Health evaluation of African penguins (*Spheniscus demersus*) in southern Africa

The African penguin (*Spheniscus demersus*) is an endangered seabird that breeds along the coast of Namibia and South Africa, and disease surveillance was identified as a priority for its conservation. Aiming for the establishment of baseline data on the presence of potential pathogens in this species, a comprehensive health assessment (blood smear examination, haematology, biochemistry and serology) was conducted on samples obtained from 578 African penguins at 11 breeding colonies and a rehabilitation centre. There were 68 penguins that were seropositive for at least one of seven pathogens tested: avian encephalomyelitis virus, avian infectious bronchitis virus, avian reovirus, infectious bursal disease virus, Newcastle disease virus, *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. All samples were seronegative for avian influenza virus subtypes H5 and H7 and infectious laryngotracheitis virus. The apparent prevalence of *Babesia* sp. and *Borrelia* sp. in blood smears was consistent with previous studies. *Babesia*-infected individuals had a regenerative response of the erythrocytic lineage, an active inflammatory response and hepatic function impairment. These findings indicate that African penguins may be exposed to conservation-significant pathogens in the wild and encourage further studies aiming for the direct detection and/or isolation of these microorganisms.

**Introduction**

The African penguin (*Spheniscus demersus*) is considered an endangered species (BirdLife International 2015) that breeds from central Namibia to South Africa’s Eastern Cape Province (Hockey, Dean & Ryan 2005) (Figure 1). There has been more than a 60% decrease in the population between 2001 and 2009, mainly attributable to changes in overall abundance and local availability of prey (Crawford et al. 2006, 2011; Sherley et al. 2013). The levels of breeding success were deemed inadequate to sustain the African penguin population, and among other conservation efforts, limiting mortality through controlling the spread of disease was suggested to try to maintain an equilibrium situation (Crawford et al. 2006).

Disease is a major ecological force that has the potential to cause significant effects especially in threatened populations (Friend, McLean & Dein 2001) and Heard et al. (2013) showed that the threat of disease increases with the level of extinction risk in all species. However, there is limited knowledge on the effects of disease on population dynamics of seabirds (Lewison et al. 2012) or even for the role of disease as a major threat to species at risk of extinction (Heard et al. 2013). While a single disease outbreak could decimate a population, the true cost of disease may be associated with chronic attrition of the population (Friend et al. 2001) and thereby influence metabolic rate, life history traits and social status (Barbosa & Palacios 2009).

Comprehensive health assessments of free-ranging avian species have rarely been reported in the literature (Smith et al. 2008). Modern conservation efforts can be enhanced by the availability of comprehensive health assessment data at a population level (Karesh & Cook 1995). Disease is often listed as a predicted threat to threatened species but this is generally a precautionary approach because there is a lack of surveillance data necessary to fully evaluate the threat (Heard et al. 2013). Therefore, health assessments and the compilation of baseline data on the presence of parasites and potential pathogens fill a critical data gap, particularly for endangered species. If a species is negatively affected by a major threat other than disease, that species is more likely to be simultaneously threatened by disease (Heard et al. 2013).

Several parasites have been recorded from the African penguin: seven nematode species, two larval trematode species, one argasid tick species and two louse species (Brandão, Moreira & Luque 2014). Only the larval trematode *Cardiocephaloides physalis* has caused mortality in the African penguin (Randall & Bray 1983; Horne, Bray & Bousfield 2011); however, all parasite species may affect the fitness of the host, predispose the individual to disease, cause poor breeding productivity and...
nest desertion (Brandão et al. 2014; Duffy 1983; Kanarek, Horne & Zalesny 2013).

A large-scale health assessment was conducted on the African penguin following the methods reported by Karesh et al. (1999), Smith et al. (2008) and Travis et al. (2006) on other penguin species, using blood smear examination, haematology, biochemistry and serology. Adult penguins in the breeding season on the colonies in South Africa as well as penguins admitted for rehabilitation were sampled. Additional samples included banked serum samples from penguins previously admitted to the Southern African Foundation for the Conservation of Coastal Birds (SANCCOB) and previous colony samples.

**Methods**

**Sampling procedures**

A total of 578 samples from the breeding range were analysed in this study. These samples were obtained from African penguins bled at Western Cape and Eastern Cape breeding colonies as well as from African penguins bled on admission for rehabilitation to SANCCOB (Cape Town, Western Cape); collected from various Namibian colonies in 2009 and from areas in the Western Cape from 2010 to 2013 (Figure 1). African penguins are admitted for rehabilitation because of oiling, debilitation, injuries, arrested moult and eggs and chicks admitted for hand-rearing (Parsons & Underhill 2005). Table 1 summarises the distribution of the sampling effort in relation to the sample collection site, month and year, clinical history and laboratory examinations.

Colony samples were collected from penguins that were visually healthy on examination by a veterinarian. All penguins sampled at breeding colonies were adults, with the exception of 11 chicks and 1 juvenile sampled in the Western Cape 2007–2008 group. Birds selected were either resting in the colony or sitting on nests with medium to large chicks. Handling time was 5–10 min per bird, and the birds were released near their nest sites. Samples collected from penguins in the Namibia 2009 group were obtained on the second day of admission to the centre, and all were adults. Samples collected from penguins in the rehabilitation 2010–2013 group were obtained within the first 3 days of admission to the centre and comprised 53 chicks, 25 juveniles and 87 adults. For 365 of the total samples, sex was determined through routine DNA analysis by Molecular Diagnostic Services (Pty) Ltd (Durban, South Africa): 186 male penguins (51%) and 179 female penguins (49%).
TABLE 1: Summary of the sampling effort and tests evaluated during this health assessment of African penguins.

| Group          | Location       | Sampling period | Clinical history | Sample size | Blood smear | Haematology | Serum chemistry | Serology | Sexing |
|----------------|----------------|-----------------|------------------|-------------|-------------|-------------|----------------|----------|--------|
| Western Cape  | Dassen Island  | December 2007   | Healthy          | 38          | -           | -           | -              | X        | -      |
|                | Dassen Island  | December 2008   | Healthy          | 41          | -           | -           | -              | X        | -      |
|                | Robben Island  | December 2007   | Healthy          | 17          | -           | -           | -              | X        | -      |
|                | Robben Island  | December 2008   | Healthy          | 38          | -           | -           | -              | X        | -      |
|                | Boulders       | February 2008    | Healthy          | 10          | -           | -           | -              | X        | -      |
|                | Dyer Island    | January 2008     | Healthy          | 23          |            | -           | -              | X        | -      |
|                | Dyer Island    | December 2008    | Healthy          | 38          | -           | -           | -              | X        | -      |
| Namibia 2009   | Halifax Island | April 2009       | Oiled            | 9           | X           | -           | -              | X        | X      |
|                | Ichabee Island  | April 2009       | Oiled            | 12          | X           | -           | -              | X        | X      |
|                | Mercury Island | April 2009       | Oiled            | 15          | X           | -           | -              | X        | X      |
| Western Cape  | Dassen Island  | December 2011    | Healthy          | 20          | X           | X           | X              | X        | X      |
|                | Robben Island  | June 2011        | Healthy          | 20          | X           | X           | X              | X        | X      |
|                | Boulders       | August 2012      | Healthy          | 20          | X           | X           | X              | X        | X      |
|                | Betty’s Bay     | August 2010      | Healthy          | 20          | X           | X           | X              | X        | X      |
|                | Dyer Island     | August 2010      | Healthy          | 20          | X           | X           | X              | X        | X      |
| Eastern Cape   | Bird Island     | July 2012        | Healthy          | 50          | X           | X           | X              | X        | X      |
|                | St. Croix Island| July 2012        | Healthy          | 17          | X           | X           | X              | X        | X      |
| Rehabilitation | SANCCOB         | 2010–2013        | Oiled            | 60          | X           | X           | X              | X        | X      |
|                | SANCCOB         | 2010–2013        | Chick            | 53          | X           | X           | X              | X        | X      |
|                | SANCCOB         | 2010–2013        | Moulting         | 17          | X           | X           | X              | X        | X      |
|                | SANCCOB         | 2010–2013        | Weak or wounded  | 35          | X           | X           | X              | X        | X      |

SANCCOB, Southern African Foundation for the Conservation of Coastal Birds.

Haematology

Blood (5 mL – 20 mL) was collected through veni-puncture of the jugular vein using a 21-G needle (25 mm × 0.8 mm), immediately transferred into ethylenediaminetetraacetic acid and serum clot activator tubes (Vacuette®; Greiner Bio-One, Austria) and stored at 4 °C for up to 60 h until being analysed. Serum clot activator tubes were centrifuged and serum transferred into separate eppendorf tubes and immediately frozen at -20 °C. Blood smears were prepared, air-dried, fixed in methanol and stained with modified Wright–Giemsa stain (Kyro-Quick®; Kron Laboratories [Pty] Ltd, Benrose, South Africa). All slides were examined for blood parasites for 10 min using a 50× oil immersion lens with a 10× eyepiece. Haematology and biochemistry analyses were performed following routine laboratory procedures at IDEXX Laboratories (Pty) Ltd (Cape Town, South Africa) (see Parsons et al. 2015b for details).

Serology

The frozen serum samples were submitted to the Western Cape Provincial Veterinary Laboratory (Stellenbosch, South Africa) for haemagglutination inhibition assay (HIA) for avian influenza virus subtypes H5 and H7 (AIV H5, AIV H7) and Newcastle disease virus (NDV) and for serum plate agglutination (SPA) testing for Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS). The HIA testing for avian influenza virus was done according to the protocol for non-chicken species (World Organisation for Animal Health 2014). Additionally, samples were submitted to IDEXX Laboratories (Pty) Ltd (Johannesburg, South Africa) for indirect enzyme-linked immunosorbent assay (ELISA) testing for avian infectious bronchitis virus (IBV), avian encephalomyelitis virus (AEV), avian reovirus (ARV), infectious bursal disease virus (IBDV), MG and MS (Table 2). ELISA testing used secondary antibodies targeting chicken IgY. In the case of Mycoplasma spp., SPA and ELISA were used to test different subsets of samples. Because of the occurrence of herpesvirus respiratory infections at the same facility (Parsons et al. 2015a), a limited number of samples were submitted to Agrilabs (Pioneerfoods [Pty] Ltd, Malmesbury, South Africa) to be tested for infectious laryngotracheitis virus (ILTV, also referred to as gallid herpesvirus 1) through indirect ELISA.

Data analysis

Statistical significance was set at 0.05 and tests were two-tailed using SPSS 21 for Windows (IBM Corp., 2011, Armonk, NY, USA). Fisher’s exact test was used to evaluate if the seroprevalence (number of positive samples/number of samples tested) for Mycoplasma spp. was different in relation to the serological test (SPA or ELISA). The data set presented by Parsons et al. (2015b) was used as haematological reference values for comparison with seropositive individuals; this data set comprises the seronegative and blood parasite-negative, apparently healthy adult African penguins sampled at colonies in this study. Mann-Whitney tests were used to compare haematological results between reference values and individuals that were seropositive for AEV, MG (SPA test) or two or more pathogens. Haematological results of individuals seropositive for other pathogens were not included in this analysis because of insufficient sample size (less than five samples).

Because c. 60% of the blood parasite-positive individuals were chicks, a different data set had to be used as reference values to evaluate the haematological results of these individuals; seronegative and blood parasite-negative apparently healthy African penguin chicks admitted to...
TABLE 2: Diagnostic results for pathogens tested during this health assessment of African penguins.

| Pathogen                     | Test       | Western Cape 2007–2008 | Western Cape 2010–2012 | Eastern Cape 2012 | Namibia spill 2009 | Rehabilitation 2010–2013 | Total |
|------------------------------|------------|------------------------|------------------------|-------------------|-------------------|--------------------------|-------|
|                              | %          | n                      | %                      | %                 | %                 | %                        | %     |
| Avian encephalomyelitis virus| ELISA      | 0.93                   | 107                    | 5.00              | 0.00              | 0.00                     | 10.00 |
| Avian infectious bronchitis virus| ELISA    | 6.54                   | 107                    | 0.00              | 0.00              | 0.00                     | 40.00 |
| Avian Influenza Virus subtype H5 | HIA       | 0.00                   | 98                     | 0.00              | 0.00              | 0.00                     | 0.00  |
| Avian Influenza Virus subtype H7 | HIA       | 0.00                   | 98                     | 0.00              | 0.00              | 0.00                     | 0.00  |
| Avian reovirus               | ELISA      | 2.80                   | 107                    | 0.00              | 0.00              | 0.00                     | 5.00  |
| Infectious bursal disease virus| ELISA   | 4.67                   | 107                    | 2.00              | 0.00              | 0.00                     | 15.00 |
| Infectious salpingitis virus  | ELISA      | Not tested             | Not tested             | Not tested        | Not tested        | Not tested               | 0.00  |
| Newcastle Disease Virus      | HIA        | 2.04                   | 98                     | 3.00              | 0.00              | 0.00                     | 0.00  |
| Mycoplasma gallisepticum     | SPA        | 5.26                   | 95                     | 12.64             | 53                | 0.00                     | 3.08  |
| Mycoplasma gallisepticum     | ELISA      | Not tested             | Not tested             | Not tested        | Not tested        | Not tested               | 0.00  |
| Mycoplasma synoviae          | SPA        | 9.47                   | 95                     | 1.72              | 58                | 0.00                     | 2.04  |
| Mycoplasma synoviae          | ELISA      | Not tested             | Not tested             | Not tested        | Not tested        | Not tested               | 0.00  |
| Total                        | Blood smear| 2.50                   | 100                    | 0.00              | 67                | 34.15                    | 6.06  |

Blood smear examination

| Babesia sp.                     | Not tested | 3.00 | 100 | 1.52 | 66 | 2.44 | 41 | 17.68 | 164 | 9.16 | 371 |
| Borrelia sp.                    | Not tested | 0.00 | 100 | 0.00 | 66 | 0.00 | 41 | 1.83  | 164 | 0.81 | 371 |

SPA, serum plate agglutination; HIA, haemagglutination inhibition assay; ELISA, enzyme-linked immunosorbent assay.

SANCCOB were used as a reference data set. Mann–Whitney tests were used to compare haematological results between these reference values and individuals positive for Babesia sp. On the other hand, Borrelia sp.—positive and mixed infection—positive individuals were not included in this analysis because of insufficient sample size.

Results

A total of 578 individuals were screened; of those, 68 penguins were seropositive for at least one of the nine pathogens tested (Table 2); of these, 12 individuals were seropositive for more than one of the diseases tested: AEV + IBDV (2 samples), AEV + IBV (1), AEV + IBDV + IBV (1), AEV + MG (1), ARV + IBV (1), IBDV + IBV (2), IBDV + MS (1), MG + MS (2), and MG + NDV (1). All samples were seronegative for AIV H5, AIV H7 and ILTV. Samples tested for antibodies against Mycoplasma spp. using SPA were more frequently positive (4.2% for MG and 8.1% for MS) than those tested using ELISA (0.5% for both MG and MS); this occurred for both MG (p < 0.01) and MS (p = 0.03). Haematological results for seropositive individuals are presented in Table 3.

Blood smears revealed 33 samples were positive for Babesia sp., 2 individuals were positive for Borrelia sp. and 1 individual was positive for both Babesia sp. and Borrelia sp. (Table 2); no other blood parasites were observed. These blood parasites were morphologically consistent with those documented by Earle et al. (1993) and Yabsley et al. (2012). Only two blood smear–positive individuals (Babesia-positive) were also found to be seropositive: one was seropositive to MG (SPA test) and the other was seropositive to both AEV and IBDV. Haematological results for blood smear–positive individuals are presented in Table 4.

Of the positive individuals, 66 (97%) were adults compared to two (3%) chicks. There was no difference across genders. Of the positive adults, there were 49 (74%) that were sampled as healthy individuals in wild colonies (90% unknown breeding status, 10% sitting with chicks) and 17 (26%) sampled when admitted for rehabilitation (94% oiled, 6% injured). There was a significant difference in the prevalence of seropositive individuals between the three geographical areas: Namibia, Western Cape and Eastern Cape. Complete details on the sampling effort and serological and blood smear results in relation to age group and sex are provided in Table 1-A1, and in relation to breeding colony in Table 2-A1.

Ethical considerations

Research permits to conduct this work were obtained by the Department of Environmental Affairs (DEA) (RES2012/61 EXT, RES2011/19, and RES2010/58), CapeNature (AAA007-00047-0056, AAA004-0508-0035, AAA004-000120-0035 and AAA007-00040-0035) and South African National Parks (PARSN1027). Procedures were approved by the Animal Ethics Committee of the DEA, and all blood samples were collected by veterinarians (N.J.P., T.A.G.) registered with the South African Veterinary Council. Where applicable, ARRIVE guidelines for reporting *in vivo* animal experiments (Kilkenny et al. 2010) have been adhered to.

Discussion

Our results should be interpreted taking into account the characteristics and inherent limitations of the serological tests used in this study. Because serological tests specifically designed for African penguins are not currently available, we used commercial tests designed for poultry. The indirect
| Parameter                  | Unit            | Reference values (healthy adults) mean ± s.d. | Avian encephalomyelitis virus mean ± s.d. | Mycoplasma galliseptum (serum plate agglutination test) mean ± s.d. | Two or more pathogens mean ± s.d. | Infectious bursal disease virus mean ± s.d. | Infectious bronchitis virus mean ± s.d. | Newcastle disease virus mean ± s.d. |
|---------------------------|-----------------|---------------------------------------------|-------------------------------------------|---------------------------------------------------------------|-------------------------------|-------------------------------------------|----------------------------------------|----------------------------------------|
| Head length               | mm              | 121.1 ± 3.9                                | 120.0 ± 5.9                               | 120.1 ± 3.2                                                   | 118.4 ± 7.4                  | 127.2 ± 1                             | 117.9 ± 1                          | 118.3 ± 2                             |
| Body mass                 | kg              | 2.86 ± 0.37                                | 2.62 ± 0.40                               | 2.76 ± 0.30                                                  | 2.71 ± 0.50                  | 2.84 ± 1                              | 2.43 ± 0.10                         | 2.62 ± 2                             |
| Haematocrit               | %               | 46.0 ± 5.7                                 | 46.1 ± 4.6                                | 45.1 ± 3.3                                                   | 46.6 ± 7.9                   | 38.0 ± 1                              | 50.6 ± 3.5                          | 51.0 ± 2                             |
| Haemoglobin               | g/dL            | 18.4 ± 2.4                                 | 19.2 ± 0.6                                | 18.9 ± 1.8                                                   | 19.1 ± 1.6                   | 16.1 ± 1                              | 19.3 ± 1                            | 19.3 ± 2                             |
| Red blood cell count      | 10⁹/L           | 1.82 ± 0.09                                | 1.94 ± 0.09                               | 1.83 ± 0.19                                                  | 1.93 ± 0.15                  | 1.65 ± 1                              | 1.99 ± 1                            | 1.88 ± 2                             |
| MCV                       | fl              | 251.0 ± 35.6                               | 243.1 ± 13.9                              | 248.5 ± 27.8                                                | 246.3 ± 13.1                 | 230.3 ± 1                             | 236.2 ± 1                           | 271.9 ± 2                            |
| MCH                       | pg              | 101.1 ± 14.3                               | 99.0 ± 2.7                                | 104.0 ± 11.5                                                | 98.8 ± 4.6                   | 97.6 ± 1                              | 97.0 ± 1                            | 102.7 ± 2                            |
| Sodium                    | mmol/L          | 154 ± 6                                    | 146 ± 9                                   | 145 ± 5                                                      | 151 ± 5                      | 148 ± 1                               | 146 ± 1                             | 158 ± 1                              |
| Potassium                 | mmol/L          | 5.09 ± 2.5                                 | 5.00 ± 1.4                                | 10.05 ± 4.3                                                 | 4.85 ± 1.12                  | 6.20 ± 1                              | 5.86 ± 1                            | 3.65 ± 1                             |
| Chloride                  | mmol/L          | 1.21 ± 6                                   | 1.15 ± 7                                  | 1.15 ± 5                                                    | 1.16 ± 4                     | 1.14 ± 1                              | 1.09 ± 1                            | 1.28 ± 1                             |
| Calcium                   | mmol/L          | 2.77 ± 0.82                                | 2.53 ± 0.19                               | 2.43 ± 0.24                                                 | 2.50 ± 0.09                  | 1.96 ± 1                              | 2.35 ± 1                            | 2.58 ± 1                             |
| Inorganic phosphate       | mmol/L          | 1.53 ± 0.62                                | 1.35 ± 0.46                               | 1.65 ± 0.13                                                 | 1.48 ± 0.55                  | 1.20 ± 1                              | 1.80 ± 1                            | 1.69 ± 1                             |
| Creatinine                | mmol/L          | 24.1 ± 11.9                                | 20.0 ± 17.0                               | 12.6 ± 9.9                                                  | 16.8 ± 8.5                   | 5.0 ± 1                               | 4.0 ± 1                             | 16.0 ± 1                             |
| Cholesterol               | mmol/L          | 5.36 ± 1.36                                | 5.41 ± 1.20                               | 6.12 ± 1.37                                                 | 5.32 ± 0.67                  | 5.70 ± 1                              | 4.30 ± 1                            | 6.10 ± 1                             |
| Glucose                   | mmol/L          | 11.8 ± 2.2                                 | 12.5 ± 1.4                                | 12.6 ± 1.2                                                  | 12.2 ± 1.5                   | 11.1 ± 1                              | 11.9 ± 1                            | 10.0 ± 1                             |
| Uric Acid                 | mmol/L          | 394 ± 221                                  | 604 ± 482                                 | 345 ± 280                                                   | 539 ± 186                    | 73 ± 1                                | 448 ± 1                             | 263 ± 1                              |
| Bile Acids                | mmol/L          | 9.53 ± 16.75                               | 18.26 ± 15.33                             | 9.26 ± 13.30                                                | 17.58 ± 21.15               | 1.64 ± 1                              | 7.20 ± 1                            | 2.35 ± 2                             |
| Total serum protein       | g/L             | 59.0 ± 9.6                                 | 53.7 ± 7.0                                | 70.0 ± 5.8                                                  | 61.8 ± 8.6                   | 5.37 ± 1                              | 5.10 ± 1                            | 4.90 ± 1                             |
| Albumin                   | g/L             | 19.3 ± 4.0                                 | 16.6 ± 3.2                                | 21.0 ± 3.2                                                  | 20.5 ± 3.0                   | 16.6 ± 1                              | 16.0 ± 1                            | 15.0 ± 1                             |
| Globulin                  | g/L             | 39.8 ± 6.3                                 | 37.1 ± 3.9                                | 49.0 ± 3.5                                                  | 41.3 ± 5.8                   | 37.1 ± 1                              | 35.0 ± 1                            | 35.0 ± 1                             |
| Albumin / globulin        |                | 0.48 ± 0.05                                | 0.44 ± 0.05                               | 0.50 ± 0.06                                                 | 0.46 ± 0.04                  | 0.46 ± 1                              | 0.43 ± 1                            | 0.43 ± 1                             |
| Aspartate transaminase    | U/L             | 218 ± 90                                   | 279 ± 35                                  | 247 ± 107                                                   | 184 ± 44                     | 151 ± 1                               | 326 ± 1                             | 120 ± 1                              |
| Creatine kinase           | U/L             | 419 ± 272                                  | 461 ± 498                                 | 561 ± 358                                                   | 365 ± 187                    | 188 ± 1                               | 375 ± 1                             | 385 ± 1                              |

Source: Reference values obtained from Parsons et al. (2015b); all other values from this study.

MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration.

*, indicate groups that were significantly different from the reference values (only evaluated when sample size was ≥5).
| Parameter               | Unit         | Reference values | Healthy chicks | Healthy adults | Babesia sp. | Chicks | Juveniles and adults | Barreloa sp. | Co-infection by Babesia sp. and Barreloa sp. |
|-------------------------|--------------|------------------|----------------|---------------|-------------|--------|----------------------|-------------|---------------------------------------------|
| Head length             | mm           | mean ± s.d.      | 106.7 ± 4.8    | 30            | 121.1 ± 3.9 | 108    | 103.9 ± 5.2          | 119.3 ± 4.3 | 104.1 ± 2.0                                  |
| Body mass               | kg           | mean ± s.d.      | 2.32 ± 0.30    | 30            | 2.86 ± 0.37 | 108    | 2.20 ± 0.20          | 2.36 ± 0.50 | 1.67 ± 2.0                                   |
| Haematocrit             | %            | mean ± s.d.      | 3.17 ± 6.4     | 30            | 46.0 ± 5.7  | 107    | 29.3 ± 5.0           | 40.5 ± 10.7 | 35.5 ± 2.0                                   |
| Haemoglobin             | g/dL         | mean ± s.d.      | 12.6 ± 1.9     | 22            | 18.4 ± 2.4  | 103    | 9.3 ± 2.3            | 15.1 ± 5.4  | 12.2 ± 1.8                                   |
| Red blood cell count    | 10^9/L       | mean ± s.d.      | 1.55 ± 0.18    | 22            | 1.82 ± 0.26 | 103    | 1.15 ± 0.32          | 1.65 ± 0.37 | 1.61 ± 1.0                                   |
| MCV                     | ft           | mean ± s.d.      | 220.8 ± 14.7   | 22            | 251.0 ± 35.6| 103    | 275.5 ± 41.6         | 235.0 ± 30.3| 211.2 ± 1.0                                  |
| MCH                     | pg           | mean ± s.d.      | 8.13 ± 4.8     | 22            | 101.1 ± 14.3| 103    | 81.5 ± 4.3           | 88.3 ± 19.3 | 75.8 ± 1.0                                   |
| MCHC                    | g/dL         | mean ± s.d.      | 3.72 ± 3.0     | 22            | 40.0 ± 3.5  | 103    | 30.1 ± 4.1           | 37.2 ± 5.3  | 35.9 ± 1.0                                   |
| White blood cell count  | 10^9/L       | mean ± s.d.      | 15.0 ± 5.2     | 22            | 17.7 ± 8.4  | 103    | 21.2 ± 5.0           | 31.4 ± 23.4 | 24.6 ± 1.0                                   |
| Sodium                  | mmol/L       | mean ± s.d.      | 146 ± 5        | 27            | 154 ± 6    | 105    | 148 ± 3              | 144 ± 13    | 140 ± 2                                      |
| Potassium               | mmol/L       | mean ± s.d.      | 5.45 ± 0.71    | 27            | 5.09 ± 2.52 | 105    | 5.67 ± 0.74          | 5.57 ± 159  | 4.91 ± 2.0                                   |
| Chloride                | mmol/L       | mean ± s.d.      | 113 ± 5        | 27            | 121 ± 6    | 104    | 115 ± 3              | 112 ± 9     | 108 ± 1                                      |
| Calcium                 | mmol/L       | mean ± s.d.      | 2.58 ± 0.15    | 27            | 2.77 ± 0.82| 105    | 2.57 ± 0.11          | 2.38 ± 0.35 | 2.53 ± 2.0                                   |
| Inorganic phosphate     | mmol/L       | mean ± s.d.      | 1.89 ± 0.28    | 26            | 1.53 ± 0.62| 105    | 2.31 ± 0.49          | 2.08 ± 1.55 | 2.35 ± 2.0                                   |
| Creatinine              | mmol/L       | mean ± s.d.      | 17.6 ± 12.6    | 27            | 24.1 ± 11.9| 105    | 20.6 ± 8.4           | 41.6 ± 44.8 | 41.5 ± 2.0                                   |
| Cholesterol             | mmol/L       | mean ± s.d.      | 4.45 ± 1.03    | 27            | 5.36 ± 1.36| 105    | 4.61 ± 0.96          | 4.95 ± 2.13 | 5.00 ± 2.0                                   |
| Glucose                 | mmol/L       | mean ± s.d.      | 12.3 ± 1.3     | 27            | 11.8 ± 2.2 | 105    | 12.2 ± 1.0           | 10.4 ± 4.7  | 10.9 ± 2.0                                   |
| Uric Acid               | mmol/L       | mean ± s.d.      | 652 ± 3.19     | 27            | 394 ± 22.1 | 104    | 542 ± 436            | 562 ± 488  | 460 ± 2.0                                    |
| Bile Acids              | mmol/L       | mean ± s.d.      | 25.47 ± 14.25  | 22            | 95.3 ± 16.75| 87     | 14.19 ± 11.20        | 9.16 ± 13.37| 43.10 ± 2.9                                   |
| Total serum protein     | g/L          | mean ± s.d.      | 43.1 ± 5.4     | 25            | 59.0 ± 9.6 | 105    | 46.3 ± 4.6           | 48.2 ± 17.7 | 45.5 ± 2.0                                   |
| Albumin                 | g/L          | mean ± s.d.      | 13.7 ± 1.4     | 27            | 19.3 ± 4.0 | 105    | 14.1 ± 1.6           | 15.5 ± 5.8 | 13.5 ± 2.0                                   |
| Globulin                | g/L          | mean ± s.d.      | 29.6 ± 4.4     | 25            | 39.8 ± 6.3 | 105    | 31.8 ± 4.0           | 32.7 ± 12.3 | 32.0 ± 2.0                                   |
| Albumin / globulin      | -            | mean ± s.d.      | 0.57 ± 0.05    | 25            | 0.48 ± 0.05| 105    | 0.45 ± 0.04          | 0.49 ± 0.10 | 0.42 ± 0.2                                   |
| Aspartate transaminase  | U/L          | mean ± s.d.      | 146 ± 5        | 27            | 218 ± 90  | 104    | 151 ± 38             | 376 ± 247  | 797 ± 2.0                                    |
| Creatine kinase         | U/L          | mean ± s.d.      | 392 ± 124      | 27            | 419 ± 272 | 105    | 368 ± 153            | 1275 ± 2283| 1559 ± 2.0                                   |

Source: Reference values for adults obtained from Parsons et al. (2015b), all other values from this study.

MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration.

*, indicate groups that were significantly different from the reference values of their age class (only evaluated when the sample size was ≥ 5).
ELISA tests used in this study rely on the basic assumption that antibodies against chicken IgY can also reliably recognise penguin IgY. While these specific commercial tests have not undergone thorough validation to estimate their sensitivity and specificity when applied to samples from African penguins, other studies on the antigenic properties of penguin immunoglobulins corroborate the validity of their basic methodological assumption (Bizelli et al. 2015; Graczyk et al. 1994, 1995). Unfortunately, the lack of serological tests specifically designed or validated for penguins is a recurrent methodological limitation of serological inquiries in these species (Karesh et al. 1999; Nunes et al. 2012; Smith et al. 2008; Travis et al. 2006; Uhart et al. 2004), which hopefully will be overcome through ongoing research aiming at the production of secondary antibodies specifically targeting penguin IgY (Bizelli et al. 2015). On the other hand, the HLA used to test for NDV, AIV H5 and AIV H7 is not subject to this limitation because it does not rely on the recognition by secondary antibodies.

It is also worth noting that this is not a comprehensive study into all pathogens and parasites that can affect the health of African penguins on an individual or population level. Further studies looking at epidemiology as well as interaction between parasites, pathogens and fitness of individuals are encouraged.

**Avian encephalomyelitis virus (Picornaviridae)**

Seropositivity to AEV was identified in the Namibian and the Western Cape samples and in penguins admitted for rehabilitation at SANCCOB; overall seroprevalence was relatively low (2.9%). AEV has been documented in domestic birds in South Africa (Odend’hal 1983), but it has never been demonstrated to infect penguins by direct diagnostic methods. Serological surveys examining penguins in Peru and at the Falkland and Galapagos Islands have only found negative results (Smith et al. 2008; Travis et al. 2006; Uhart et al. 2004), whereas Karesh et al. (1999) found antibodies against AEV in southern rockhopper penguins (Eudyptes chrysocome) in Argentina, with seroprevalence (3%) similar to that observed in this study.

AEV infections seldom cause clinical disease in adult chickens, but can lead to significant decreases in egg production and hatchability; however, in young chickens, AEV can produce paralysis, ataxia and muscular dystrophy (Tannock & Shafren 1994). In this study, AEV seropositive penguins had slightly lower serum sodium and chloride concentrations; this cannot be explained by the pathogenesis of AEV infection and is therefore interpreted as an incidental finding.

**Avian infectious bronchitis virus (Coronaviridae)**

Seropositivity to IBV was identified in the Namibian and the Western Cape samples and in penguins admitted for rehabilitation at SANCCOB; overall seroprevalence was relatively low (3.6%). Few studies have tested penguins for antibodies against IBV. Karesh et al. (1999) found a seroprevalence between 23% and 47% (depending on the titre cutpoint) in southern rockhopper penguins in Argentina, whereas Smith et al. (2008) did not detect antibodies against this pathogen in Humboldt penguins (Spheniscus humboldti) in Peru. DNA from coronaviruses has been detected in the tissues of beachcast carcasses of Magellanic penguins (Spheniscus magellanicus) in Brazil; however, it is unclear whether these viruses were associated with disease (Niemeyer et al. 2012).

Coronaviruses such as IBV are known to cause respiratory, intestinal and reproductive diseases in both domestic and wild birds (Gerlach 1994). However, the significance of this infection in penguins is unclear. Individuals that were seropositive for IBV had significantly lower body mass but not head length than otherwise healthy adults, suggesting poorer body condition compared to those that were seronegative. However, this result should be interpreted with caution considering the low sample size.

**Avian influenza virus (Orthomyxoviridae)**

We found no serological evidence of highly pathogenic influenza A virus (subtypes H5 and H7), despite past evidence of their circulation in wild birds in South Africa (Abolnik et al. 2012; Cumming et al. 2011). Penguin seropositivity to AIV has been demonstrated by studies in the Antarctic (Abad et al. 2013; Morgan & Westbury 1981; Wallensten et al. 2006) and Subantarctic (Abad et al. 2013), and Hurt et al. (2014) have demonstrated that the AIV H11N2 present in penguins on the Antarctic Peninsula is an evolutionarily distinct lineage, not closely related to AIV strains from migratory flying birds. On the other hand, the few serological studies on penguins at lower latitudes conducted to date have failed to demonstrate exposure to AIV (Karesh et al. 1999; Smith et al. 2008; Travis et al. 2006). However, this is unlikely to result from an absence of circulation of these viruses, as their worldwide distribution has been extensively documented (Olsen et al. 2006). It is likely that these negative results reflect the fact that AIV occurrence is highly variable and species and location dependent (Hanson et al. 2008). It must also be considered that antibodies against AIV subtypes other than H5 and H7 would have gone undetected by the tests used in this study.

**Avian reovirus (Reoviridae)**

Antibodies against ARV were detected in wild African penguins sampled in Namibia and the Western Cape, with a low overall seroprevalence (0.9%). Reovirus-like agents with some similarity to reference chicken reovirus strain were isolated in African penguins that died at a zoo in the United Kingdom (Gough et al. 2002). However, in that case, the birds were seronegative to the one-way neutralisation test, and it was unclear what role the virus played in their deaths (Gough et al. 2002). Surveys in Peru and on the Falkland and Galapagos Islands have found only seronegative penguins (Smith et al. 2008; Travis et al. 2006;
Uhart et al. (2004). On the other hand, Karesh et al. (1999) detected antibodies against ARV in 23% of southern rockhopper penguins sampled in Argentina. ARV has been documented in domestic birds worldwide, including South Africa, and may lead to a broad variety of clinical presentations (Gerlach 1994; Van Loon et al. 2001).

**Infectious bursal disease virus (Birnaviridae)**

Antibodies against IBDV were detected in wild African penguins sampled in Namibia and the Western Cape and in penguins admitted for rehabilitation at SANCCOB; overall seroprevalence was relatively low (2.7%). Antibodies against IBDV have been demonstrated in penguins by studies using ELISA in Brazil (Nunes et al. 2012) and virus neutralisation tests in Crozet Archipelago and at various locations in Antarctica (Gardner, Kerry & Riddle 1997; Gauthier-Clerc et al. 2002; Watts, Miller & Shellam 2009), whereas studies using agar-gel diffusion tests have failed to obtain positive results in South America (Karesh et al. 1999; Smith et al. 2008; Travis et al. 2006). Watts et al. (2009) argue that IBDV serotype 1 is endemic and widespread in Antarctic birds, with Emperor penguins (Aptenodytes forsteri) playing a key role in the virus’ persistence in Antarctica.

IBDV is known to cause disease in young chickens, in which it can produce bursal lymphoid depletion and high mortality (World Organisation for Animal Health 2008). No clinical signs of disease have been observed in any of the seropositive penguin species in the wild (Gardner et al. 1997; Gauthier-Clerc et al. 2002; Nunes et al. 2012; Watts et al. 2009). Gough et al. (2002) reported the isolation of IBDV serotype 2 from the tissues of African and Macaroni penguins (Eudyptes chrysocome) deceased at a zoo in the United Kingdom and considered that although the infection was not primarily responsible for the deaths, it may have exacerbated concurrent disease conditions. Unfortunately, in this study, we did not have a sufficient number of seropositive penguins to evaluate the potential health effects of exposure to IBDV.

**Infectious laryngotracheitis virus (Herpesviridae)**

There were no positive samples in serology testing for ILTV (also known as gallid herpesvirus 1) despite previous evidence that African penguins are susceptible to herpesvirus-like infections (Kincaid, Bunton & Cranfield 1988; Parsons et al. 2015a). Previous studies on other penguin species have also failed to identify antibodies against this virus (Karesh et al. 1999; Smith et al. 2008). Wild African penguin chicks have presented herpesvirus-like respiratory infections, which were not detected by molecular or serological tests targeting ILTV, suggesting that a different herpesvirus was involved (Parsons et al. 2015a).

**Newcastle disease virus (Paramyxoviridae)**

Five individuals were seropositive to NDV (also known as avian paramyxovirus type 1), all of which were sampled in the Western Cape. Penguins that were seropositive for NDV have been demonstrated in the Antarctic (Morgan & Westbury 1981), Argentina (Karesh et al. 1999), Macquarie Island (Morgan et al. 1981) and South Shetland Islands (Thomazelli et al. 2010). Thomazelli et al. (2010) determined that the strains detected in penguins at the South Shetlands Islands had low pathogenicity. NDV infection has also been demonstrated in captive penguins in the United States (Pierson & Pflow 1975), where a velogenic neurotropic strain was identified, and in Israel (Haddas et al. 2014), where the pathogenicity of the strain could not be determined. It is clear that penguins are susceptible to this virus and that some NDV strains, presumably those with low pathogenicity, circulate in wild penguin populations. NDV has also been demonstrated in great white pelicans (Pelecanus onocrotalus) in the Western Cape (Assunção et al. 2007).

It is interesting to note that one of the individuals identified as seropositive was a penguin that had been rehabilitated at SANCCOB 7 years earlier and, at that time, received vaccination for NDV. The vaccination consisted of an initial ocular spray vaccination on admission to the centre with live Lasota strain (Nobilis™ ND LASOTA, Kempton Park, South Africa) followed by an intramuscular injection of inactivated Lasota strain (Lomovac, TAD, Germany) (N.J. Parsons, unpublished data). There is no literature, to our knowledge, that determines how long the vaccination antibodies remain detectable in a penguin following vaccination. Although it is unlikely that antibodies are still circulating 7 years after vaccination, it is possible that vaccination may have interfered with the results. SANCCOB stopped routinely marking all penguins before release into the wild in August 2005, but routinely vaccinated for NDV up until August 2008.

**Mycoplasma spp.**

Serological tests for MG and MS have not been routinely used in wild penguin species. There was inconsistency between the serological tests, with a higher frequency of positives when samples were tested with SPA compared to ELISA testing. While different subsets of samples were tested with each test, this discrepancy suggests an inherent difference in the sensitivity and specificity of the two tests. It is also important to consider that cross-reactivity with other Mycoplasma spp. from African penguins in this study is possible. Multiple Mycoplasma spp. (excluding MG and MS) have been demonstrated to occur in penguins (Banks, Cary & Hogg 2009; Buckle et al. 2013; Dewar et al. 2013; Frasca et al. 2005). Furthermore, Frasca et al. (2005) found cross-reactivity of antibodies against Mycoplasma sphenisci to antibodies against MG and MS in agglutination tests. Therefore, caution should be used when interpreting these results.

MG and MS potentially cause respiratory disease, sinusitis, conjunctivitis and synovitis in domestic and wild birds (Jordan 1975). Mycoplasma sphenisci was described in an African penguin showing signs of upper respiratory tract disease in a North American aquarium (Frasca et al. 2005) and M. lipofaciens was identified from the lungs of a Fiordland penguin (Eudyptes pachyrhynchus) after post-mortem
examination showed bronchopneumonia (Buckle et al. 2013). On the other hand, *M. sphenisci* and other *Mycoplasma* spp. have been detected in the faeces of apparently healthy penguins in Antarctica and subantarctic islands (Banks et al. 2009; Dewar et al. 2013). In this study, African penguins seropositive to MG in the SPA test had considerably lower serum concentrations of sodium, chloride and creatinine and higher concentrations of potassium, suggesting impairment of kidney function. Although MG and MS are known to produce renal lesions, these tend to be less prominent than respiratory and articular lesions (Jordan 1975; Lockaby et al. 1998). Future studies will be necessary to identify which species of *Mycoplasma* occurs in African penguins and to confirm if it produces significant renal disease.

It is worth noting that great white pelicans have been shown to have high prevalence (98%) of *Mycoplasma* spp. in South Africa (Assunção et al. 2007). This species breeds sympatrically with and often predate on African penguins (Mwema, de Ponte Machado & Ryan 2010). Furthermore, because great white pelicans are known to feed on avian offal in agricultural areas (Crawford, Cooper & Dyer 1995), they could play a key role in spreading pathogens such as *Mycoplasma* spp. from domestic animals to seabirds (Assunção et al. 2007).

**Babesia sp. and Borrelia sp.**

Although we did not fully characterise the blood parasites, their morphology was consistent with *Babesia peircei* and relapsing fever *Borrelia* as previously described in African penguins in the same region (Earlé et al. 1993; Yabsley et al. 2012). The apparent prevalence of *Babesia* sp. in wild African penguins in this study (1.5% – 3.0%) is similar to that observed in previous studies, as is the higher frequency of *Babesia* sp. and *Borrelia* sp. among chicks and individuals undergoing rehabilitation (Brossy et al. 1999; Earlé et al. 1993; Yabsley et al. 2012).

The pathological significance of *Babesia* sp. to penguins is not clear, and so far, this parasite has only been associated with only mild regenerative anaemia (Brossy et al. 1999; Cunningham et al. 1993; Vanstreels et al. 2015). In this study, African penguin chicks with *Babesia* sp. had significantly different haematological and serum chemistry values compared to healthy chicks. *Babesia*-infected penguins had abnormalities in erythrocyte size and lower haemoglobin concentration, suggesting a regenerative response of the erythrocytic lineage, presumably to the haemolysis caused by the parasite. Higher white blood cell counts in *Babesia*-infected penguins indicate an active inflammatory response to the parasite and/or a stress response. Finally, higher serum levels of creatinine kinase and lower serum levels of uric acids and albumin indicate impairment of hepatic function and may also be partly related to haemolysis (see Harrison & Lightfoot 2006).

**Conclusion**

Considering the decreasing trend of the African penguin population (Crawford et al. 2011), disease is yet another significant threat to the species in addition to poor nutrition, environmental degradation and anthropogenic impacts (Woods et al. 2009). Serological surveillance can be a powerful tool to track the prevalence of pathogens that are otherwise difficult to detect in wildlife populations (Gilbert et al. 2013). The reported seroprevalence in this study is consistent with previously reported studies on wild penguins, suggesting that these are endemic pathogens or natural, apathogenic flora. It must also be borne in mind that the presence of antibodies indicates past exposure to a pathogen and does not necessarily indicate presence of the organism or active infection. In addition, cross-reaction of tests with other antigens and microorganisms may interfere with specificity of the results (Barbosa & Palacios 2009). Studies addressing the direct detection and isolation of pathogenic organisms in penguins are encouraged and, in combination with serological investigations, should provide deeper insight on their epidemiology in these birds.

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**Competing interests**

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this article.

**Authors’ contributions**

N.J.P. and T.A.G. designed and coordinated the study, collected samples and compiled results. A.M.S. and R.E.T.V. conducted epidemiological and statistical analyses. N.J.P., T.A.G., A.M.S. and R.E.T.V. made conceptual contributions, assisted with data interpretation and directly contributed to the preparation and revision of the manuscript.

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### Appendix 1

**Details of sampling effort and serological results in relation to age group and sex.**

| Group         | Age       | Sex       | Infectious bronchitis virus | Avian encephalomyelitis virus | Avian reovirus | AIV H5 | AIV H7 | Infectious laryngotracheitis virus | Newcastle disease virus | Mycoplasma gallisepticum (SPA) | Mycoplasma gallisepticum (ELISA) | Mycoplasma synoviae (SPA) | Mycoplasma synoviae (ELISA) | Babesia sp. | Borrelia sp. |
|---------------|-----------|-----------|-----------------------------|-------------------------------|----------------|--------|--------|-----------------------------------|------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------|-------------|
|               |           |           | n Positve | n                       | n Positve | n        | n Positve | n Positive | n Positive | n Positive | n Positive | n Positive | n Positive | n Positive | n Positive |
| **Western Cape** | **Chick** | Unknown  | 4         | - 4                   | - 4      | 7         | 7 4      | 0         | 7         | 7         | 0          | 7         | 1         | 0            | 0          | 0          |
|               | **Juvenile** | Unknown | 1         | - 1                   | - 1      | 0         | 0 1      | 0         | 0         | 0         | 0          | 0         | 0          | 0            | 0          | 0          |
|               | **Adult** | Unknown  | 102       | 7 102                 | 1 102    | 3 91      | 91 102   | 5 0       | 91 2      | 2 88      | 5 0         | 88         | 8 0        | 0            | 0          | 0          |
| **Namibia 2009** | **Adult** | Male     | 10         | 5 10                  | 1 10     | 1 9       | 9 10      | 2 0       | 9         | 9         | 0          | 9          | 9          | 0            | 0          | 19         |
|               | **Female** |          | 10         | 3 10                  | 1 10     | 11 11     | 10 11    | 1 0       | 11        | 11        | 0          | 11         | 0          | 0            | 0          | 21         |
|               | **Unknown** |         | 0          | 0                    | - 0      | 0         | 1 1      | 0         | 1         | 1         | 0          | 1          | 0          | 0            | 0          | 1          |
| **Western Cape 2010–2012** | **Adult** | Male     | 53         | - 53                  | 5 53     | 53 53     | 2 23     | 53 1      | 28 5      | 20 1       | 32          | 20         | 1          | 53          | 1          | 53         |
|               | **Female** |          | 47         | - 47                  | - 47     | 47 47     | 47 47    | 14       | 47 2      | 25 7      | 20          | 26         | 1          | 20          | 47         | 2          |
| **Eastern Cape 2012** | **Adult** | Male     | 26         | - 26                  | - 26     | 26 26     | 26 26    | 0        | 26        | - 26      | 0           | 26         | - 26       | 26          | - 26       | 26         |
|               | **Female** |          | 37         | - 37                  | - 37     | 39 39     | 37 37    | 0        | 39        | - 37      | 0           | 37         | - 37       | 39          | - 37       | 39         |
|               | **Unknown** |         | 2          | - 2                   | - 2      | 2 2       | 2 2      | 0        | 2         | - 0       | - 2         | 0          | - 2        | 0            | - 2        | 1          |
| **Rehabilitation** | **Chick** | Male     | 24         | - 24                  | - 24     | 25 25     | 24 24    | 19       | 25        | - 14      | - 10       | 15          | - 10       | 31          | 31         |
|               | **Female** |          | 18         | - 18                  | - 18     | 16 16     | 18 18    | 17       | 16        | - 11      | - 4        | 12          | - 4        | 20          | 7          | 20         |
|               | **Unknown** |         | 1          | - 1                   | - 1      | 1 1       | 1 1      | 0        | 1         | - 0       | - 1        | 0           | - 1        | 1           | 1          |
|               | **Juvenile** |         | 14         | - 14                  | - 14     | 14 14     | 14 14    | 0        | 14        | - 8       | - 6        | 8           | - 6        | 14          | 3          |
|               | **Adult** | Male     | 41         | - 41                  | 41 41    | 42 42     | 41 1     | 0        | 42        | - 10      | - 31       | 9           | - 31       | 43          | - 43       |
|               | **Female** |          | 39         | - 39                  | 39 39    | 40 40     | 39 39    | 0        | 40        | - 18      | - 21       | 17          | - 21       | 40          | 3          |
|               | **Unknown** |         | 4          | - 4                   | - 4       | 4 4       | 4 4      | 0        | 4         | - 0       | - 4        | 0           | - 4        | 4            | - 4        |

SPA, serum plate agglutination; ELISA, enzyme-linked immunosorbent assay.
Table 2A1: Details of sampling effort and serological results in relation to sampling location and/or clinical history.

| Group               | Location or clinical history | Infectious bronchitis virus | Avian encephalomyelitis virus | Avian reovirus | AIV H5 | H7 | Infectious bursal disease virus | Infectious laryngotracheitis virus | Newcastle disease virus | Mycoplasma gallisepticum (ELISA) | Mycoplasma synoviae (ELISA) | Mycoplasma synoviae (ELISA) | Babesia sp. | Borrelia sp. |
|---------------------|-------------------------------|-----------------------------|-------------------------------|---------------|--------|----|-------------------------------|-------------------------------|----------------------|--------------------------------|---------------------------|--------------------------|-------------|--------------|
| Western Cape 2007–2008 | Dassen Island                 | 41                          | 2                             | 41            | 2       | 38  | 38                           | 41                           | 1                   | 0                               | 37                        | 6                        | 0            | 0            |
|                     | Robben Island                 | 27                          | 5                             | 27            | 1       | 28  | 28                           | 27                           | 1                   | 0                               | 28                        | 2                        | 0            | 0            |
|                     | Boulders                      | 0                           | 0                             | 0             | 0       | 10  | 10                           | 0                            | 0                   | 0                               | 10                        | 1                        | 0            | 0            |
| Namibia 2009        | Dyer Island                   | 39                          | -                             | 39            | -       | 22  | 22                           | 39                           | 3                   | 0                               | 21                        | 1                        | 0            | 0            |
|                     | Halifax Island                | 3                           | -                             | 3             | -       | 6   | 6                            | 3                            | 0                   | 0                               | 6                         | 6                        | 0            | 0            |
|                     | Ichaboe Island                | 8                           | 2                             | 8             | 1       | 1   | 4                            | 8                            | -                   | 0                               | 4                         | -                        | 0            | 0            |
|                     | Mercury Island                | 7                           | 6                             | 7             | 1       | 1   | 8                            | 8                            | 3                   | 0                               | 8                         | -                        | 0            | 0            |
|                     | Possession Island             | 2                           | -                             | 2             | -       | 3   | 3                            | 2                            | 0                   | 0                               | 3                         | -                        | 0            | 0            |
| Western Cape 2010–2012 | Dassen Island                 | 20                          | -                             | 20            | 1       | 20  | 20                           | 20                           | 0                   | 0                               | 20                        | 0                        | 20           | 0            |
|                     | Robben Island                 | 20                          | -                             | 20            | -       | 20  | 20                           | 20                           | 0                   | 0                               | 20                        | 1                        | 20           | 0            |
|                     | Boulders                      | 20                          | -                             | 20            | -       | 20  | 20                           | 20                           | 2                   | 0                               | 20                        | 1                        | 20           | 0            |
|                     | Betty’s Bay                   | 20                          | -                             | 20            | -       | 20  | 20                           | 20                           | 2                   | 0                               | 20                        | 0                        | 20           | 0            |
|                     | Dyer Island                   | 20                          | -                             | 20            | -       | 20  | 20                           | 20                           | 2                   | 0                               | 20                        | 1                        | 20           | 0            |
| Eastern Cape 2012   | Bird Island                   | 48                          | -                             | 48            | -       | 50  | 50                           | 48                           | -                   | 0                               | 48                        | 0                        | 48           | 0            |
|                     | St. Croix Island              | 17                          | -                             | 17            | -       | 17  | 17                           | 17                           | 0                   | 0                               | 17                        | 0                        | 17           | 0            |
| Rehabilitation 2010–2013 | Oiled Chick                 | 59                          | 1                             | 59            | 3       | 59  | 59                           | 59                           | 1                   | 0                               | 29                        | 2                        | 26           | 2            |
|                     | Chick                         | 43                          | -                             | 43            | 1       | 43  | 42                           | 43                           | 1                   | 36                              | 15                        | 27                       | 17           | 16           |
|                     | Moulting                      | 16                          | -                             | 16            | -       | 17  | 17                           | 16                           | -                   | 0                               | 14                        | 3                        | 14           | 17           |
|                     | Weak/ wounded                 | 33                          | -                             | 33            | 1       | 33  | 35                           | 35                           | 33                  | -                               | 35                        | 26                       | 9            | 26           |

SPA, serum plate agglutination; ELISA, enzyme-linked immunosorbent assay.