Chapter 4
Infectious Bursal Disease Virus and Antarctic Birds

J.M. Watts, G.D. Miller, and G.R. Shellam

4.1 Introduction

The geographic isolation of the Antarctic continent coupled with the extreme climate has historically been assumed to protect the indigenous Antarctic wildlife from exposure to infectious agents found among animals in more temperate regions. However, with the number of tourists visiting the region more than doubling in the last 10 years (IAATO 2004) and climate change predicted to enhance the success of alien micro-organisms (Frenot et al. 2005), the threat of introduced disease to Antarctic wildlife has become a concern (Kerry and Clarke 1995; Kerry et al. 1999). Emergence of disease in naïve bird populations can reduce both population abundance and geographical distribution (Friend et al. 2001).

This concern is highlighted by recent evidence of introduction of infectious organisms to isolated wildlife populations in the sub-Antarctic and southern Indian Ocean. The isolation of *Salmonella* serotypes normally associated with human activity from penguins, fur seals and albatross on sub-Antarctic Bird Island (Olsen et al. 1996; Palmgren et al. 2000) and *Campylobacter jejuni* subsp. *jejuni* with high genetic similarity to Northern Hemisphere strains from macaroni penguins *Eudyptes chrysolophus* at the same location (Broman et al. 2000) suggests recent introduction of these bacteria. The introduction of avian cholera, caused by *Pasteurella multocida,* to Amsterdam Island is thought to be a major cause of population decline in the now-endangered large yellow-nosed albatross *Diomedea chlororhynchos* (Weimerskirch 2004). Avian cholera has also been implicated as the cause of death in rockhopper penguins *Eudyptes chrysocome* on sub-Antarctic Campbell Island (de Lisle et al. 1990); brown skuas *Catharacta lönnbergi* on Litchfield Island, Antarctica (Parmelee et al. 1979); and a single southern giant petrel *Macronectes giganteus* on King George Island, Antarctica (Leotta et al. 2003).

J.M. Watts, G.D. Miller, and G.R. Shellam
Microbiology and Immunology, School of Biomedical, Biomolecular and Chemical Sciences, University of Western Australia, WA 6009, Australia
e-mail: djawatts@bigpond.net.au; gshellam@cyllene.uwa.edu.au

K.R. Kerry and M.J. Riddle (eds.), *Health of Antarctic Wildlife: A Challenge for Science and Policy*, DOI: 10.1007/978-3-540-93923-8_5,

However, the identification of introduced disease is difficult without baseline information on infectious disease and organisms native in a population. While both Antarctic and sub-Antarctic penguins in captivity are susceptible to a range of pathogenic avian diseases (Clarke and Kerry 1993; Penrith et al. 1996; Fielding 2000), to date very little is known of the disease status or the impact of disease on free-living Antarctic penguin populations (Clarke and Kerry 1993; Kerry and Clarke 1995). There is serological evidence of exposure of free-living penguins to potentially pathogenic avian paramyxo- and influenza viruses (Morgan and Westbury 1981,1988; Austin and Webster 1993), and *Chlamydia* species (Moore and Cameron 1969) and *Salmonella* serotypes have been isolated from Adélie penguins, *Pygoscelis adeliae*, on Ross Island (Oelke and Steiniger 1973) although no associated disease has been described. There are two instances in which an infectious agent has been suspected as responsible for an observed mass mortality in Antarctic penguins in the wild. Symptoms resembling those of the viral disease puffinosis were observed in gentoo penguins, *Pygoscelis papua*, on Signy Island where several hundred chicks died, although no infectious agent was isolated (MacDonald and Conroy 1971), and large numbers of apparently well-nourished Adélie penguin chicks were found dead at a colony near Mawson Station in 1972 (Kerry et al. 1996). Approximately 65% of chicks were recently dead and many surviving chicks were ataxic and unable to stand. The cause of these deaths remains unknown.

In 1997 Gardner et al. reported the presence of antibodies to a pathogenic poultry virus, infectious bursal disease virus (IBDV), in two Antarctic penguin species breeding in the vicinity of the Australian Antarctic research station of Mawson (67°36′S, 62°52′E). High-titre neutralising antibodies to serotype 1 virus were detected in up to 2.6% of 133 adult Adélie penguins from two breeding colonies and 65.4% of 52 emperor penguin chicks (*Aptenodytes forsteri*) from a single breeding site in the 1995/96 summer. Antibody was also found in 1.5% (n = 136) of adult Adélie penguins from the same area in 1991. The absence of antibodies in 26 adult and 17 chick Adélie penguins from a remote colony (74°21′S, 165°03′E) in the Ross Sea area of Antarctica led these authors to suggest that the virus may have been introduced into penguin populations in the Mawson region by human activity. A possible mechanism of introduction proposed was the inappropriate disposal of chicken products, allowing infection of migratory scavenging birds such as the south polar skua *Catharacta maccormicki* and subsequent transmission to penguin colonies (Gardner et al. 1997).

IBDV was identified in 1962 (Winterfield et al.) as the causative agent of a newly described, acute, highly infectious disease of chickens (Cosgrove 1962). The virus has subsequently been found to be ubiquitous within the poultry industry worldwide (Leong et al. 2000). IBDV is a non-enveloped virus with a bi-segmented double-stranded RNA genome of approximately 6,300 base pairs (Kibenge et al. 1988) and is classified as a member of the family Birnaviridae, genus *Avibirnavirus*. The Birnaviridae family also contains viruses in other genera which infect fish (infectious pancreatic necrosis virus, *Aquabirnavirus*), bivalve molluscs (tellina and oyster virus, *Aquabirnavirus*) and insects (*Drosophila* X virus, *Entomobirnavirus*)

but there is no serological cross-reaction between these and avian birnaviruses (Kibenge et al. 1988). Two serotypes of IBDV are recognised, designated serotype 1 and 2, and are differentiated by the virus neutralisation assay (McFerran et al. 1980), a specific and sensitive functional assay that utilises antibodies to inhibit virus infection of cells. IBDV has been isolated from chickens, ducks and turkeys, although clinical disease has been described only in chickens and is associated exclusively with serotype 1 strains (Lukert and Saif 1997). Serological studies have shown that antibodies to serotype 2 IBDV are widespread in turkeys, chickens and ducks although all strains isolated are non-pathogenic (Lukert and Saif 1997).

IBDV is a very stable virus that is highly resistant to disinfection and adverse environmental conditions (Kibenge et al. 1988). It can persist for months in poultry pens, feed and faeces (Lukert and Saif 1997). Once introduced to a susceptible flock, transmission is via the faecal–oral route, with virus excreted in the faeces for up to 14 days post infection (Fenner et al. 1987). There is no known vertical transmission via eggs or carrier state in recovered birds (Lukert and Saif 1997).

The most striking feature of virulent IBDV infection in chickens is the selective targeting of lymphoid tissue resulting in B lymphocyte depletion, particularly in the bursa of Fabricius. Infection in young chicks (0–3 weeks) is subclinical but results in severe immunosuppression, leaving the birds highly susceptible to secondary infection with a wide range of avian pathogens (Sharma et al. 2000). Acute disease is seen in 3–8-week-old chicks, the time of maximal bursal development, and commonly results in 100% morbidity and up to 30% mortality. Clinical signs include diarrhoea, anorexia, trembling and prostration (Lukert and Saif 1997). Infection in older chickens is generally subclinical; however, a broader age range of susceptibility to acute disease and increased mortality rates has been seen with the emergence of very virulent IBDV strains (vvIBDV) (van den Berg 2000). Phylogenetic studies have shown that vvIBDV strains belong to the same genetic lineage as the classical serotype 1 virus, although mortality rates following infection with vvIBDV can reach 100% (van den Berg 2000). The high mortality and morbidity along with worldwide distribution has led the Office International des Epizooties (OIE) to consider infectious bursal disease (IBD) a disease of considerable socio-economic importance (van den Berg 2000).

Although IBDV has been isolated only from chickens, turkeys and ducks, there is now accumulating serological evidence of widespread infection in wild bird populations. Antibody to IBDV has been detected by the agar gel immunodiffusion (AGID) test in a number of free-living wild bird species in Nigeria (Nawathe et al. 1978) and Western Australia (Wilcox et al. 1983). Common eiders *Somateria mollissima* and herring gulls *Larus argentatus* in the Baltic Sea, along with spectacled eiders *Somateria fischeri* in Alaska, displayed neutralising antibody to serotype 1 IBDV (Hollmen et al. 2000), while virus-neutralising antibody against both serotype 1 and 2 viruses has been found in 14 species of wild birds in Japan (Ogawa et al. 1998) and king penguins, *Aptenodytes patagonicus*, from sub-Antarctic Possession Island (Gauthier-Clerc et al. 2002). Most of these studies sampled birds at only a single time point in each location; however, antibody was found to persist across years in king penguins breeding on Possession Island (Gauthier-Clerc et al. 2002).

With the exception of the isolated spectacled eider colony in Alaska (Hollmen et al. 2000), exposure of the studied populations or closely associated scavenging birds to poultry or poultry waste was thought to be a possible source of infection in each case.

4.2 Case Study

The aim of this study was to investigate IBDV in Antarctic birds by establishing both the prevalence and persistence of the virus in populations of emperor penguins, Adélie penguins and migratory south polar skuas breeding in regions around three Antarctic research stations: the Australian stations of Mawson and Davis (68°34′S, 77°58′E) in East Antarctica and the Italian station Terra Nova Bay (74°41′S, 164°07′E) in the Ross Sea. Blood samples were collected from birds over five summers (Table 4.1). Adult Adélie penguins and south polar skuas were sampled across the breeding season, from November to February. Chicks of both species were sampled in February when 2–3 months of age. Adélie penguin samples were obtained from breeding colonies within a maximum of 80 km of each research station. All south polar skuas sampled were breeding in the vicinity of Adélie penguin colonies in the Vestfold Hills region surrounding Davis Station. Emperor penguin chick sera were obtained at three different colonies: Auster Rookery (50 km east of Mawson Station), Amanda Bay Rookery (90 km south west

Table 4.1 Serum samples collected from Antarctic bird species

Species	Age	Location	Sampling season	Number
Emperor penguin (*Aptenodytes forsteri*)	Chick	Auster	1996/97	31
		Amanda Bay	1997/98	17
		Cape Washington	2000/01	49
				Total 97
Adélie penguin (*Pygoscelis adeliae*)	Adult	Mawson coast	1996/97	127
			1997/98	66
		Davis coast	1997/98	369
			1999/00	186
			2001/02	40
		Terra Nova Bay	2000/01	84
				Total 872
	Chick	Mawson coast	1996/97	44
			2000/01	24
		Davis coast	2001/02	56
				Total 124
South polar skua (*Catharacta maccormicki*)	Adult	Vestfold Hills, Davis	1999/00	118
			2001/02	128
				Total 246
	Chick	Vestfold Hills, Davis	2001/02	56

of Davis) and Cape Washington Rookery (40 km east of Terra Nova Bay). Birds were bled in November/December of each year when approximately 4–5 months old.

Antibody titres to IBDV serotype 1 in serum were measured using a standard virus neutralisation test (Westbury and Fahey 1993). IBDV serotype 1 strain GT101 was used as antigen. Antibody titres are expressed as the reciprocal of the highest dilution that completely inhibited virus. Antibody titres of ≥16 were regarded as positive, with titres >64 deemed highly significant (Giambrone 1980). Results were compared using the chi-square test with statistical significance determined as $P < 0.05$.

Sera from 1,395 birds were tested, with 189 (13.5%) exhibiting neutralising antibodies to serotype 1 IBDV. Results of the virus neutralisation tests for each species are summarised in Table 4.2. The seroprevalence in adult Adélie penguins was 7.7%. There was no significant difference in prevalence between locations or between years at each location (data not shown). Highly significant titres were recorded in 1.8% of birds, with a maximum neutralising antibody titre of 512 and a geometric mean titre of 57.6. A single Adélie chick (prevalence 0.8%) possessed IBDV antibody with a titre of 16.

Neutralising antibody was detected in a total of 11.8% of adult south polar skuas in the Vestfold Hills region but there was a significant difference ($P < 0.05$) in prevalence between sampling periods. In the 1999/2000 summer, 16.9% of birds were antibody positive with only 7% prevalence in 2001/02. This difference was reflected in the antibody titres, with a maximum titre of 256 in 1999/2000 and highly significant titres in 9.3% of birds. In 2001/02, a maximum titre of 80 was recorded with highly significant titres in only 3.1%. All south polar skua chicks tested in 2001/02 were negative for antibody, although it should be noted that chicks were tested only in a year of low prevalence and antibody titres in adults.

A very different pattern was found in emperor penguin chicks, with an overall seroprevalence of 95.9%. There was no significant difference in prevalence between each of the three locations, with 93.5, 100 and 95.9% recorded at Auster, Amanda Bay and Cape Washington, respectively. Antibody titres were higher than in the other two species of birds (Table 4.2), with highly significant titres detected in 100% of chicks at Amanda Bay, 85.7% at Cape Washington and 67.7% at Auster,

Table 4.2 Prevalence of IBDV serotype 1 antibodies in Antarctic bird species. Antibody titres ≥ 16 positive

Species	Age	Number tested	Number positive	Prevalence (%)	Max. titre	Geometric mean titre
Emperor	Chick	97	93	95.9	10,240	1,757.4
Adélie	Adult	872	67	7.7	512	57.6
	Chick	124	1	0.8	16	16.0
South Polar skua	Adult	246	29	11.8	256	68.3
	Chick	56	0	0	0	0

and a maximum titre of 10,240. Results at Auster are consistent with the 65.4% prevalence (titres ≥ 80) recorded previously at this location (Gardner et al. 1997).

The detection of IBDV RNA in emperor penguin chick tissue samples was attempted by reverse transcriptase polymerase chain reaction (RT PCR). Bursa of Fabricius, spleen, kidney, liver and intestinal tissue were extracted from 23 emperor penguin chick carcasses collected frozen off the ice at Auster Rookery throughout the 2000 winter. While the ages of chicks collected are unknown, the weights of the carcasses ranged from 302 g to 1.5 kg, indicating that all chicks were below the creching age of 45–50 days (Williams 1995). A nested RT PCR was performed as described by Liu et al. (1998), using primers flanking the hypervariable region of the VP2 structural protein of IBDV between nucleotides 703 and 1,193. Bursae from IBDV type 1 vaccine strain V877 infected chickens was used as a positive control. Products of the predicted size were amplified from three emperor penguin bursae samples. All other organ samples were RT PCR negative. On sequencing, the RT PCR products from each penguin were found to be identical at the nucleotide level both to each other and to the positive control strain V877, a classical Australian strain of low pathogenicity used as a live vaccine in Australia, Europe and Asia (Firth 1974; Proffitt et al. 1999).

Isolation of IBDV from RT PCR positive penguin bursae samples was attempted in specific pathogen free (SPF) chicks. Intraocular inoculation of 3–4-week-old and 1-day-old chicks was performed with bursal homogenates prepared as described by Westbury and Fahey (1993). Three chicks from each group were killed, and the bursae examined on days 3, 5, 7, 14 and 21 post infection. Throughout the experiments no evidence of clinical disease was seen in inoculated SPF chicks. All bursae appeared normal, displaying no signs of atrophy, and there was no significant difference in bursal to body weight ratios between infected and control chicks. RT PCR on all bursal samples was negative. Serum was also extracted from day 14 and 21 chicks, and virus neutralisation tests performed as previously described. IBDV neutralising antibody was not detected in any of the SPF chicks.

4.3 Discussion

The presence of neutralising antibody in all three species of birds, from all locations and time points, indicates that IBDV serotype 1 is endemic and widespread in Antarctic bird populations. The large distances between the sampling sites (Terra Nova Bay is approximately 4,500 km via the coast from Davis, the closest Australian station) suggest that the virus would not spread readily between these sites by either direct contact between penguins or movement of migratory birds. It is therefore unlikely that IBDV serotype 1 has been introduced into Antarctic bird populations by human activity in the Mawson Station region. Detection of neutralising antibodies in king penguins at Possession Island (Gauthier-Clerc et al. 2002) suggests that the virus may also be endemic in sub-Antarctic bird populations, although rockhopper penguins in Argentina (Karesh et al. 1999) and southern giant

petrels from Patagonia (Uhart et al. 2003), two species which frequent the sub-Antarctic, were tested negative for IBDV antibodies by AGID. AGID is less sensitive than virus neutralisation, however (Weisman and Hitchner 1978); so further investigation of sub-Antarctic birds is required.

The generally low titres of neutralising antibody in Adélie penguin and south polar skua adults are similar to titres reported in other adult wild bird species. A maximum titre of 128 was recorded in common and spectacled eiders (Hollmen et al. 2000), 256 in king penguins (Gauthier-Clerc et al. 2002) and 269 in wild birds from Japan (Ogawa et al. 1998). While the prevalence recorded in adult birds varies widely between studies, the results in Adélie penguins compare well with those previously recorded in this species, where a high-titre antibody (≥80) was found in 1.5–2.6% of birds (Gardner et al. 1997), and with the 4% prevalence in adult king penguins (Gauthier-Clerc et al. 2002).

The high prevalence and titre of antibody recorded in emperor penguin chicks is greater than reported in any other wild bird species. Up to 96% prevalence was recorded in common eider adults but high-titre antibody (≥128) was seen in only 15% of these birds (Hollmen et al. 2000). Two studies have investigated chicks of other bird species; in the closely related king penguin, only 3% of 3-month-old and 5% of the 1-year-old chicks were positive with a maximum titre of 64 (Gauthier-Clerc et al. 2002), while 45% prevalence was seen in herring gull chicks from the Baltic Sea (Hollmen et al. 2000). Interestingly, rapid transmission of virus through entire flocks of domestic chickens resulting in seroconversion of 100% of birds and very high antibody titres is a common pattern following field exposure to IBDV (Lukert and Saif 1997).

The almost complete absence of neutralising antibody in Adélie and south polar skua chicks is in stark contrast with results recorded in emperor penguin chicks and suggests a very different epidemiology among these species. While neutralising antibody results indicate that Adélie penguin and south polar skua adults are exposed to IBDV during their life cycle, the lack of antibody in chicks from both species suggests that infection of adults occurs remote from breeding sites with no subsequent transmission to chicks. There are a number of possible routes of infection in each species. South polar skuas are known predators of weakened emperor penguin chicks (Williams 1995), which have a high seroprevalence of IBDV serotype 1, and may also have contact with infected birds or material during their annual northern migration. Adélie penguins are also occasional visitors to emperor penguin colonies, and while adult penguins generally spend the winter non-breeding months dispersed within the Antarctic pack-ice, they are occasional vagrants to South America, Australia, New Zealand and sub-Antarctic islands (Williams 1995) where exposure may occur.

The high antibody prevalence in emperor penguin chicks indicates that infection occurs each year at the breeding sites. While IBDV can survive for extended times in the environment (Kibenge et al. 1988), emperor penguins breed on sea-ice which often melts during summer, and so persistent contamination of breeding sites is not possible. Introduction of virus from an outside source into each breeding colony every year is unlikely, especially since migratory birds are absent during winter

when chicks are young. These results therefore suggest that emperor penguins are carriers of IBDV. As virus transmission in domestic chickens is via the faecal–oral route, emperor chicks are most likely infected by contaminated faecal material while brooding on the feet of adults or drinking snow. Vertical transmission through the egg cannot be excluded. The possibility of species of wild birds acting as carriers or reservoirs and playing a role in the epidemiology of IBDV has previously been suggested. Detection of antibodies in migratory birds led to the speculation that they may have been responsible for the introduction and spread of very virulent IBDV among domestic chickens in Japan (Ogawa et al. 1998), whereas scavenging herring gulls are the suspected source of infection in eider ducks in the Baltic Sea (Hollmen et al. 2000). Persistence of virus in spectacled eiders was suggested following the detection of antibody in 70% of adults in a remote Alaskan colony where recent introduction of virus from poultry waste or migratory birds was unlikely (Hollmen et al. 2000).

While the presence of neutralising antibody implies exposure to the virus, it does not necessarily indicate the presence of disease. IBDV serotype 1 strains differ markedly in virulence in chickens and, although all strains appear non-pathogenic in turkeys and ducks (Lukert and Saif 1997), very little is known of the effect on wild bird species. The presence of IBDV neutralising antibody in common and spectacled eiders raises concern as the populations of both species are in decline with low duckling survival rates a primary cause, particularly in the Baltic Sea common eider colonies where IBDV antibody prevalence in adult birds was highest (Hollmen et al. 2000). The presence of IBD with a pathology similar to that seen in chickens would directly affect chick and fledgling survival. However, no evidence of immunosuppression was found in herring gull chicks following sheep red blood cell immunisation, despite increasing antibody titres indicating active IBDV infection (Hollmen et al. 2000). The lack of any observed clinical symptoms along with the detection of a classical Australian low pathogenic strain of IBDV in emperor penguin bursal tissue suggests that IBDV does not cause disease in Antarctic bird species.

All Australian IBDV strains isolated are genetically distinct from strains isolated in other parts of the world (Proffitt et al. 1999; Ignjatovic and Sapats 2002). The presence of a classical Australian strain of IBDV serotype 1 in emperor penguin chick samples therefore suggests a common evolution or source of Australian and Antarctic IBDV strains, although this has yet to be confirmed. The detection of IBDV RNA in bursal samples by RT PCR but a failure to isolate the virus in SPF chicks is not unusual, as IBDV RNA persists in bursal tissue from domestic chickens for substantially longer than infectious virus (Abdel-Alim and Saif 2001); however, 100% nucleotide similarity with the positive control virus means that contamination of samples cannot be conclusively ruled out.

These results contribute to the understanding of the health and survival of Antarctic penguins by establishing that IBDV serotype 1 is endemic in Antarctic bird populations and has not been introduced by recent human activity. However, as the threat to the Antarctic wildlife is amplified through increasing human activity and environmental change, this study also highlights the need for further information

on the endemic microflora of Antarctic birds and surveillance to allow the identification of introduced disease events. This is also the first study to demonstrate persistence of IBDV neutralising antibody in wild bird populations across both geographic location and time and to establish the role of a species as a carrier of the virus. Further work is now required to fully understand the epidemiology of IBDV in emperor penguins, with the prevalence of antibodies in adult emperor penguins to be determined, a virus isolate obtained and mode of transmission examined.

References

Abdel-Alim GA, Saif YM (2001) Detection and persistence of infectious bursal disease virus in specific-pathogen-free and commercial broiler chickens. Avian Dis 45:646–654

Austin FJ, Webster RG (1993) Evidence of ortho- and paramyxoviruses in fauna from Antarctica. J Wildl Dis 29:568–571

Broman TB, Bergstrom S, On SLW, Palmgren H, McCafferty DJ, Sellin M, Olsen B (2000) Isolation and characterisation of *Campylobacter jejuni* subsp. *jejuni* from macaroni penguins (*Eudyptes chrysolophus*) in the subantarctic region. Appl Environ Microbiol 66(1):449–452

Clarke JR, Kerry KR (1993) Diseases and parasites of penguins. Kor J Polar Res 4:79–96

Cosgrove AS (1962) An apparently new disease of chickens – avian nephrosis. Avian Dis 6:385–389

de Lisle GW, Stanislawek WL, Moors PJ (1990) *Pasteurella multocida* infections in Rockhopper penguins (*Eudyptes chrysocome*) from Campbell Island, New Zealand. J Wildl Dis 26:283–285

Fenner F, Bachman P, Gibbs E, Murph F, Studder M, White D (1987) Birnaviridae. Veterinary virology. Academic, London.

Fielding MJ (2000) Deaths in captive penguins. Vet Rec 146:199–200

Firth GA (1974) Occurrence of an infectious bursal syndrome within an Australian poultry flock. Aust Vet J 50:128–130

Frenot Y, Chown SL, Whinam J, Selkirk PM, Convey P, Skotnicki M, Bergstrom DM (2005) Biological invasions of the Antarctic: extent, impacts and implications. Biol Rev 80:45–72

Friend M, McLean RG, Dein FJ (2001) Disease emergence in birds: challenges for the twenty-first century. The Auk 118:290–303

Gardner H, Kerry K, Riddle M, Brouwer S, Gleeson L (1997) Poultry virus infection in Antarctic penguins. Nature 387:245

Gauthier-Clerc M, Eterradossi N, Toquin D, Guittet M, Kuntz G, Le Maho Y (2002) Serological survey of the king penguin, *Atenodytes patagonicus*, in Crozet Archipelago for antibodies to infectious bursal disease, influenza A and Newcastle disease viruses. Polar Biol 25:316–319

Giambrone JJ (1980) Microculture neutralisation tests for serodiagnosis of three avian viral infections. Avian Dis 24: 284–287

Hollmen T, Franson JC, Docherty DE, Kilpi M, Hario M, Creekmore LH, Petersen M (2000) Infectious bursal disease virus antibodies in eider ducks and herring gulls. Condor 102:688–691

IAATO (2004) International Association of Antarctic Tour Operators. http://www.IAATO.org

Ignjatovic J, Sapats S (2002) Confirmation of the existence of two distinct genetic groups of infectious bursal disease virus in Australia. Aust J Vet 80:689–694

Karesh WB, Uhart MM, Frere E, Gandini P, Braselton E, Puche H, Cook RA (1999) Health evaluation of free-ranging rockhopper penguins (*Eudyptes chrysocomes)* in Argentina. J Zoo Wildl Med 30:25–31

Kerry K, Clarke J (1995) Time for concern: is the health of Antarctica's wildlife in danger? Antarct Sci 7:343

Kerry KR, Gardner HG, Clarke JR (1996) Penguin deaths: diet or disease? Microbiol Aust 17:16

Kerry K, Riddle M, Clarke J (1999) Diseases of Antarctic wildlife: report to the Scientific Committee on Antarctic Research (SCAR) and the Council of Managers of National Antarctic Programs (COMNAP), Australian Antarctic Division, Hobart: 104 pp

Kibenge FSB, Dhillon AS, Russell RG (1988) Biochemistry and immunology of infectious bursal disease virus. J Gen Virol 69:1757–1775

Leong JC, Brown D, Dobos P, Kibenge FSB, Ludert JE, Mulleer E, Nicholson B (2000) Birnaviridae. In: van Regenmortel MHV, Fauquet CM, Bishop DHL (eds) Virus taxonomy. Classification and nomenclature of viruses. Academic, London

Leotta GA, Rivas M, Chinen I, Vigo GB, Moredo FA, Coria N, Wolcott MJ (2003) Avian cholera in a southern giant petrel (*Macronectes giganteus*) from Antarctica. J Wildl Dis 39:732–735

Liu X, Giambrone JJ, Dormitorio T (1998) Simplified sample processing combined with a sensitive nested polymerase chain reaction assay for detection of infectious bursal disease virus in the bursa of Fabricus. Avian Dis 42:480–485

Lukert PD, Saif YM (1997) Infectious bursal disease. In: Calnek BW (ed), Diseases of poultry, 10th edn. Iowa State University Press, Ames, IA, pp 721–738

MacDonald JW, Conroy JWH(1971) Virus disease resembling puffinosis in the gentoo penguin *Pygoscelis papua* on Signy Island, South Orkney Islands. British Ant Sur Bull (26):80–83

McFerran JB, McNulty MS, McKillop ER, Connor TJ, McCracken RM, Collins DS, Allan GM (1980) Isolation and serological studies with infectious bursal disease viruses from fowl, turkey and ducks: demonstration of a second serotype. Avian Pathol 9:395–404

Moore BW, Cameron AS (1969) Chlamydia antibodies in Antarctic fauna. Avian Dis XVIII:681–684

Morgan IR, Westbury HA (1981) Virological studies of Adélie penguins (*Pygoscelis adeliae)* in Antarctica. Avian Dis 25:1019–1027

Morgan IR, Westbury HA (1988) Studies of viruses in penguins in the Vestfold Hills, Antarctica. Hydrobiologica 165:262–269

Nawathe DR, Onunkwo O, Smith IM (1978) Serological evidence of infection with infection with the virus of infectious bursal disease in wild and domestic birds in Nigeria. Vet Rec 102:444

Oelke H, Steiniger F (1973) Salmonella in Adélie penguins (*Pygoscelis adeliae*) and south polar skuas (*Catharacta maccormicki*) on Ross Island Antarctica. Avian Dis 17:568–573

Ogawa M, Wakuda T, Yamaguchi T, Murata K, Setiyono A, Fukushi H, Hirai K (1998) Seroprevalence of infectious bursal disease virus in free-living wild birds in Japan. J Vet Med Sci 60:1277–1279

Olsen BB, Bergstrom S, McCafferty DJ, Sellin M, Wistrom J (1996) *Salmonella enteritidis* in Antarctica: zoonosis in man or humanosis in penguins? Lancet 348:1319–1320

Palmgren H, McCafferty D, Aspan A, Broman T, Sellin M, Wollin R, Bergstron S, Olsen B (2000) Salmonella in sub-Antarctica: low heterogeneity in salmonella serotypes in South Georgian seals and birds. Epidemiol Infect 125:257–262

Parmelee DF, Maxson SJ, Bernstein NP (1979) Fowl cholera outbreak among brown skuas at Palmer Station. Ant J US 14:168–169

Penrith M-L, Huchzermeyer FW, De Wet SC, Penrith MJ (1996) Concurrent infection with *Clostridium* and *Plasmodium* in a captive king penguin A*ptenodytes patagonicus*. Avian Pathol 23:373–380

Proffitt JM, Bastin DA, Lehrbach PR (1999) Sequence analysis of Australian infectious bursal disease viruses. Aust Vet J 77:186–188

Sharma JM, Kim I-J, Rautenschlein S, Yeh H-Y (2000) Infectious bursal disease virus of chickens: pathogenesis and immunosuppression. Dev Comp Immunol 24:223–235

Uhart MM, Quintana F, Karesh WB, Braselton WE (2003) Hematology, plasma biochemistry and serosurvey for selected infectious agents in southern giant petrels from Patagonia, Argentina. J Wildl Dis 39:359–365

van den Berg TP (2000) Acute infectious bursal disease in poultry: a review. Avian Pathol 29:175–194

Weimerskirch H (2004) Diseases threaten Southern Ocean albatrosses. Polar Biol 27:376–379
Weisman J, Hitchner SB (1978) Virus-neutralization versus agar-gel precipitin tests for detecting serological response to Infectious Bursal Disease. Avian Dis 22:598–603
Westbury HA and Fahey KJ (1993) Infectious bursal disease. Virology and serology. In: Corner LA, Bagust TJ (eds) Australian sandard diagnostic techniques for animal diseases. Standing Committee on Agriculture and Resource Management, Australia
Wilcox GE, Flower RLP, Baxendale W, Mackenzie JS (1983) Serological survey of wild birds in Australia for the prevalence of antibodies to egg drop syndrome 176 (EDS-76) and infectious bursal disease viruses. Avian Pathol 12:135–139
Williams TD (1995) The penguins. Oxford University Press, London
Winterfield RW, Hitcher SB, Appleton GS, Cosgrove AS (1962) Avian nephrosis, nephritis and Gumboro disease. L & M News and Views 1:103