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EPIDEMIOLOGY, PATHOLOGY, AND GENETIC ANALYSIS OF A CANINE DISTEMPER EPIDEMIC IN NAMIBIA

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ABSTRACT: Severe population declines have resulted from the spillover of canine distemper virus (CDV) into susceptible wildlife, with both domestic and wild canids being involved in the maintenance and transmission of the virus. This study (March 2001 to October 2003) collated case data, serologic, pathologic, and molecular data to describe the spillover of CDV from domestic dogs (*Canis familiaris*) to black-backed jackals (*Canis mesomelas*) during an epidemic on the Namibian coast. Antibody prevalence in jackals peaked at 74.1%, and the clinical signs and histopathologic observations closely resembled those observed in domestic dog cases. Viral RNA was isolated from the brain of a domestic dog from the outbreak area. Sequence data from the phosphoprotein (P) gene and the hemagglutinin (H) genes were used for phylogenetic analyses. The P gene sequence from the domestic dog shared 98% identity with the sequence data available for other CDV isolates of African carnivores. For the H gene, the two sequences available from the outbreak that decimated the lion population in Tanzania in 1994 were the closest match with the Namibian sample, being 94% identical across 1,122 base pairs (bp). Phylogenetic analyses based on this region clustered the Namibian sample with the CDV that is within the morbilliviruses. This is the first description of an epidemic involving black-backed jackals in Namibia, demonstrating that this species has the capacity for rapid and large-scale dissemination of CDV. This work highlights the threat posed to endangered wildlife in Namibia by the spillover of CDV from domestic dog populations. Very few sequence data are currently available for CDV isolates from African carnivores, and this work provides the first sequence data from a Namibian CDV isolate.

Key words: Black-backed jackal, canine distemper virus, *Canis mesomelas*, domestic dog, epidemic, hemagglutinin gene, phosphoprotein gene, sequence analysis.

INTRODUCTION

Canine distemper virus (CDV) is a member of the morbillivirus genus (*Paramyxoviridae*) and has a negative-strand nonsegmented RNA genome. CDV is antigenically and genetically closely linked to the other members of the morbillivirus genus, which includes measles virus, Rinderpest virus which causes cattle plague, Peste des petits ruminants virus (affecting small ruminants), porpoise and dolphin morbilliviruses, and Phocine distemper virus (PDV), a virus of seals that is very closely related to CDV. Canine distemper virus is highly contagious and has been responsible for severe population declines in both terrestrial (Murray et al., 1999) and aquatic wildlife (Kennedy et al., 2000). Canine distemper continues to be a major disease of domestic dogs (*Canis familiaris*), re-occurring despite vaccination efforts (Pardo et al., 2005; Norris et al., 2006).

An emerging disease in a wide range of wildlife species (Harder and Osterhaus, 1997), canine distemper spilled over from domestic dog populations to cause mass mortalities in lion (*Panthero leo*; Roelke-Parker et al., 1996) and pinniped populations (Kennedy et al., 2000). Canine distemper virus has also caused significant mortality amongst the endangered African wild dogs (*Lycaon pictus;* Alexander et al., 1994). Although CDV is thought to be maintained in domestic dog populations worldwide, the virus has a very broad, and apparently expanding, host range (Harder and Osterhaus, 1997) and may be maintained and transmitted by mixed-species

populations. For example, in the USA, CDV may be maintained in wild species (e.g., raccoon, *Procyon lotor*) from which spillover may have occurred to zoo animals such as lions, leopards (*Panthera pardus*), and tigers (*Panthera tigris*; Appel et al., 1994), as well as to the highly endangered black-footed ferret (*Mustela nigripes*; Williams et al., 1988).

The biology and ecology of many wild canids make them particularly suitable hosts for the transmission and maintenance of generalist canid pathogens such as CDV (Funk et al., 2001). The black-backed jackal (*Canis mesomelas*) is a social canid and an opportunistic predator capable of dispersing over large distances, characteristics which make it particularly suited to the dissemination of rabies (McKenzie, 1993; Bingham et al., 1999) and CDV.

The involvement of jackals (*Canis* spp.) in the transmission of CDV has been inferred from serologic surveys and population declines (Alexander and Appel, 1994; Alexander et al., 1994), but this information alone is not sufficient to discern the jackals' role in the epidemiology of CDV. Serologic data must be supplemented by age and mortality data, and clinical, pathologic, and virus characterization data, the latter providing conclusive evidence of natural infection and the identity of the virus involved (Haydon et al., 2002).

The analysis of genetic data also provides a powerful tool for understanding the transmission dynamics of a pathogen and for identifying its transmission routes. For example, molecular analyses of the CDV strain responsible for the Serengeti outbreak in 1994 demonstrated genetic clustering within geographic areas rather than within host species. This suggested that a single virus strain was causing mortality in a range of species and that the virus was freely transmissible between domestic dogs and the wild carnivore populations (Cleaveland et al., 2000). With the exception of the Serengeti isolates, very few CDV isolates from African carnivores have been characterized, and the role of wild carnivores in the epidemiology of CDV is still very poorly understood.

Canine distemper virus is well established in the Namibian domestic dog population, but cases have also been reported (although not confirmed) in black-backed jackals and aardwolfs (*Proteles cristatus*) (Schneider, 1994). Exposure has been documented in African wild dogs (*Lycaon pictus*) (Laurenson et al., 1997) and cheetahs (*Acinonyx jubatus*) (Munson et al., 2004) in Namibia. However, relatively little was known about the dynamics of infection in the Namibian wildlife, and no Namibian CDV sequence data were available until 2002–2003, when a CDV outbreak on the Namibian coast provided the opportunity to document, for the first time, infection patterns in wild and domestic canids (Gowtage-Sequeira, 2005) and to subsequently characterize the virus circulating in the area after the epidemic. This study aimed to describe the epidemic by compiling the serologic, pathologic, virus sequence, and case data: 1) to confirm that CDV was the cause of the observed morbidity and mortality; 2) to investigate the role played by the black-backed jackal in the maintenance and transmission of CDV on the Namibian coast; and 3) to determine, using sequence analyses of the phosphoprotein (P) and hemagglutinin (H) genes, whether the Namibian CDV isolate was genetically similar to other CDV strains responsible for mass mortalities in carnivores and pinnipeds.

MATERIALS AND METHODS

Study areas

The Namibian coastline is defined by the hyper-arid ecosystem of the Namib Desert (Barnard, 1998). This desert is dominated by sand dunes, saltpans, large lichen fields, and hummock vegetation and stretches the length of the Namibian coast and for between 80 to 200 km inland. In terms of large mammals, this is a relatively simple ecosystem that supports a small number of carnivore species. The major coastal towns of Swakopmund (22°40′S, 14°31′E), Walvis Bay (22°57′S,

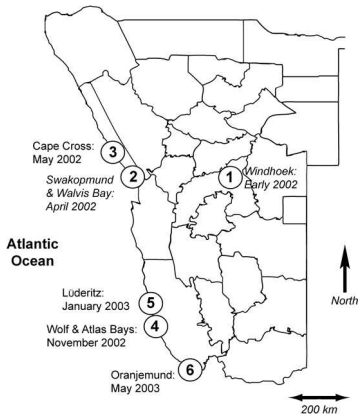

FIGURE 1. A map of Namibia's magisterial districts showing the sequence of the CDV epidemic. (1) A large epidemic in domestic dogs was reported in the capital city, Windhoek, in early 2002. (2) Clinical cases were reported in the domestic dog population of Swakopmund, 260 km west of Windhoek, in April 2002. (3) The first jackal case at the Cape Cross Seal Reserve (a 51-km^2 reserve 117 km north of Swakopmund and 331 km west of Windhoek) likely occurred in early May 2002. (4) Jackals trapped at Wolf Bay and Atlas Bay (19 km and 17 km from Lüderitz, respectively) in November 2002 exhibited clinical signs of CDV infection and were seropositive; Lüderitz is 446 km south of Swakopmund and 292 km southwest of Windhoek. (5) Peak of the outbreak amongst domestic dogs in Lüderitz in January 2003. (6) CDV outbreak reported amongst domestic dogs in Oranjemund, 245 km south of Lüderitz, May 2003.

14°30′E), and Lüderitz (26°39′S, 15°10′E) harbor a domestic dog population estimated at approximately 13,000 individuals (Gowtage-Sequeira, 2005). Numerous Cape fur seal (*Arctocephalus pusillus pusillus*) colonies are scattered between the coastal towns, the largest haul-out sites being the Cape Cross Seal Reserve colony (between 21°45′S, 13°58′E and 21°51′S, 14°03′E) and those at Wolf Bay and Atlas Bay (26°49′S, 15°07′E and 26°50′S, 15°08′E, respectively; Fig. 1). The Namibian government's Ministry of Fisheries and Marine Resources, utilizing aerial surveys and tag-recapture techniques, estimated the 2001 population of these colonies at 187,000 and 167,000 individuals (adults and pups), respectively, with the total seal population of Namibia estimated at over one million individuals (Pallett, 2000; Mukapuli, 2004). Black-backed jackals and small numbers of brown hyenas (*Hyaena brunnea*) concentrate at the seal colonies, feeding almost exclusively on seal (Hiscocks and Perrin, 1987).

Sample collection

The spread of the CDV epidemic was determined from the temporal and spatial pattern of suspected and confirmed cases and from the antibody prevalence patterns in both jackals (n=90) and dogs (n=92) that were opportunistically sampled between March 2001 and October 2003. Suspected cases were defined as those which died during the epidemic period (early 2002 to May 2003) and displayed one or more of the following clinical signs: ataxia, myoclonus, seizures, ocular-nasal discharge, dyspnea (by observation), and respiratory signs consistent with pneumonia (detected on auscultation). Confirmed cases were those that tested positive by either histopathology or immunohistochemistry. Case reports were obtained from discussions with informants including local research scientists, veterinarians, and government officials. Tissue samples from jackals (n=10) and domestic dogs (n=4) were obtained from animals in extremis which were euthanized in accordance with the requirements of the Namibian Ministry of Environment and Tourism.

Jackal capture and sampling

Serum samples were obtained from a total of 90 live-trapped anesthetized jackals and euthanized jackals sampled opportunistically between 2001 and 2003. Jackals were trapped at the Cape Cross and the Wolf Bay and Atlas Bay seal colonies using cage traps and padded steel foothold traps (No. 4 Victor Softcatch, Woodstream Corp., Lititz, Pennsylvania, USA). Individuals were anaesthetized with either tiletamine-zolazepam (10mg/kg IM, Zoletil 100, Virbac RSA (Pty) Ltd., Centurion, South Africa) or with a combination of two or more of ketamine (3–5 mg/kg, Anaket-V, Bayer (Pty) Ltd., South Africa), medetomidine (0.03–0.05 mg/kg, Domitor, Novartis (Pty) Ltd., South Africa), diazepam (0.2–0.5 mg/kg, Tranject, SCP Pharmaceuticals (Pty) Ltd., South Africa) and tiletamine-zolazepam (2–4 mg/kg). Blood was sampled from the cephalic vein and centrifuged. Sera were stored at −20 C. Individuals were aged and assigned to one of three age classes using tooth wear and body weight criteria: juvenile (<12 mo), sub-adult (12–35 mo), and adult (≥36 mo; Lombaard, 1971; Ferguson et al., 1983).

Domestic dog sampling

Serum samples were obtained from a total of 92 live and euthanized, nonvaccinated domestic dogs sourced from veterinary clinics, local Town Council roundups, and house visits

in the coastal towns of Swakopmund, Walvis Bay, and Lüderitz. Live animals were restrained and blood was sampled from the cephalic or jugular veins; euthanized animals were sampled from the heart.

Histopathology

Formalin-fixed tissue samples were routinely processed and embedded in paraffin wax. Sections were stained by hematoxylin and eosin for histopathologic examination at the Central Veterinary Laboratory (Windhoek, Namibia) and the PathCare Laboratories (Cape Town, South Africa).

Immunohistochemistry

Ethanol-fixed tissues from one jackal and one domestic dog were stained for CDV antigen at Intervet (Huntingdon, UK) in a peroxidase–anti-peroxidase reaction based on protocols described elsewhere (Catelli et al., 1998). Briefly, the tissues were dewaxed using xylene and industrial methylated spirit (IMS). Any existing peroxidase was blocked with H_2O_2 in methylated spirit and, after washing in TRIS buffered saline (TBS), the tissues were incubated with normal goat serum (blocking agent 1:10 dilution). Excess goat serum was removed and rabbit anti-CDV polyclonal antibody was added and incubated. After addition of the peroxidase-conjugated goat anti-rabbit serum, the tissues were washed in TBS and acetate-citrate buffer and placed in a nickel-enhanced diaminobenzine substrate. The reaction was stopped by washing in TBS and the tissues were counterstained with Light Green stain, dehydrated in IMS, cleared in xylene, and then mounted. Urinary bladder and small intestine tissues from a known-positive domestic dog and a known-negative dog were included as controls. Sequential sections of the jackal and domestic dog tissues, incubated with the normal rabbit serum, were used as test controls.

Virus neutralization tests

Jackal and domestic dog sera were tested for virus neutralizing antibodies to CDV as described elsewhere (Chalmers and Baxendale, 1994). Briefly, antibody titers were determined by making 4-fold serial dilutions of the sera starting at 1:8 and incubating these with an equal volume of CDV suspension (Bussel strain) at a concentration of between 32 and 316 $TCID_{50}$. After 1-hr incubation at 37 C, the virus-serum mixture was added to freshly seeded VERO cell cultures in 96-well plates. The plates were incubated for 3–5 days and the cell monolayer checked for virus-specific cytopathic effect by microscopy. The titer of neutralizing antibodies was taken to be the last dilution to inhibit a 50% cytopathic effect, and the test cut-off point ($\geq$1:32) was determined from the frequency distributions of the titers ($\log_{10}$).

CDV sequencing and analysis

Five samples of cerebrum and cerebellum brain tissue, preserved in RNAlater (Qiagen, Crawley, West Sussex, UK), were obtained from an adult male domestic dog suffering from myoclonus and chewing fits. The animal was euthanized at a veterinary clinic in Swakopmund in 2005. Tissues were macerated using the Fastprep-24 (QBiogene, Cambridge, Cambridgeshire, UK) machine, and total cellular RNA was extracted from the macerated tissue using the Trizol® technique (Invitrogen, Carlsbad, California, USA). Reverse transcription (RT) reactions using SuperScriptII RT (Invitrogen) with random hexanucleotide primers were performed and used in PCR with KOD Hot Start polymerase (Novagen, Merk Chemicals Ltd., Nottingham, Nottinghamshire, UK) as detailed below. Amplification of a segment of the P gene was initially carried out using universal morbillivirus primers P1 (5′-ATGTTTATGATCACAGCGGT-3′) and P2 (5′-ATTGGGTTGCACCACTTGTC-3′; Forsyth and Barrett, 1995). No product was detectable from the first-round PCR, but subsequent hemi-nested PCR reactions using P1 (as above) and P3 (5′-GTTCGCATGCTTGGTTCCCTATAC-3′) generated positive reaction products of the correct size (320 bp), and the resulting products were sequenced in their entirety. Amplification of a section of the H gene was carried out using the same RT protocol with random hexanucleotide primers and a pair of CDV specific primers (CDVF1 5′-TTAGGGCTCAGGTAGTCCAACA-3′ to CDV R1 5′-GACAAGGCCGACTCCAGACAA-3′) that prime genome positions 7057–7078 and 8206–8225, respectively. Amplification with these primers yielded a product of 1,122 bp that was then sequenced in its entirety. Phylogenetic analyses were conducted in MEGA4 (Tamura et al., 2007).

Statistical analyses

Logistic regression models (SPLUS 2000, MathSoft Inc., Cambridge, Massachusetts, USA) were used to investigate the effects of age on seropositivity and mortality in jackals; sampling year, location, age, and sex were the factors included in the analyses. For each analysis, a generalized linear model (GLM; Crawley 1993) was fitted to the data using a

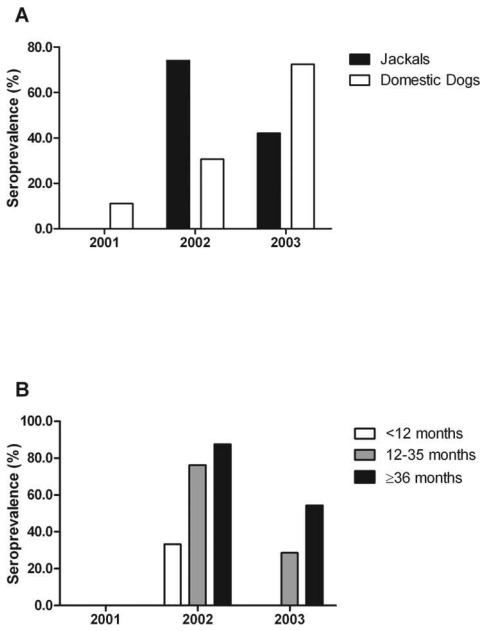

FIGURE 2. (A) Antibody prevalence for CDV in jackals (n=90) and domestic dogs (n=92) by year, 2002–2003. (B) The age-antibody prevalence trends for CDV in jackals (n=89), 2002–2003. None of the animals trapped in 2001 were seropositive, and no juvenile jackals were trapped in 2003. For jackals, significantly more deaths occurred in the younger age classes (χ^2_2=7.65, P=0.022), which is supported by the observation that no juveniles were trapped at Cape Cross after the epidemic in 2003. There was also a significant (χ^2_2=9.21, P=0.010) increase in antibody prevalence with age.

function with binomial errors, starting with the maximum model including all the terms. In a step-wise deletion, any nonsignificant variables were removed from the model to produce a minimum adequate model. The variables in the minimum model were checked for confounding effects by step-wise removal from the model. Interactions were only tested in models where the main effects were significant, starting with the interactions for all the significant effects and removing the least significant in a step-wise deletion, as was done for the main effects.

RESULTS

Prior to the epidemic, over the course of 5 mo in 2001, only seven jackal carcasses were reported or recovered from the study's seal colonies. There were no suspected cases of CDV reported in either jackals or domestic dogs, and the antibody prevalence (Fig. 2a) was 0% in jackals (0/17 tested) and a low 11% (3/27) in domestic dogs; the three seropositive domestic dogs originated from Walvis Bay, just 31 km south of Swakopmund.

In early 2002, numerous distemper cases were reported in the capital, Windhoek, where over 100 dogs were euthanized (Fig. 1, point 1). By April of the same year, veterinarians in the coastal town of Swakopmund reported an outbreak of distemper with 50 or more dogs treated, over 100 euthanized, and an estimated additional 200 unconfirmed cases (Fig. 1, point 2). In late April, the first jackal with clinical signs consistent with canine distemper infection was reported in Walvis Bay (Fig. 1, point 2), and in early May another symptomatic jackal was sighted at Cape Cross, 140 km north (Fig. 1, point 3). In June, five jackal carcasses were found at Cape Cross, and affected individuals with clinical signs such as myoclonus were observed around the seal colony. More than half of the jackals (67%, 12/18) trapped between July and August, the peak period of the epidemic, had clinical signs consistent with canine distemper infection, and seroprevalence had risen to 27% (4/15). Over the same period, 15 jackal carcasses were found at Cape Cross, but only two additional domestic dog cases were reported from Walvis Bay and Swakopmund.

The CDV outbreak at Cape Cross persisted into October 2002, when four jackal carcasses were recorded in the area, and 42% (8/19) of jackals trapped that month showed one or more clinical signs of infection and an antibody prevalence of 95% (19/20); in the nearby town of Swakopmund, 56% (9/16) of domestic dogs were seropositive in November. Further south, none of the domestic dogs sampled in Lüderitz between October and November 2002 were seropositive (0/18), although one animal had myoclonus of the hind limbs. A suspected domestic dog case was reported from the Lüderitz town kennels in late November. However,

during the same month, jackals trapped at Wolf Bay and Atlas Bays (Fig. 1, point 4; 17 km south of Lüderitz) showed clinical signs of distemper infection (30%, 3/10), and 90% were seropositive (9/10); three jackal carcasses were recovered from this area in the same month.

The outbreak in Lüderitz peaked in January 2003 (Fig. 1, point 5), when 126 carcasses were collected by the Town Council and an additional 100 cases were reported. The antibody prevalence amongst domestic dogs sampled from Lüderitz in January 2003 was 72% (21/29), with 91% (20/22) of dogs examined exhibiting clinical signs consistent with distemper infection.

The last reported domestic dog case occurred in Lüderitz in April 2003, but another domestic dog outbreak was reported in May in the coastal town of Oranjemund (Fig. 1, point 6), 245 km south of Lüderitz; this is thought to have been where the epidemic ended. By October 2003, no juvenile jackals (Fig. 2b), or individuals with clinical signs, were trapped at Cape Cross, but 42% (8/19) of trapped jackals were antibody positive.

Tissue analyses

Histopathologic examination of formalin-fixed tissues confirmed infection in both jackals (3/10; positive individuals from Cape Cross in July, August, and October 2002) and domestic dogs from Lüderitz (3/4; two positive individuals sampled in December 2002 and one in January 2003). All the jackals positive by histopathologic examination showed clinical signs of infection, but only one of these was seropositive. Serum was only available from one of the three histopathology positive domestic dogs, and this individual was also seronegative, as expected from an active infection. Histopathologic observations from the jackals included mucopurulent interstitial pneumonia with intracytoplasmic inclusion bodies, lymphoid depletion, and meningoencephalitis. Both the jackal and the domestic dog submitted for testing had CDV antigen present in the liver, lung, and mesenteric lymph node tissues. In the jackal, the cerebrum was also positive and, in the domestic dog, the small intestine and spleen were positive.

Sequence analyses

Data were generated for a region of the P gene by using a set of universal morbillivirus P gene primers (Forsyth and Barrett, 1995). The region of the phosphoprotein generated and sequenced covered positions 410–705 within the P open reading frame (CDV genome position 2153–2448). This short stretch of 295 nucleotides was compared to a selection of published canine sequences, including those from various international domestic and wild dog isolates and those from wild African carnivores, including hyenas (*Crocuta crocuta*; Haas et al., 1996) and lions infected with CDV in the 1990s (Roelke-Parker et al., 1996). Very little sequence data exists from cases of CDV in wildlife across Africa, but alignment of the Namibian domestic dog sequence reported here, with partial sequence data from a hyena isolate (position 378–649 within the P open reading frame, 271 nucleotides; Dr. T. Barrett, unpubl. data), showed 97% identity at the nucleotide level with two amino acid differences over the 90 amino acids encoded in this region (98% identity). Comparisons made with sequence data generated from lion isolates (GenBank CDU53711) showed a similar level of homology across this region. The P gene (open reading frame position 410–705, 295 nucleotides) of the domestic dog sequence presented here shared 98% identity with the lion data (Roelke-Parker et al., 1996). Phylogenetic analysis of the Namibian P gene data, with isolates from both domestic and wild dogs as well as other wild carnivores, is shown in Fig. 3. Each of the sequences in the analysis clustered as expected, with respect to geographic location. The Namibian domestic dog sequence generated here is most closely related to the sequence generated from the African lions. The low bootstrap support may be a

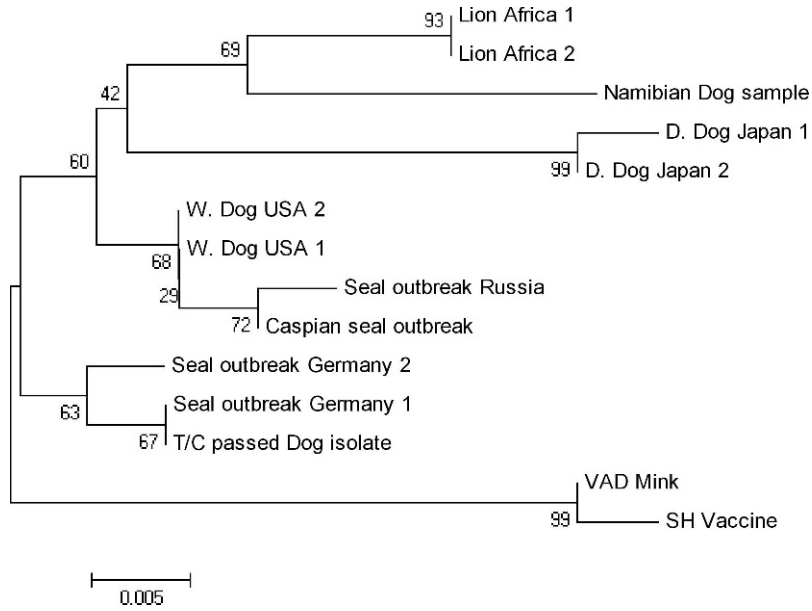

FIGURE 3. Phylogenetic analysis of the 255-nucleotide P gene sequence. The phylogenetic tree was generated using the neighbor-joining method. The bootstrap consensus tree is inferred from 10,000 replicates. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those used to infer the phylogenetic tree, computed using the Kimura 2-parameter method. There were a total of 255 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4. Key to phylogenetic tree: Lion Africa 1=CDU53711; Lion Africa 2=CDU76708; Namibian domestic dog=NJ002276; Domestic (D) dog Japan 1=AB252714; D. dog Japan 2=AB252715; Wild (W) dog USA 1=AY964107; W. dog USA 2=AY964111; Seal outbreak Russia=DQ234801; Caspian seal outbreak=DQ241386; Seal outbreak Germany 1=AJ582384; Seal outbreak Germany 2=AJ582385; T/C (Tissue culture) passed dog isolate=CDV5804p (AY386316); VAD (Vaccine Associated Disease) in mink=AY130856; SH (Synder Hill) vaccine=AY286481.

consequence of errors present in the available sequence.

The fragment of the H gene, a main determinant of intraspecific transmission for morbilliviruses amplified in this study, was 1,122 bp in length. An equivalent analysis as that carried out with the P sequence data was also carried out with the H data although, unfortunately, sequence data available for the H gene of CDV from carnivores, in particular the African lion outbreak, was only 255 nucleotides in length (amino acids 45 to 99 of the H open reading frame), and so the genetic analyses that could be performed were limited. Across the 255 bp, the Namibian domestic dog sequence showed 94% identity with the two sequences available from the outbreak in lions in 1994 (Roelke-Parker et al., 1996) and varying degrees of identity to the other strains aligned; 93% with the Onderstepoort vaccine strain (AF305419), 96% with a virulent domestic dog isolate (5804p) from Germany (AY386316), and 94% identity with CDV isolated from the brain of an infected raccoon (AY649446). Of the nine proposed N-linked glycosylation sites previously reported for the CDV H gene, this sequence includes one of these sites, position 19–21 (NSS), which is conserved within both the lion and Namibian dog isolate reported here (Fig. 4). The sequence generated also covered the major N-terminal hydrophobic region of the H protein, a highly conserved region in all morbilliviruses (amino acids 35–58). The remaining 868 nucleotides of the H gene of the Namibian isolate showed both similarities and differences with other CDV isolates, being 95% identical to the virulent

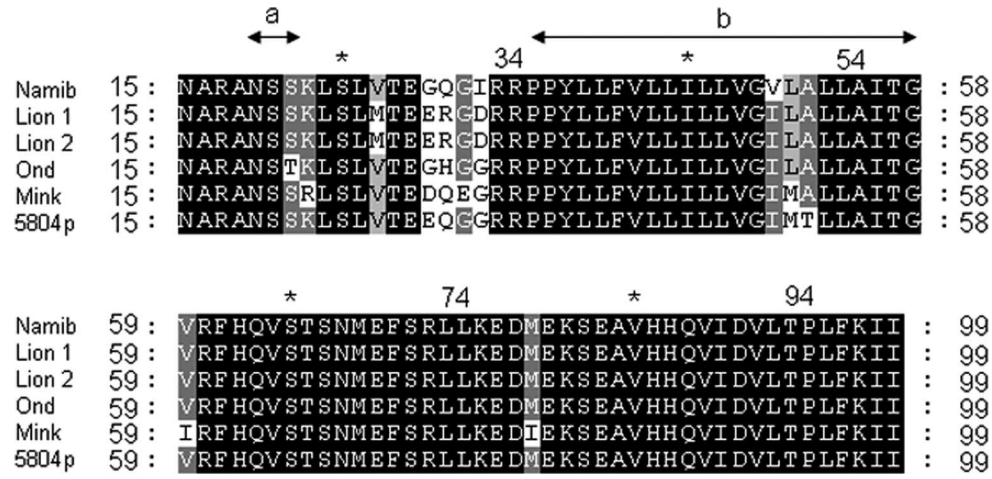

FIGURE 4. Amino acid sequence alignment of the H protein fragment from the Namibian dog virus with other CDV isolates. The sequence encompassed the open reading frame at positions 45 to 297 (84 amino acids). A potential N-linked glycosylation site (a) and the major hydrophobic transmembrane region (b) are shown. Accession numbers for sequences analyzed: Namib=NJ002277; Lion 1=CDU53716; Lion 2=CDU53717; Ond=CDV Onderstepoort vaccine strain AF378705; Mink=CDV isolate from mink, Z47759; 5804p=CDV isolated from a dog in Germany, AY386316.

5804p isolate of CDV from a German domestic dog (AY386316), 93% identical to the raccoon isolate (AY649446), and 91% similarity to the Onderstepoort vaccine strain (AF305419); similar levels of identity were observed at the amino acid level with 95, 94, and 92% sequence identity, respectively. This region contained five of the 12 conserved cysteine residues, conserved within the H open reading frame, that are known to be essential for correct folding of the mature protein. The N-linked glycosylation sites present at positions 149–151 and 309–311 were also conserved within the domestic dog sequence (Haas et al., 1996). The 1,122-bp sequence of the H gene was compared to data available from CDV and PDV strains that were responsible for seal outbreaks in other countries. The different morbilliviruses clustered as expected, with CDV and PDV forming a distinct cluster, and the Namibian dog sequence clustered most closely with CDV.

DISCUSSION

The hypothesis that CDV was responsible for the observed jackal and domestic dog morbidity and mortality on the Namibian coast between 2001 and 2003 is supported by the clinical, histopathologic, and serologic findings as well as by the definitive diagnosis obtained by immunohistochemistry. This study provides the first description of a CDV outbreak in *C. mesomelas* and the first basic description of the clinical and histopathologic findings in this species, which are very similar to those described for domestic dogs (Appel, 1987; Greene and Appel, 1998).

The pattern of increasing antibody prevalence with age in the jackal population (Fig. 2b) could be explained by: 1) a constant force of infection in an endemic area, 2) differential rates of exposure in a population experiencing sporadic outbreaks, or 3) an increase in disease resistance with age. The variation in antibody prevalence between years (Fig. 2a), and the high mortality, suggested that this was an epidemic occurrence. There was no evidence to suggest that sporadic and repeated outbreaks of CDV occur on the Namibian coast and, therefore, the increase in antibody prevalence with age may be due to either age-specific

mortality or differential exposure during the epidemic; the contact patterns of juveniles and subadults may differ from those of adults and, once they are infected, they may be more likely than adults to succumb to the disease.

The magnitude and rapid spread of the epidemic in jackals may be, in part, explained by the evident naivety of the population and the fact that the epidemic coincided with the breeding season in 2002, when increased intraspecies contacts may have augmented transmission rates. Jackals in this ecosystem facilitated the rapid and widespread dissemination of CDV well beyond the urban ranges of domestic dogs and were the likely source of CDV for domestic dogs in Lüderitz. However, it is thought that the coastal jackal population may not maintain CDV because persistence is unlikely in a population of this size, given: 1) the rapid spread of the virus through the population, 2) the likelihood that surviving jackals would show long-lived immunity, and 3) the low rate of generation of new susceptibles, given the low population turnover (jackals only breed once a year and suffer high pup mortality).

Canine distemper virus can also cause mortality in hyenas (Haas et al., 1996), and one of two brown hyenas tested was seropositive, but the effect of the epidemic on this population is unknown (Gowtage-Sequeira, 2005). In Namibia and elsewhere in sub-Saharan Africa, where endangered carnivores (e.g., *Lycaon pictus*, *Canis simensis*) are sympatric with *C. mesomelas*, the jackal may act as an important liaison host, linking domestic dog reservoirs with threatened canid species.

Although it is highly likely that domestic dogs played a role in the maintenance of CDV in the Namibian coastal ecosystem, it was not possible to determine from the data collected whether domestic dogs were solely responsible or whether they were part of a maintenance community (Haydon et al., 2002). However, with both domestic dogs and jackals being found positive for virus antigen, there was a risk of spillover into the seal population, especially because jackals are known to feed freely amongst seals at the mass haul-out sites. This would likely provide ideal conditions for the transmission of the virus across the species barrier. Previous outbreaks in seal populations have been attributed to transmission between infected terrestrial animals and seals at haul-out sites (Forsyth et al., 1998; Kennedy et al., 2000). The CDV outbreak amongst the seals of Lake Baikal was attributed to the spillover of CDV from domestic dog populations surrounding the lake (Grachev et al., 1989; Visser et al., 1990). However, spillover from terrestrial carnivores to sympatric pinniped populations does not always occur. The CDV outbreak amongst domestic dogs of the Galapagos Islands in 2001 did not spill over to the Galapagos sea lions (*Zalophus califonianus wollebacki*), as serologic testing showed no evidence of exposure in this species (Levy et al., 2008).

The lack of CDV-related mortality during this outbreak on the Namibian coast raises questions regarding the host-specificity of the particular CDV strain and the susceptibility of the Cape fur seals. The H protein, which sits in the lipoprotein envelope of CDV (Greene and Appel, 1998), mediates the attachment of the virus to the host cells and is a determinant of host range and pathogenicity (Carpenter et al., 1998). The H protein enables the virus to bind to cellular receptor molecules and, as such, shows a high degree of sequence variation. It has been well documented that small amino acid changes within the H gene can alter the ability of morbilliviruses to enter different cell types. For measles virus, a single amino acid substitution within the H gene is able to confer entry into different cell types in vitro (Hsu et al., 1998; Seki et al., 2006). The signaling lymphocyte activation molecule has long been proposed as a morbillivirus receptor (Tatsuo et al., 2001), and although exact

virus:receptor interactions are unknown, it is now generally accepted that multiple receptors are utilized by these viruses (Fujita et al., 2007) and that this must have a significant effect on the potential for species-to-species transmission (von Messling et al., 2001). For CDV, it has been demonstrated that molecular adaptation at specific receptor-binding sites of the H gene is associated with the spread of this virus from dogs to novel noncanid hosts in the wild (McCarthy et al., 2007). It is possible that the Cape fur seals lack the necessary receptors to enable infection with the Namibian CDV strain, as it is known that different seal species vary in their susceptibility to PDV (Hall, 1995). Unfortunately, our H gene sequence data did not encompass the region that is associated with receptor binding for other morbilliviruses; therefore, conclusions regarding potential nucleotide and amino acid changes reflecting possible species-to-species transmission are not possible. It is possible that the CDV strain isolated from the domestic dog in 2005 was the same strain which affected the coastal region in 2002–2003 and that this strain was simply not able, either through genetic incompatibility or environmental factors, to infect Cape fur seals.

The effects of the CDV epidemic on the Namibian coast may have been exacerbated by environmental or other cofactors that were not investigated in this study. The presence of cofactors, the effects of which could be magnified by the immunosuppressive effects of CDV rather than by changes in the virus itself, may be critical in determining the levels of morbidity and mortality caused by CDV. For example, it has been demonstrated that elevated levels of the blood parasite, *Babesia*, were correlated with the unusually high mortality caused by CDV amongst the lions of the Serengeti (Munson et al., 2008). In this case, the periods of drought preceding each outbreak led to high tick burdens on the herbivores, predated by the lions, which in turn resulted in an increased prevalence of *Babesia* infections in these predators; these infections were exacerbated by the immunosuppressive effects of CDV.

It is likely that the timing of the CDV outbreak, which occurred during a period of low food availability, did contribute to the observed morbidity and mortality. The nutritional status of domestic dogs is thought to play a role in the outcome of infection (Appel, 1970), and recent studies with CDV in ferrets have suggested that vitamin A status may modulate the clinical presentation of infection (Rodeheffer et al., 2007). Jackals on the Namibian coast are highly dependent on the seals as a food source (Hiscocks and Perrin, 1987) and are restricted to killing young seal pups, before they grow too large, or to scavenging seals. Therefore, the jackals are subjected to a period of low food availability during the months immediately preceding the seal birthing season in November and December. Individuals that lose body condition and, therefore, a possible reduction in immune competence during this period, may have been more likely to succumb to the disease.

This study provides the first data on a CDV strain circulating in Namibia and also demonstrated its close phylogenetic relationship to CDV strains from other outbreaks. Unfortunately, outbreaks of CDV are generally very poorly characterized, and only rarely are genetic data generated from such events. Even where data exist, the genetic sequences generated are often limited, making phylogenetic analyses difficult, and making the task of determining possible mutations that may be important in species to species transmission almost impossible. Over the last 15 yr, improved diagnostic techniques have shown that the host range of CDV has dramatically increased (Harder and Osterhaus, 1997). Retrospective studies using serologic samples (Bengston et al., 1991) and postmortem tissues (Myers et al., 1997) have implicated CDV as the cause of mortalities across several different species. The se-

quence data from this study may assist future research in the identification of CDV strains and, therefore, aid in the protection of valuable wildlife resources, as it is possible that nucleotide mutations may be identified that could explain the transmission status of a virus within, and between, populations.

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