DIPHTHERITIC STOMATITIS IN YELLOW-EYED PENGUINS
(MEGADYPTES ANTIPODES) IN NEW ZEALAND

Maurice R. Alley,1,5 Rod B. Suepaul,2 Bruce McKinlay,3 Melanie J. Young,3 Jianning Wang,4
Kerri J. Morgan,1 Stuart A. Hunter,1 and Brett D. Gartrell1

1 Wildbase Research, Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11222,
Palmerston North 4442, New Zealand
2 School of Veterinary Medicine, Faculty of Medical Sciences, The University of the West Indies, St. Augustine,
Trinidad and Tobago
3 Coastal Otago Area Office, Department of Conservation, PO Box 5244, Moray Place, Dunedin 9058, New Zealand
4 Australian Animal Health Laboratory, 5 Portarlington Road, Geelong, Victoria 3219, Australia
5 Corresponding author (email: M.R.Alley@massey.ac.nz)

ABSTRACT: Diphtheritic stomatitis is a seasonal disease that has been recognized as a syndrome in
Yellow-eyed Penguin (Megadyptes antipodes) chicks in New Zealand for >10 yr. It was present in about
50% of 234 chicks examined since 2002 and is characterized by a thick serocellular exudate in the oral
cavity of 1–4-wk-old chicks. The syndrome includes inanition, weight loss, and death in many affected
birds. Microscopically, the lesions varied in severity. Most affected chicks had severe, locally extensive,
ulcerative stomatitis with large amounts of exudate containing numerous bacteria; a smaller number
had mild focal lesions with smaller amounts of exudate and bacteria. Although Corynebacterium
amycolatum has been consistently isolated from the oral lesions, it was also present in the oral cavity
of 34% of normal adult penguins and their chicks and is not known to possess diphtheritic toxins. A
primary viral pathogen was therefore suspected, and intracytoplasmic inclusion bodies were
occasionally seen in oral mucosal epithelial cells. No herpesvirus DNA was detected with PCR.
Avipoxvirus DNA and an unidentified virus-like agent were detected in some early oral lesions,
but could not be confirmed in subsequent testing. Electron microscopy on early affected epithelium with
intracytoplasmic inclusion bodies was unrewarding. Our findings raise the possibility that the disease is
caused by an unknown primary virus infection followed by secondary Corynebacterium invasion, but
this requires confirmation. The means of transmission has not been established but insect vectors are
suspected.

Key words: Corynebacterium, oral cavity, poxvirus, ulceration, Yellow-eyed Penguin chicks.

INTRODUCTION

The Yellow-eyed Penguin or hoiho (Megadyptes antipodes) is only found in New Zealand territories. It is one of the world’s
rarest penguins, and the only extant member of the genus Megadyptes (Seddon et al. 2013). It is currently listed as endangered on the
IUCN Red List and ranked as Category B for conservation priority because of its restricted geographic range and the continuing decline
in numbers of mature adults. The birds nest a short distance inland on lower parts of the South Island, on southern off-shore islands,
and on subantarctic islands, with nesting starting in September and fledging in February–March. Breeding success is variable,
however, and serious population declines (Heather and Robertson 2005) have continued. The main threats to these penguins are
destruction of breeding habitat and predation of adults and chicks by dogs (Canis lupus familiaris), cats (Felis catus), and mustelids
(McKinlay 2001). There is also evidence that the capture of foraging adults in gill nets may be a substantial problem particularly in North
Otago and around the Otago Peninsula (Darby and Dawson 2000). However, in addition to these anthropogenic factors, sporadic mass mortality events such as that affecting the Otago population in the summer of 1990 (Gill and Darby 1993) have caused significant losses from which the population
has taken several years to recover.

In the breeding season of 2002–03, a new disease syndrome called diphtheritic stomatitis was identified in chicks from the Otago
Peninsula (Alley et al. 2004). The disease was characterized by weight loss and exudative or
erosive lesions in the oral cavity. Although no cases were seen in the following season, high mortality from this syndrome occurred in the 2004–05 breeding season in chicks from the Otago, eastern Southland, and Stewart Island coastlines, and these mortalities have continued intermittently. There have been few previous reports of oral or pharyngeal lesions in penguins in captivity or in the wild. In a North American zoo, Jackass Penguins (Spheniscus demersus) developed lesions resembling those seen with infectious laryngotracheitis in poultry and other birds. Polyhedral viral particles measuring 80–140 nm, a size consistent with Herpetoviridae, were found in the lesions with the use of electron microscopy (Kincaid et al. 1988). In Argentina, an avipoxvirus (Avipoxviridae) caused sporadic wart-like lesions around the beak, flippers, cloaca, feet, and eyes of Magellanic Penguin (Spheniscus magellanicus) chicks (Kane et al. 2012). This avipoxvirus was also the suspected cause of mortality in Gentoo Penguins (Pygoscelis papua) in West Falkland in 2006 (Munro 2007). However, in South Africa, no disease has been reported in wild Jackass Penguins with poxvirus infection (Stannard et al. 1998).

In New Zealand, avipoxvirus infections are common in many bird species (Ha et al. 2013). Since its recognition in 2002, diphtheritic stomatitis has remained an important cause of mortality in Yellow-eyed Penguin chicks. We reviewed all archived case reports and tissues held at Wildbase Pathology, Institute of Veterinary Animal and Biomedical Sciences, Massey University (Palmerston North, New Zealand) over the last 12 yr, to record and understand the nature of the disease better. We describe the pathology of the lesions and correlate this with the available microbiologic and epidemiologic findings.

**MATERIALS AND METHODS**

During the 20-yr period 1994–2014, carcasses of more than 550 Yellow-eyed Penguin adults and chicks were retrieved by Department of Conservation workers from nest sites and coastal areas of the South Island, Stewart Island, and occasionally from subantarctic islands such as Auckland and Campbell Islands. The dead chicks were collected from the mainly solitary nest sites that occupy the breeding territories located in coastal scrubland, forest, or pasture, and transported in chilled containers to Massey University for postmortem examination.

We performed detailed necropsies on all birds and diagnosis of cause of death was based on gross pathologic findings and the examination of selected tissues fixed in 10% buffered formalin and processed routinely for histopathology. Four-millimeter sections were cut and stained with H&E; where bacterial colonies were detected, staining with Gram Twort (Twort 1924) was undertaken. Where possible, sterile swabs of oral lesions or fresh samples of oral exudate, together with samples of fresh liver and lung tissue, were cultured by routine methods (Quinn et al. 1994). Colombia 5% sheep blood agar (CBA) (Fort Richard Laboratories, New Zealand) was inoculated and incubated aerobically at 35°C for 3 d (all cases) and anaerobically at 37°C for 2 d (three cases). Corynebacterium spp. colonies were subcultured onto CBA and sent to the Environmental Science and Research, Reference Laboratory, Porirua, New Zealand, for identification.

For trichomonas investigation, oral swabs were obtained from 10, 1–2-wk-old chicks from Katiti Point and Barracluda Bay with the use of the InPouch TF system (Biomed Diagnostics, White City, Oregon, USA) developed for testing bovine genital and feline fecal samples for Trichomonas foetus. This test was used because it may have greater sensitivity in detecting low-intensity infections (Bunbury et al. 2005).

We performed electron microscopy on samples of formalin-fixed and paraffin-embedded oral epithelium selected from five cases that had the highest number of intracytoplasmic inclusions. Blocks of epithelium from affected areas were deparaffinated, postfixed in osmium tetroxide, and embedded in epoxy resin before thin sections were cut and examined under a Philips 200 transmission electron microscope.

Testing for avipoxviruses was initially carried out at the National Investigation and Diagnostic Centre, Biosecurity New Zealand, Ministry of Agriculture and Forestry. DNA from three penguin chicks affected by diphtheritic stomatitis in 2002–05 was extracted from paraffin-embedded sections (cases 34152, 34171) and fresh frozen tissue (34228) using Qiap DNA Mini Kit (Qiagen, Victoria, Australia), according to manufacturer’s instructions with modifications. Five samples of fresh oral tissue were tested in the 2006 breeding season. Two PCR assays targeting different regions of the 4b core protein gene of avipoxviruses were applied for detection of avipoxvirus DNA (Lee and Lee 1997). Restriction fragment length polymorphism and nucleotide sequence analysis were then performed on the
amplified PCR products. These allowed further
differentiation of the virus from those in other
species of birds.

The method used for herpesvirus identification
was the consensus primer PCR method for
amplification of a region of the genome described
by VanDevanter et al. (1996), which will detect
and partially identify herpesviruses in tissue
samples, cell culture, and samples with no prior
DNA sequence information.

RESULTS

Epidemiology

The affected penguin chicks were aged 1–9
wk with most cases in the 2–4-wk age group,
although the exact date of hatching for >60% of
the chicks was not recorded. The disease
was first observed in the 2002–03 breeding
season (November to January) but since then
there has been considerable variation in
annual seasonal incidence based on field
observations (B.M. unpubl. data) and labora-
tory records (Table 1). Because of inaccessi-
bility, however, not all nest sites were
monitored regularly, and the data shown are
therefore likely to underestimate the number
of cases. During the 12-yr period, 113 affected
chicks were found from 234 chicks examined
in the laboratory. All these birds came from
nest sites on the southeast coast of the South
Island or from the northern beaches of
Stewart Island. The largest number of affect-
ed birds came from the Otago Peninsula, but
this likely reflects the ease of observer access
to nest sites and the amount of research
project activity undertaken in the area. There
was no difference between sexes in disease
occurrence. The main presenting signs were
inanition and loss of weight and, on close
examination, the presence of crusty yellow
exudate was often visible at the commissures
of the mouth and occasionally on the beak. In
some cases, the chicks were emaciated and
moribund or were found dead in the nest.
When two chicks were present both usually
showed lesions.

Gross pathology

The majority of carcasses showed severe
loss of body condition with lower-than-normal
body weights (200–500 g) compared to
unaffected chicks (300–1,000 g). A caseous
thick yellow–gray diphtheritic membrane was
attached to various areas of the oral cavity,
including the hard palate, dorsal and rostral
surfaces of the tongue, sublingual mucosa,
and the buccal mucosa. In severe cases
diphtheritic membranes covered areas of
mucosal ulceration in the upper and lower
oral cavity, the tongue, and sometimes the
pharynx (Fig. 1A). In many cases the exudate
covered the entire tongue and in some cases a
caseous exudate was visible in the gapes of the

<table>
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<tr>
<th>Breeding season</th>
<th>North Otago</th>
<th>Otago Peninsula</th>
<th>Catlins</th>
<th>Stewart Island</th>
<th>Total cases</th>
<th>No. chicks examined</th>
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<td>3</td>
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<tr>
<td>Total</td>
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<td>95</td>
<td>1</td>
<td>8</td>
<td>113</td>
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FIGURE 1. Lesions in the oral cavity and lungs of Yellow-eyed Penguin (*Megadyptes antipodes*) chicks in New Zealand with diphtheritic stomatitis. (A) Oral cavity of chick showing severe exudative lesions. Ulcerated areas of mucosa were present when exudate was lifted. (B) Oral cavity of chick with mild lesions. Note focal areas of erosion on roof and walls (arrows). (C) Histopathology of typical severe lesion on floor of oral cavity with thick, layered, serocellular exudate (s) covering an extensive area of ulceration and erosion (u). H&E. Bar=200 μm. Inset: Gram-positive pleomorphic bacteria associated with the exudate. Gram Twort stain. Bar=30 μm. (D) Typical mild lesion (m) in oral cavity with thin exudate containing few bacteria overlying area of epithelial thickening of underlying mucosa. H&E. Bar=100 μm. (E) Focal area of hyperplastic epithelium in oral cavity with swollen and vacuolated cells containing numerous basophilic to amphophilic intracytoplasmic inclusions (arrows) that displace the nuclei eccentrically. H&E. Bar=40 μm. (F) Lung of affected penguin showing early granuloma (g) within parenchyma containing numerous colonies of pleomorphic bacteria. H&E. Bar=200 μm.
mouth and at the commissures of the beak. Mild lesions were localized plaques, thickenings, or erosions of the oral mucosa, that were often difficult to detect, as they were sometimes covered with mucus or food debris (Fig. 1B). In mild cases it was concluded that death was due to a variety of other causes.

We recorded the distribution of gross lesions in detail in 57 chicks examined during 2002–13. Lesions were disseminated mainly on the floor and walls of the oral cavity (Fig. 1A) in 48 (84%) chicks, mainly on the tongue of 27 (47%) chicks, and in the commissures of the mouth and the margins of the beak in 20 (35%). The lesions on the tongue often covered the entire organ, but in some cases they were confined to the lateral margins and frenulum. Other locations for gross lesions included the hard or soft palate and the choana, pharynx, glottis, gapes, and the base of the bill. Localized areas of consolidation or nodules were observed in the lungs of five birds.

**Histopathology**

The severity of histologic lesions in the oral cavity varied among chicks and often within the oral cavity of the same chick. In the 103 chicks examined histologically the major differences between chicks were in the severity of the exudate, the extent of erosion or ulceration, and the amount of submucosal inflammation.

The oral inflammatory exudate was usually composed of thick layers of degenerate inflammatory and epithelial cells separated by eosinophilic necrotic debris, fibrin, and keratin. Gram-positive bacteria were detected in this exudate in the majority of chicks (Fig. 1C). These were pleomorphic or rod-shaped and were predominantly located in the middle to superficial areas of the exudate. Large numbers of bacteria were found in the chicks with severe exudate, and moderate numbers were present in those chicks with mild exudate.

In the epidermis, the main difference between lesions was the extent of erosion or ulceration. The most extensive ulceration occurred in those chicks with severe exudate. Nevertheless mild erosion with occasional small foci of ulceration was present in many chicks with a thin exudate. Moderate cellular proliferation, swelling, vacuolation, and both orthokeratotic and parakeratotic hyperkeratosis occurred predominantly at the margins of all erosive and ulcerative lesions. In a few chicks, however, these were the only epithelial changes present with no accompanying exudate or submucosal inflammation. The more severe lesions (Fig. 1C) had extensive erosion and ulceration associated with moderate to thick exudate, containing moderate to large amounts of bacteria and a moderate to severe submucosal inflammatory infiltrate. This pattern was observed in 22 (73%) of affected chicks in the 2008 breeding season. In chicks with mild gross lesions there was mild focal erosion (Fig. 1D) with little or no ulceration of the epithelium. This was accompanied by thin exudate, with small numbers of bacteria and mild submucosal inflammation. Occasionally, there would only be proliferative changes in the epithelium, with no exudate or submucosal inflammation.

Intracytoplasmic inclusion bodies were found in the oral mucosal epithelium in 10/103 chicks examined histologically. They were usually basophilic or occasionally amphophilic and seen most often in vacuolated cells in hyperplastic areas (Fig. 1E). They were present in the basal cells of a few sections with severe lesions. Occasional eosinophilic/amphophilic intranuclear inclusion bodies were found in one section with ulceration. Only one of the chicks with mild changes had small numbers of basophilic intranuclear inclusion bodies.

Microscopic lung lesions were present in three chicks, two of which had mild erosive oral lesions and the other had gross lesions on the roof of the oral cavity. The lesions were small to large foci, in which the parabronchi and small bronchioles contained necrotic debris (Fig. 1F) within which were colonies of Gram-positive short rods in two chicks and Gram-positive cocci in one chick. The necrotic center was surrounded by moderate numbers of both degenerate and nondegenerate heterophils and macrophages, with a variable amount of loose connective tissue and fibrosis at the periphery. The pleura was thickened.
with fibrous granulation tissue in one section. In a section through the head of one chick with moderate oral lesions, there were extensive amounts of thick cellular exudate containing Gram-positive bacterial colonies that occluded the lumen of the inner/middle ear.

**Microbiology**

*Corynebacterium* sp. was the most common organism isolated from the oral cavity of affected chicks. It was cultured in 12/17 (71%) chicks from the 2002–03 to the 2005–06 breeding seasons, with none or different organisms being found in the other five cases. Growth was heavy in five chicks with three of these associated with severe epithelial lesions. There was moderate growth from three chicks, one associated with severe epithelial lesions and large numbers of bacterial colonies on histopathology and two with mild focal ulceration and erosion and moderate numbers of bacterial colonies on histopathology. Two *Corynebacterium* sp. isolates sent to the reference laboratory were identified as *Corynebacterium amycolatum*.

No *Corynebacterium* sp. was cultured from postmortem and field swabs submitted from three chicks diagnosed with diphtheritic stomatitis during the 2006–07 season. From 2008–13, the oral lesions from another 12 chicks were cultured, and eight of these grew *Corynebacterium* sp., but these organisms were seldom isolated in pure culture. The next most common organism recovered was *Streptococcus* sp. (n=5) and *Escherichia coli* (n=3), which were found in moderate numbers.

*Corynebacterium* spp. were also isolated from the oral cavities of 30/88 (34%) of normal Yellow-eyed Penguins (adults and chicks) and from 5/10 (50%) Little Penguins (*Eudyptula minor*), 8/11 (73%) Northern Royal Albatrosses (*Diomedea exulans*), and 1/8 (13%) Red-billed Gulls (*Chroicocephalus scopulinus*) sampled on the Otago coastline in 2005.

**Electron microscopy**

No discernible viral particles were detected by electron microscopy of formalin-fixed tissues from the oral cavity of five chicks. The main findings were moderate numbers of necrotic epithelial cells showing typical ultrastructural degenerative and necrotic features such as mitochondrial swelling, ribosomal detachment, the formation of myelin figures and pyknotic and karyorrhectic nuclei. The intracytoplasmic inclusions noted with light microscopy were membrane-bound structures that contained structureless, stippled, variably electron-dense material that could not be conclusively interpreted as viral particles and bore resemblance to degraded cellular material.

**Parasitology**

Ten oral swab samples from chicks in 2005 (four affected and six normal) were tested for *Trichomonas* sp. with the use of the InPouch TF culture system. All were negative.

**PCR analysis**

The results of PCR analysis for viruses are shown in Table 2. Sequence analysis of PCR products demonstrated up to 91% (case 34152) and 97% (case 34171) nucleotide identity to avipoxviruses of different species.

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**Table 2. Results of PCR analysis for viruses in tissue from affected Yellow-eyed Penguin chicks in New Zealand.**

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Sample</th>
<th>Poxvirus PCR</th>
<th>Herpesvirus PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>34152</td>
<td>Paraffin-embedded tissue (cloaca)</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>34171</td>
<td>Paraffin-embedded tissue (oral lesion)</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>34228</td>
<td>Fresh frozen tissue (oral lesion)</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>38015</td>
<td>Fresh frozen tissue (oral lesion)</td>
<td>Not tested</td>
<td>Negative</td>
</tr>
<tr>
<td>Five samples 2006–07</td>
<td>Fresh frozen tissue (oral lesions)</td>
<td>Negative</td>
<td>Not tested</td>
</tr>
</tbody>
</table>

* In this sample an unidentified virus-like agent was detected with the use of avian cell culture. Electron microscopy showed a small spherical virus-like particle (20–30 µm).
The assay amplifying the 667 nucleotide 4b core protein gene detected poxviruses from all of a variety of avian species tested, whereas the other, which amplified a 578 base-pair fragment of a different portion of the gene, detected poxviruses in all but one of the species tested.

**DISCUSSION**

Diphtheritic stomatitis can be an important cause of mortality in Yellow-eyed Penguin chicks found in breeding areas of both the lower South Island and southern offshore islands of New Zealand. Since its initial recognition in 2002–03, the disease has been responsible for heavy mortalities occurring in alternate years. However, because of the remote location of many nest sites, it has not been possible to obtain reliable epidemiologic data, and the prevalence of the disease can only be estimated from its occurrence in accessible coastal localities. The disease is thought to be caused by an infectious agent, most likely a poxvirus, with secondary bacterial infection playing an important role. Other possibilities that have been considered are a severely pathogenic strain of *C. amycolatum*, an unidentified herpesvirus, circovirus, polyomavirus, or *Trichomonas* infection. Because Yellow-eyed Penguins are solitary nesters (Heather and Robertson 2005) the direct transmission of an infectious agent between birds is unlikely. However, biting black flies (*Austrosimulium* sp.) are common around nest sites and these are thought to be the insect vector responsible for transmitting *Leucocytozoon* sp. between Yellow-eyed Penguin chicks (Hill et al. 2010).

The exudative oral lesions were similar in many respects to the diphtheritic lesions seen in humans (Hadfield et al. 2000) and other mammals and the fact that *C. amycolatum* was cultured from 70% of the cases from the 2002–03 to the 2005–06 breeding seasons, closely associates the bacteria with this disease. However, unlike *Corynebacterium diphtheria*, *C. amycolatum* does not secrete an exotoxin and is a common component of the normal flora of the human skin and mucus membranes. Serious opportunistic infections may still occur (Esteban et al. 1999), and our studies suggest that in Yellow-eyed Penguins the organism has a role as a secondary bacterial invader that is carried in the oral cavity of normal birds but still contributes significantly to the lesions. Because no studies have been done on the pathogenic properties of *C. amycolatum* in Yellow-eyed Penguins, its role in the pathogenesis of the disease needs to be investigated.

The majority of gross lesions were confined to the oral cavity extending back to the soft palate. Many lesions also occurred on or beneath the tongue, suggesting that they should be easily identified once the beak is opened. However, this necessitates the capture and handling of live chicks, and familiarity with normal appearance of the oral mucosa. It is therefore likely that many cases are not recognized by field workers because mild cases particularly were difficult to differentiate from regurgitated food material and mucous. Discospondylitis has also been reported as a possible sequel to stomatitis on two occasions. One was following a stomatitis outbreak in 2004 (Bergen and Gartrell 2010) and a second in 2009 (M.R.A. unpubl. data). Although bacteria were seen in the lesions histologically, no organisms could be cultured.

In the Stewart Island region during the 2006–07 breeding season 11/32 chicks were diagnosed with diphtheritic stomatitis. This contributed to the season’s poor reproductive success of Yellow-eyed Penguins in this region. The other causes of mortality diagnosed in the chicks submitted included trauma (*n* = 5), leucocytozoonosis (*n* = 5) (Hill et al. 2010), and starvation (*n* = 4). In the previous season (2005–06), there were only 1/5 cases of diphtheritic stomatitis in this region. This increase was in keeping with the biennial fluctuation in frequency of the disease noted elsewhere.

The mild lesions, seen most easily on histopathology, are likely to represent either the beginning of the larger, “severe” lesions or areas in which the effect of the pathogen(s) was not as marked. Gram-positive coccobacilli
resembling *Corynebacterium* spp. were invariably present within the fibrinocellular exudate of the severe lesions but not always in the mild lesions, suggesting they have a role in promoting tissue damage and progression of the lesions. Although thick necrotic exudate formation is a characteristic of oral *Corynebacterium* infections in many species it is found in the oral cavity of birds with infection by poxviruses, herpesviruses, bacteria, *Candida albicans*, and *Trichomonas* sp. (Schmidt et al. 2003).

The epithelial lesions observed, such as erosion, ulceration, hyperplasia, swelling, and vacuolation, are similar to those associated with avian poxviral or herpesviral infection (Schmidt et al. 2003). However, poxviruses tend to cause more proliferative epithelial changes with marked swelling, vacuolation and prominent eosinophilic, intracytoplasmic inclusion bodies (Bollinger bodies). In domestic animals these typical inclusion bodies are termed Type A inclusions, but poxviruses also produce smaller basophilic intracytoplasmic inclusions termed Type B inclusions (Ginn et al. 2007), which are similar to those seen in the current study. The detection of poxvirus DNA in two chicks from the early 2002 cases supports the possibility that a poxvirus is the initiating agent and the subsequent failure to confirm this has been disappointing.

Herpesviral infection produces necrosis, ulceration, and erosion of epithelium with basophilic intranuclear inclusions in existing epithelial cells adjacent to the necrotic areas (Schmidt et al. 2003). An eosinophilic intranuclear inclusion detected in one case in the 2004–05 breeding season raised the possibility of herpesvirus infection, but no inclusions of this type were seen in subsequent cases and PCR analyses for herpesvirus has been negative.

The lung lesions were not prominent grossly, and histologically they were similar in appearance in all three affected chicks. They were small to large necrotic foci containing Gram-positive bacterial colonies, which may have arisen secondarily to a direct pulmonary infection with a primary viral pathogen (Schmidt et al. 2003). Herpesvirus and poxvirus infection are known to cause primary lung lesions in birds. Herpesviruses primarily affect the respiratory tract resulting in lesions ranging from proliferative bronchitis to severe hemorrhagic or fibrinonecrotic tracheitis with intranuclear inclusions and syncytiar cell formation as reported in Jackass Penguins (Kincaid et al. 1988; Schmidt et al. 2003).

We observed basophilic intracytoplasmic inclusion bodies in the oral mucosal lesions in 12 cases. Electron microscopy of three cases showed that these were composed of intracytoplasmic, membrane-bound electron-dense, structureless, stippled material that could represent degraded viral particles or cellular organelles. The tissues were too poorly fixed for accurate morphologic interpretation. Although there were no significant findings on electron microscopy, the possibility of a primary viral agent cannot be ruled out, as these inclusions were not present in the epithelial mucosa of chicks without the disease and were primarily located at the margins of the lesions or associated with epithelial cell degenerative changes such as vacuolation and swelling. These inclusions were not characteristic of a poxvirus or herpesvirus and there is the possibility that other viruses, such as a circovirus or polyomavirus, may have been involved (Schmidt et al. 2003).

The samples tested for *Trichomonas* sp. culture were all negative. However, these were from only a few penguin colonies, and the number and distribution may not be representative of the entire Yellow-eyed Penguin population. This sampling was carried out in November 2005, the beginning of the breeding season following the 2004–05 season in which there was a high number of cases. It is therefore possible that at the time of sampling, *Trichomonas* organisms may have been at low prevalence and could have been missed. Nevertheless, there has been no indication from histopathology or direct smears made from lesions at the time of necropsy that protozoal organisms have a role in this disease.

The fluctuating seasonal occurrence of the disease may be explained by changes in vector...
numbers or movement or possibly even environmental factors such as temperature and humidity. Marked biennial fluctuations in prevalence are common in many poxvirus infections, including avipox lesions in captive shore plovers and scabby mouth lesions in lambs (M.R.A. unpubl. data). This is presumably related to virus carriage and fluctuating levels of immunity in the adult population.

This disease syndrome currently represents a serious threat to Yellow-eyed Penguins on the New Zealand mainland, as the affected chicks usually succumb to starvation and seldom recover fully without antibiotic treatment and careful nursing. Further research is urgently needed on both its epidemiology and etiology with a priority on the virology of early cases. This would improve our understanding and management of the disease and help reduce its impact on Yellow-eyed Penguin populations.

ACKNOWLEDGMENTS

We thank Department of Conservation personnel Kate McGiness, Dave Houston, Dave Agnew, Sandy King, Helen Jones, and Phred Dobbins, who collected and submitted affected birds. Technical assistance was provided by Pat Davey, Evelyn Lupton, Doug Hopcroft, Ann Midwinter, and Hamish Mack. The project was partially supported by the Yellow-eyed Penguin Trust.

LITERATURE CITED


Submitted for publication 21 July 2015. Accepted 29 July 2016.