in that year were seropositive. The majority of the baby seals (26 of the 34) and all of the juvenile (n=4) and subadult (n=1) seals admitted in 1992 had morbillivirus specific serum antibodies upon arrival.

These data show that morbillivirus specific serum antibodies were detected in a large proportion of the seals admitted to the SRRC at Pieterburen. Although the animals presented to this centre are certainly not representative of the whole population in the Dutch Wadden Sea, the data from the juvenile group, especially, indicate that active PDV-1 infections continue to occur in this area at present. This raises questions about the persistence of PDV-1 in individual animals and about the duration of the virus excretion by infected animals. In most of the morbilliviruses of terrestrial mammals, the virus may persist in some animals for prolonged periods but is rarely excreted for more than a few weeks (Fenner and others 1987). Given the limited size of the seal populations in the Dutch Wadden Sea, and the frequency of their contacts with animals from neighbouring groups (Reijnders 1989), it may be speculated that the virus is excreted by the seals for longer periods. Support for this assumption came from the authors' observations after a vaccination-challenge experiment in 1988 (Visser and others 1989). Six harbour seals protected from fatal PDV-1 challenge by vaccination were reintroduced into a vaccinated group that had been kept in isolation and had remained seronegative until vaccination. The six pups born in this group about half a year later, all died of phocine distemper within two weeks after birth. This was probably due to persistence of the virus in this group which had remained free from signs during this period. In subsequent years all the pups in this group were vaccinated directly after birth, and no more cases of distemper have been observed in this group since.

Taken together, these data indicate that PDV-1 has continued to circulate among both harbour and grey seals in the Dutch Wadden Sea since its introduction in 1988. It is not clear why on the one hand PDV-1 still appears to circulate among the seals in the Dutch waters while on the other hand the virus has disappeared from certain populations in UK waters (Hughes and others 1992). However, during the recent oil spill accident near the Shetland islands, the authors noted that several grey seals admitted to a local sanctuary, including juvenile animals, did have morbillivirus specific serum antibodies indicating the continued presence of PDV-1 in this area. Although PDV-1, due to different population densities and behavioural patterns, may not have persisted in all populations in different areas, more extensive screening would be needed to obtain a clearer picture of the present epizootiology of PDV-1 infections in the respective seal populations. Furthermore, studies on the pathogenesis of this virus in its natural host species are needed before a mathematical model can be established that may indicate whether PDV-1 will continue to be present among the seal populations of northwest Europe.

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Seminal vesiculitis and epididymitis in an Anglo-Nubian buck

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THOUGH seminal vesiculitis is one of the most common inflammatory causes of infertility in male animals (Carroll and others 1963) with an incidence ranging from 0·85 to 9·0 per cent in bulls (Blom and Christensen 1965, Bagshaw and Ladds 1974), the condition is reportedly rare in rams and goats (Roberts 1986).

In this case, a two-year-old Anglo-Nubian buck, with a history of partial penile protrusion and infertility, was studied over a four-week period before he was killed and the reproductive tract examined. Four single semen samples were collected from the buck at weekly intervals using an artificial vagina warmed to 41° to 44°C (Sharma and others 1957) and assessed using conventional methods (Hafez 1987).

Ultrasound imaging was carried out by using a B-mode, real time portable scanner (Concept 2, Dynamic Imaging) fitted with a 7·5 MHz linear-array transducer and connected to a video graphic printer (UP-850, Sony). Both the testes and the epididymides were imaged in the transverse and longitudinal plane in the live animal and immediately after removal following slaughter, as previously described (Ahmad and others 1991). Each testis and epididymis was sectioned longitudinally and examined for gross lesions. Tissue samples (2 to 4 mm thick) from each testis, the head and the tail of the epididymis, and the seminal vesicles were preserved in 10 per cent buffered formol saline before processing and staining of tissue sections with haematoxylin and eosin.

Gross examination of the testes and epididymides revealed no abnormality. The buck showed normal libido when exposed to the teaser. However, upon mounting, the glans penis could not reach the vulva, although erection appeared to be normal. When the artificial vagina was placed over the protruded penis, normal ejaculation occurred with a good pelvic thrust. There appeared to be normal movement of the erect penis within the prepuce with no evidence of any lesions which might interfere with protrusion.

Although all ejaculates were of a normal creamy white colour, their consistency was gelatinous and stringy and, as a consequence, the sperm cell concentration could not be determined. The volume of the ejaculate ranged from 0·8 to 1·3 ml. All the spermatozoa were dead and, among sperm abnormalities, 70 per cent degenerated detached heads were seen.

Both testes were normal echogenically, the testicular parenchyma was moderately homogeneous and echogenic throughout, with one or two more echogenic areas present. The epididymal tail, which is normally more heterogeneous than the testis and also less echogenic, was moderately homogeneous and more echogenic than normal. The head of the epididymis was normal except for a small non-echogenic area with enhanced through transmission and specular reflection, present on the left side (Fig 1). The latter artifacts are commonly associated with fluid-filled cavities.

At necropsy, bilateral, fibrous testicular adhesions were seen between the parietal and visceral layers of the testicular tunic. The parenchyma of each testis showed white, granular foci presumably representing mineralisation, towards the mediastinum testis. A small cyst, containing clear watery fluid without any spermatozoa, was found close to the proximal end of the left epididymal head. The tail of the epididymis appeared whitish in colour with tiny tubules bulging from the cut surface rather like a cauliflower in appearance.

Histopathologically, there was bilateral, moderate-to-severe, diffuse seminal vesiculitis. The interstitial tissue was heavily infiltrated with plasma cells together with some neutrophils and occasionally macrophages. A few acini contained a suppurative (neutrophilic) exudate mixed with glandular secretion (Fig 2). Similarly, the tail of the epididymis showed bilateral, diffuse, chronic epididymitis with heavy infiltration of plasma cells beneath the epithelial lining. No histological abnormality was detected in the epididymal head. The large majority of the seminiferous tubules of each testis showed evidence of spermatogenesis with spermatocytes and spermatids present. Occasionally, tubules showing degenerative changes with vacuolation and sloughing of the epithelium into the lumen were found. Surprisingly, there was no evidence of tubular calcification which might have been expected from gross examination of the testicular parenchyma.

Seminal vesiculitis is the most common inflammatory cause of infertility in bulls and is usually accompanied by lesions in other genital organs including the epididymides, ampullae and prostate, when it is termed 'the seminal vesiculitis syndrome' (Ball and others 1964). However, in this buck additional lesions were found only in the epididymal tail. The semen quality in this buck was very poor with all the spermatozoa dead and 70 per cent having degenerated detached heads. Since histologically there were no significant testicular lesions, the high incidence of detached heads was probably associated with the epididymitis as observed in bulls (Carroll and others 1968). While the large number of dead spermatozoa may have been related to the seminal vesiculitis as has

FIG 1: Longitudinal sonograph of the left epididymal head (E) showing a non-echogenic area (large arrow) with enhanced through transmission (small arrows) and specular reflection (arrow head). A small part of the testis (S) is also visible

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