A longitudinal study on avian polyomavirus-specific antibodies in captive Spix's macaws (Cyanopsitta spixii)

Deb, A; Foldenauer, U; Borjal, R J; Streich, W J; Lüken, C; Johne, R; Müller, H; Hammer, S
A longitudinal study on avian polyomavirus-specific antibodies in captive Spix’s macaws (Cyanopsitta spixii)

Amrita Deb\textsuperscript{1,*}, BVSc, M.Sc., Ulrike Foldenauer\textsuperscript{2}, Dr. med. vet., Raffy Jim Borjal\textsuperscript{1}, DVM, W. Jürgen Streich\textsuperscript{4}, Dr. rer. nat., Caroline Lüken\textsuperscript{3}, med. vet., Reimar Johne\textsuperscript{3}, PD, Dr. med. vet., Hermann Müller\textsuperscript{3}, Prof. Dr. med. vet., Sven Hammer\textsuperscript{1}, Dr. med. vet.

From
\textsuperscript{1}the Al Wabra Wildlife Preservation, P.O. BOX 44069, Doha, State of Qatar, awwp.vet@alwabra.com, Phone: +9744718708, Fax: +9744718707
\textsuperscript{2}the Clinic of Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, CH-8057 Zurich, Switzerland
\textsuperscript{3}the Institute for Virology, Faculty of Veterinary Medicine, University of Leipzig, An den Tierkliniken 29, 04103 Leipzig, Germany and
\textsuperscript{4}the Leibniz-Institute for Zoo and Wildlife Research (IZW), Alfred-Kowalke-Str. 17, 10315 Berlin, Germany

*corresponding author
ABSTRACT

Avian polyomavirus (APV) causes a range of disease syndromes in psittacine birds ranging from acute fatal disease to subclinical infections depending on age, species and a number of other unidentified factors. This study describes a survey of APV-specific antibodies in a captive population of 54 Spix’s macaws (Cyanopsitta spixii). A prevalence of 48.1% for APV-antibodies, indicating viral exposure, was found using a blocking enzyme-linked immunosorbent assay. Out of 36 Spix’s macaws that were serially tested over a period of four years, 50.0% were consistently positive, 36.1% were consistently negative, 5.5% had permanently declining antibody levels and 2.8% showed variable results. Using the PCR technique on whole blood, an apparent viraemia was detected in 2.3% of the birds, although contamination provides a likely explanation for this isolated positive PCR result in a hand-reared chick. A significantly higher white blood cell count was recorded in antibody-positive birds as compared to antibody-negative birds (p<0.05). As antibody-positive and antibody-negative birds were housed together without a change in their respective antibody status, transmission of APV within the adult breeding population appears to be a rare event.

Key words: Spix’s macaws, Cyanopsitta spixii, avian polyoma virus, antibodies, blocking enzyme-linked immunosorbent assay
INTRODUCTION

Avian polyomavirus (APV) was first described as the causative agent of budgerigar fledgling disease, an acute fatal disease of budgerigars (*Melopsittacus undulatus*) causing up to 100% mortality in neonates, and was subsequently designated as budgerigar fledgling disease virus (BFDV). Thereafter, APV has been detected repeatedly in other psittacine birds and sporadically in non-psittacine bird species.

In non-budgerigar psittacine species, APV infection of hand-reared nestlings can lead to either sudden death without any premonitory signs, or to acute fatal disease characterized by a brief period of lassitude, anorexia, delayed crop emptying and extensive subcutaneous, epicardial and serosal haemorrhages prior to death. Among parrots; macaws, conures, Eclectus parrots (*Eclectus roratus*) and lovebirds display a particular susceptibility to APV disease.

Conversely, infection of parent-raised chicks and adult birds - in most cases - is not synonymous with disease. It has been suggested that asymptomatic, juvenile and young adult budgerigars are responsible for transmission of virus through droppings, skin and feather dander to neonatal birds - which are at a substantially higher risk of fatal disease. In case of non-budgerigar psittacines, however, the lack of consistent epidemiological data and the variation observed between species precludes a definite conclusion regarding persistence of antibody titres, duration of viraemia and virus shedding in natural APV infections.

APV is a polyomavirus known to be highly stable in the environment. All APV strains characterized so far belong to one genotype and one serotype. Diagnosis of APV infection in live birds can be done by a polymerase chain reaction (PCR) on blood, feathers and cloacal swabs. Additionally, detection of APV-specific antibodies can be performed using either a serum neutralisation test (SNT) or a blocking enzyme-linked immunosorbent assay (BELISA); the results of both tests have been shown to correlate well with each other.
The Spix’s macaw (Cyanopsitta spixii; Psittaciformes, Aves) is often considered to be the most threatened parrot in the world and duly classified as “Critically Endangered (possibly extinct in the wild)” after the last known male disappeared from its native habitat in late 2000.\textsuperscript{29,30}

Out of a captive population of 78 Spix’s macaws, which are part of an international recovery effort, 50 birds, representing almost 65% of the extant population, are kept at Al Wabra Wildlife Preservation (AWWP), Qatar. In total, AWWP has been home to 62 birds up until 2007.

**MATERIALS AND METHODS**

The 54 Spix’s macaws in this study included 23 males and 31 females. Most of the birds had been acquired by AWWP from captive populations in the Philippines and Switzerland between 2000 and 2004. Unfortunately, no reliable health or management records existed for these birds prior to their transfer to AWWP. Between 2004 and 2007, 16 chicks hatched at AWWP and all chicks were hand-reared and housed in isolation from the adult birds. The Spix’s macaws at AWWP are maintained in aviary complexes separate from the other avian species kept at the facility.

The age of the birds tested ranged from 1 to 22 years. All birds were housed in aviaries with inside air-conditioned rooms and outdoor flights with artificial rainfall systems. They were usually kept either individually or in pairs, except in some cases where juveniles were flocked. The birds had been subjected to annual health checks since 2004, during which they were tested for APV among other pathogens. AWWP had no history of APV vaccination, clinical APV infection, or evidence of APV-associated mortality in the Spix’s macaw population.

Approximately 2.5 ml of blood was collected by jugular venipuncture from each bird under isoflurane anaesthesia and stored in lithium-heparin micro tubes. A fresh thin blood smear was prepared immediately from each bird using the slide to slide method. Plasma was always
separated within 4 hours of collection and frozen (-20°C) until dispatch to the laboratory for
detection of APV antibodies. For detection of APV DNA, approximately 0.2 ml of blood was
also stored immediately after collection in micro tubes of 1.3 ml capacity containing potassium
EDTA and frozen (-20°C) until dispatch to the laboratory. Feather samples for APV DNA
analysis were stored in re-sealable plastic bags.

The presence of APV-specific antibodies was tested using a blocking enzyme-linked
immunosorbent assay (BELISA). The test employs a monoclonal antibody directed against the
major structural protein VP1 of APV in an ELISA format with purified APV particles as antigen.
It measures the inhibition of color reaction of a test serum as compared to a defined negative
control serum. According to results of SNT, more than 50% inhibition is considered positive,
whereas less than 50% inhibition is considered negative. The BELISA was validated for a
number of psittacine species. 36 Spix’s macaw individuals were re-sampled in subsequent
years. Testing interval for an individual ranged from 1 month to 28 months with a median of 10
months between consecutive tests.

Testing for the presence of the APV genome in blood or feathers was performed by PCR.
The DNA was isolated from blood samples using the DNeasy Tissue Kit® (Qiagen GmbH,
40724 Hilden, Germany) as recommended by the supplier. For DNA preparation from feather
samples, a proteinase K digestion followed by phenol/chloroform/isoamylalcohol extraction and
ethanol precipitation was performed as described previously. PCR analysis was done by
amplification of a 310 bp fragment of the APV genome, as described previously, but by
addition of SYBR Green I dye (Invitrogen BV, 9351 NV Leek, The Netherlands) and PCR
product melting curve analysis in a Rotorgene 2000 Thermal Cycler® (Corbett Research
Australia, Mortlake, NSW 2137, Australia). Precautions were taken to prevent laboratory
contaminations and all reactions were controlled by the use of negative DNA isolation controls
as well as negative PCR reaction controls. PCR reactions were first analysed by melting curve analysis, and samples showing a melting peak between 80°C and 85°C were further analysed by electrophoresis on ethidium bromide-stained 2% agarose gels. PCR products with a length of 310 bp were considered positive.

Fresh thin blood smears stained with Diff-Quick® stain (Medion Diagnostics AG, 3186 Duedingen, Switzerland) were evaluated for Total White Blood Cell counts (WBC) and Differential Leukocyte Count following the methods described previously. Plasma biochemistry and enzymes were processed on a dry automated analyzer (Spotchem EZ SP-4430, A. Menarini Diagnostics, 41460 Neuss, Germany).

The haematological and biochemical data from 2004 to 2006 were evaluated separately for juveniles (birds less than 4 years; n=16 testing occasions) and adults (n=89 testing occasions). Only decreased or increased parameters, as compared to reference ranges recently established for the species, were examined. Differences between birds with positive and negative APV titres were assessed by t-test for parameters with normal distribution. As the percentage of monocytes was not normally distributed, differences in this parameter were tested by U-test. Statistical analyses were performed using the statistical package SPSS 14.0 (SPSS Inc., Chicago, IL 60606-6307, USA), with p < 0.05 as significance threshold.

RESULTS

All birds were found to be normal on clinical examination for parameters such as body condition, general appearance, heart and respiratory rate, palpation of abdominal organs and appearance of choanal and cloacal mucous membranes.

From 2004 to 2007, a total of 144 plasma samples from 54 individuals were tested for APV-specific antibodies. Of these, 48.1% (26/54) of the birds were found to be positive on at least one
sampling occasion in the BELISA, whereas 51.9% (28/54) always tested negative. Out of 36
Spix’s macaws that were tested again in subsequent years for APV, 91.7% (33/36) displayed a
consistent APV-BELISA result, with 18 birds showing consistently positive and 15 showing
consistently negative results (Table 1). Only three birds showed changing APV-BELISA results
in the study period: two had initial positive results followed by a gradual decline to negative
results, and one showed high percentages of inhibition (positive results) in three tests interspersed
with one negative result (0% inhibition).

A closer examination of the BELISA percentages of inhibition revealed some fluctuations
for each individual over the time period; however, they remained well above 70% in all of the
consistently seropositive birds. The mean percentage of inhibition (with 95% CI) of the APV-
BELISA positive subpopulation ranged from 86.8% (82.2% - 91.4%, n=13) in 2005, to 98.8%
(98.2% - 99.7%, n=16) in 2007. In case of the APV-BELISA negative birds, mean percentage of
inhibition remained consistently between 16.7% (9.5% – 23.9%, n=10) to 13.3% (9.4% – 17.3%,
n=14) in 2004 and 2006, respectively. To date all AWWP hatched and hand-reared chicks tested
(n=10), have always shown negative APV-BELISA results in repeated samples. However, this
was not the case with juveniles imported from Switzerland or the Philippines, with the youngest
bird showing a positive APV_BELISA result at the age of one year (Table1; Bird Number 35). In
addition, investigation into the origin of all APV-antibody positive birds indicated that, with the
exception of one bird of Swiss origin, all seropositive birds originated from the Philippines.

Between 2004 and 2006, whole blood and/or feather samples were also tested for the
presence of APV DNA using PCR. In total, 81 samples (69 only blood; 11 combined blood and
feather; 1 only feather) from 44 birds were tested over the period of three years. Of these, 2.3%
(1/44) birds tested positive for APV specific DNA in whole blood samples. All feather samples
were negative. A comparison of results of PCR testing with APV-BELISA results of individual birds revealed that PCR-positive bird (Bird number 31) tested negative in BELISA (Table 1).

A summary of mean, standard deviation (SD), range of hematologic values and AST is shown in Table 2. No statistically significant differences were found between APV-BELISA positive and negative juvenile birds for white blood cell count, percentage of lymphocytes, percentage of monocytes, or AST (data not shown). However, statistically significant differences were found between APV-BELISA positive and negative adult birds: white blood cell count (p = 0.02) was higher in positively tested birds as compared to negatively tested birds, and AST concentration (p = 0.014) was higher in APV negatively tested birds as compared to positively tested birds.

DISCUSSION

The purpose of this study was to perform a retrospective evaluation of data recording clinical health status, APV-specific antibody titers, and PCR results for APV-DNA in a closed captive population of unvaccinated adult Spix’s macaws over a period of 4 years. The results should contribute to the understanding of the nature and course of natural APV infection within this population at AWWP and, possibly, to augment recent efforts in determining the duration of persistence of antibodies following naturally acquired APV infections in macaw species.

Attempts have been made to characterize the persistence of antibodies and the duration of cloacal virus shedding for APV in a number of psittacine species; the variation observed has been attributed to the species, age at the time of exposure, concurrent infections like psittacine beak and feather disease (PBFD), and certain unidentified individual factors. The susceptibility of macaws to APV infections has been previously noted and this study presents evidence that the Spix’s macaw is no exception. As a majority of the birds that tested positive were
acquired as adults, the age of exposure or history of clinical illness cannot be elucidated. Notably, PBFD has never been detected during routine annual testing for the virus since 2004. In addition, this study confirms the conclusion of several authors that antibodies to APV can be detected in adult non-budgerigar psittacine birds in the absence of clinical symptoms. In case of budgerigars, antibody titers are sustained for periods of up to 5 years- presumed to be life-long, and viral shedding from the cloaca ceases at the onset of sexual maturity. Recent investigations have contributed to the hitherto scarce data on the course of infection in non-budgerigar psittacines. In macaws, the susceptibility to potentially fatal clinical disease is pronounced between 4-14 weeks of age; thereafter exposure leads to inapparent infection. Initial serosurveys in non-budgerigar psittacines suggested that antibody titers are transient following APV infection and decline within weeks of infection. Subsequent research has demonstrated that antibodies can persist for up to 2 years; that viraemia, when present, is always detected and does not necessarily correlate with antibody status. Also, cloacal shedding of virus can occur irregularly for up to a year. Our retrospective analysis of testing results for APV-specific antibodies in the Spix’s macaw population at AWWP revealed that most of the birds showed consistently positive or negative results over the period of four years when sampled repeatedly. Only three birds demonstrated an observable changing pattern of results, of which two had declining antibody levels. In case of the third bird, a deterioration of one sample during storage or shipment provides a likely explanation for the result of 0% inhibition at one time-point and three strong positive results at the remaining time-points of analysis. In general, the extremely low detection rate of the APV genome suggests that the isolated positive PCR result was a result of contamination either at the time of sampling or at the laboratory. This is supported by our observation that three pairs comprising one APV-antibody
positive and one negative bird were housed together with no change in antibody status during the period of four years, indicating that virus transmission did not occur. This drawback of the extremely high sensitivity of the PCR has been previously acknowledged. Although viraemia cannot be completely ruled out, it is highly unlikely given that the bird (Bird number 31) was a young hand-reared bird that had not been exposed to the adult population, compounded by the fact that the bird was seronegative at the time of sampling and did not seroconvert in subsequent tests (Table 1). In this case sequencing of the PCR products would have been invaluable in order to arrive at a conclusion, however, the retrospective nature of this study denied the authors this possibility.

Blood and serum biochemistry parameters for APV-infected birds have only been described for non-budgerigar psittacines with active and fatal infections. In our study, significantly higher total WBC counts were found in the APV antibody positive adult birds, as compared to seronegative birds. The distinct reasons for this finding are not known; nevertheless, it could be speculated that it reflects a stimulation of the immune system by APV infection. In apparently healthy budgerigars, APV had a higher detection rate in tissues as compared to serum, and the possibility that the virus persists in other tissues of the Spix’s macaws, as well, will remain a hypothesis that cannot be substantiated at this point. The finding of lower AST levels in APV antibody positive birds as compared to negative birds is in marked contrast to previously reported studies. However, these studies were performed on acutely diseased birds and despite the statistical significance recorded here, the probability of it being an unrelated incidental finding is high.

The Spix’s macaw population at AWWP plays a significant role in the international species recovery and rehabilitation program. As determined in this study, the likelihood of virus transmission within the adult population is low. However, in future PCR screening of cloacal
swabs from these birds would be an essential step in bridging the gap necessary to gauge the potential risk for infection of Spix’s macaw chicks, since it has been demonstrated that cloacal shedding can continue after viraemia ceases in macaws.8,14,40

At present all eggs from Spix’s macaws are pulled from the nest and chicks hand-reared before introduction to the breeding population. There remains a possibility that some of the adult birds might be shedding the virus. In the absence of this data, however, precautions to ensure that the chicks are not exposed to the breeding population between 4 to 14 weeks should be adequate to protect them against clinical APV disease. In conclusion, this study demonstrates the persistence of antibodies to APV for up to four years in Spix’s macaws, with little evidence of viraemia or virus transmission between seropositive and seronegative birds, thereby indicating a low level of risk for the hand-reared juvenile Spix’s macaw- a valuable conservation resource.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to Sheikh Saoud Bin Mohd. Bin Ali Al Thani, owner of the Al Wabra Wildlife Preservation, for his continued interest in, and support of, scientific research. Marcus Clauss, for suggestions and comments on the manuscript and various Al Wabra staff, especially Dr. Abdi Arif, Mr. Abid Taha, Dr. Julia Schulz and Mr. Ryan Watson.
REFERENCES


TABLE 1. Summary of serological results using the BELISA of 36 Spix’s macaws tested repeatedly for APV-specific antibodies between 2004 and 2007. The percentage of inhibition is shown; percentages >50% are considered positive, percentages <50% are considered negative. Positive results are highlighted by shading the cell. APV-PCR positive results are indicated with (*). The cage mate at the time of testing, where available, is indicated in superscript and corresponds to the “Bird Number” column (eg. bird number 7 was housed with bird number 28 and 29, in 2004 and 2006, respectively).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent BELISA-Positive Results (n=18)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>89%⁴</td>
<td>85%⁴</td>
<td>100%⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>89%³⁶</td>
<td>73%³⁶</td>
<td>96%³⁶</td>
<td>100%²⁹</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>95%⁵</td>
<td>79%⁵</td>
<td>100%⁵</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>98%</td>
<td>90%¹</td>
<td>100%¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>95%³</td>
<td>89%³</td>
<td>100%³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>95%</td>
<td>88%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>90%²⁸</td>
<td>100%²⁹</td>
<td>90%²⁹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>95%</td>
<td>97%¹⁵</td>
<td>100%¹⁵</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>90%¹⁰</td>
<td>96%¹⁰</td>
<td>100%¹⁰</td>
<td>100%¹⁴</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>87%⁹</td>
<td>94%⁹</td>
<td>100%⁹</td>
<td>89%⁹</td>
<td>100%²⁷</td>
</tr>
<tr>
<td>11</td>
<td>100%</td>
<td>100%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>93%</td>
<td>99%¹³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>13</td>
<td>92%&lt;sup&gt;14&lt;/sup&gt;</td>
<td>100%&lt;sup&gt;12&lt;/sup&gt;</td>
<td>13%</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>94%&lt;sup&gt;13&lt;/sup&gt;</td>
<td>100%&lt;sup&gt;18&lt;/sup&gt;</td>
<td>99%&lt;sup&gt;9&lt;/sup&gt;</td>
<td>90%&lt;sup&gt;8&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>90%&lt;sup&gt;8&lt;/sup&gt;</td>
<td>97%&lt;sup&gt;34&lt;/sup&gt;</td>
<td>100%&lt;sup&gt;8&lt;/sup&gt;</td>
<td>100%&lt;sup&gt;32&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>95%&lt;sup&gt;34&lt;/sup&gt;</td>
<td>76%&lt;sup&gt;34&lt;/sup&gt;</td>
<td>88%&lt;sup&gt;34&lt;/sup&gt;</td>
<td>97%</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>88%</td>
<td>83%</td>
<td>100%&lt;sup&gt;32&lt;/sup&gt;</td>
<td>90%&lt;sup&gt;32&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>90%&lt;sup&gt;14&lt;/sup&gt;</td>
<td>100%&lt;sup&gt;14&lt;/sup&gt;</td>
<td>100%&lt;sup&gt;32&lt;/sup&gt;</td>
<td>97%</td>
<td></td>
</tr>
</tbody>
</table>

**Persistent BELISA-Negative Results (n=15)**

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>13%</td>
<td>20%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>16%&lt;sup&gt;28&lt;/sup&gt;</td>
<td>16%&lt;sup&gt;28&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>11%&lt;sup&gt;25&lt;/sup&gt;</td>
<td>13%&lt;sup&gt;25&lt;/sup&gt;</td>
<td>47%&lt;sup&gt;25&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>13%&lt;sup&gt;23&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>24%&lt;sup&gt;22&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>16%&lt;sup&gt;26&lt;/sup&gt;</td>
<td>23%&lt;sup&gt;26&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>15%&lt;sup&gt;21&lt;/sup&gt;</td>
<td>22%&lt;sup&gt;21&lt;/sup&gt;</td>
<td>16%&lt;sup&gt;21&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>11%</td>
<td></td>
<td></td>
<td></td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>26%&lt;sup&gt;25&lt;/sup&gt;</td>
<td>13%</td>
<td></td>
<td></td>
<td>0%&lt;sup&gt;10&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>1%&lt;sup&gt;7&lt;/sup&gt;</td>
<td>21%&lt;sup&gt;20&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>19%&lt;sup&gt;7&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td>0%&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>13%</td>
<td></td>
<td></td>
<td></td>
<td>0%&lt;sup&gt;31&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>12%&lt;sup&gt;*&lt;/sup&gt;</td>
<td>14%&lt;sup&gt;30&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>0%&lt;sup&gt;30&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>1%&lt;sup&gt;17&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>16%</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>35</td>
<td>36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>67%&lt;sup&gt;16&lt;/sup&gt;</td>
<td>50%&lt;sup&gt;16&lt;/sup&gt;</td>
<td>23%&lt;sup&gt;16&lt;/sup&gt;</td>
<td>9%&lt;sup&gt;16&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>78%</td>
<td>36%</td>
<td>26%</td>
<td>14%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>75%&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0%&lt;sup&gt;2&lt;/sup&gt;</td>
<td>94%&lt;sup&gt;2&lt;/sup&gt;</td>
<td>64%&lt;sup&gt;7&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Mean, standard deviation (SD) and range of haematologic values as well as AST concentration for adult Spix’s macaws (*Cyanopsitta spixii*) with regard to positive or negative result in APV-BELISA testing from 2004-2006.

Note: a,b: differences between means are significant (p = 0.02), c,d: differences between means are significant (p = 0.014).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Positive APV titre</th>
<th>Negative APV titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[mean ± SD and range]</td>
<td>[mean ± SD and range]</td>
</tr>
<tr>
<td>White blood cells (x 10^3/µl)</td>
<td>10.8 ± 4.4 [3-25]^a</td>
<td>8.6 ± 4 [2.8-17.2]^b</td>
</tr>
<tr>
<td>Heterophils (%)</td>
<td>59.9 ± 15.9 [26-83]</td>
<td>61.7 ± 13.2 [32-85]</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>37 ± 16.5 [8-73]</td>
<td>35.2 ± 13.1 [14-67]</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>3.2 ± 2.6 [1-12]</td>
<td>3.5 ± 2.4 [1-13]</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>136 ± 49.9 [21-364]^c</td>
<td>172.9 ± 77.1 [82.8-471]^d</td>
</tr>
</tbody>
</table>