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INVESTIGATION OF NDV IN SOUTHERN AFRICAN WATERFOWL REVEAL INSIGHTFUL GEOGRAPHIC AND BIODIVERSITY TRENDS TO HELP CONTAIN THE VIRUS

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NOMINATED BY: Dr. Chad Campbell

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Author Notes:

This study assesses the infection rates and trends of Newcastle disease in southern African waterfowl, a project carried out by Emily Murphy in Dr. Jeffrey Peters' laboratory. Thanks to the wonderful work of Emily Murphy, whom I shadowed as an undergraduate researcher, I assessed a portion of her data in this paper to showcase my skills in scientific writing.

Emily Murphy Notes:

This data was collected from samples collected by Dr. Jeffrey Peters. The data set used in this paper was originally used in a presentation given at the 2023 Celebration of Research. The original study assesses the NDV infection rates among southern African waterfowl by geographical area and species.

Faculty Notes:

Clear and concise scientific communication is the key to informing others about the research that occurs in our labs. This paper is a great example of writing which takes the often messy and confusing obtained research data and distills it down into a meaningful message that is easy to comprehend. Lauren's writing tells a compelling story about how the spread of Newcastle Disease affects wild bird populations in Africa and further connects this to potential ramifications in the poultry industry.

Investigation of NDV in southern African waterfowl reveal insightful geographic and biodiversity trends to help contain the virus

ABSTRACT

Newcastle disease is a highly contagious and lethal disease affecting thousands of birds around the globe (Wang *et al.*, 2018). A variant of the avian paramyxovirus (APMV-1), Newcastle disease virus (NDV) causes symptoms that range from respiratory and neurological complications to death (Wang *et al.*, 2018; Abolnik, 2017). NDV is readily and often undetectably transmitted via inhalation or ingestion of the virus between wild and domestic bird populations, causing devastating consequences within the poultry industry (Abolnik, 2017; Wang *et al.*, 2018). Insufficient reporting and data collection in some regions, including southern Africa, have hampered efforts to track and contain NDV. To control the spread of the virus, it is crucial to understand the geographic and biodiversity trends underlying NDV prevalence in these underreported regions. In this study, 418 waterfowl of various species were screened for NDV in four southern African sites: Barberspan, South Africa; Lake Ngami, Botswana; Strandfontein, South Africa; and Lakes Manyame and Chivero, Zimbabwe. Both the fusion and matrix proteins, key components of APMV-1, were amplified by PCR to detect the presence of NDV in each sample (Seal *et al.*, 2000). The samples then underwent gel electrophoresis, with a positive NDV result being represented by a band. Based on the results, Lake Ngami experienced the highest rate of NDV infection. To gain further insight into the species trends in Lake Ngami, NDV infection rates were assessed in Egyptian geese, Hottentot teals, red-billed teals, and white-faced whistling ducks. Following this investigation, the white-faced whistling ducks were found to hold the highest infection rate.

INTRODUCTION

Newcastle disease is a highly infectious and deadly disease impacting numerous bird species worldwide (Wang *et al.*, 2018). Newcastle disease virus (NDV) is a variant of the avian paramyxovirus 1 (APMV-1), a negative-sense, single-stranded RNA virus (Wang *et al.*, 2018). APMV-1 viruses have diverse strains that can be categorized as Class I or Class II, depending on whether the strains predominate in wild birds or poultry, respectively (Abolnik, 2017). The RNA virus can be further divided into other genotypes due to its highly mutagenic and rapidly evolving nature (Abolnik, 2017). Moreover, depending on the strain, infection with APMV-1 can present with different symptoms that range in severity. While asymptomatic strains cause no symptoms, lentogenic strains lead to mild respiratory conditions, and mesogenic strains are linked with acute respiratory and neurological issues but with low mortality rates (Abolnik, 2017). Velogenic APMV-1 contributes to the most severe, complicated, and lethal symptoms of the strains, including lethargy, conjunctivitis, and ruffled feathers (Abolnik, 2017). Oftentimes, birds infected with velogenic NDV experience watery discharge, respiratory distress, and inflamed heads (Abolnik, 2017). Neurological effects of NDV infection can range from tremors and spasms to paralysis of the legs and wings (Abolnik, 2017). Diminished egg production and other abnormalities, such as misshapen and tinted eggs, may also arise from velogenic NDV infection (Abolnik, 2017). Most significantly, infection with this strain typically leads to sudden death in birds, even if they exhibit little to no symptoms (Abolnik, 2017).

NDV transmission can occur via inhalation or ingestion of viral remnants shed from feces or respiratory discharge (Wang *et al.*, 2018). Due to the highly infectious nature of the disease, along with its mode of transmission, NDV spreads easily from wild bird populations to poultry flocks (Abolnik, 2017). Chickens are especially vulnerable to contracting the disease, with as much as a 100% annual mortality rate resulting from outbreaks (Wang *et al.*, 2018). Additionally, NDV strains frequently infect waterfowl species, including geese and ducks, which serve as undetected carriers of the virus as they present with minimum to no symptoms (Abolnik, 2017). This further complicates containment efforts as wild bird populations can transmit the disease to local poultry flocks, leading to catastrophic effects within the poultry industry. The economic consequences are especially damaging to developing nations in Africa, where appropriate vaccination measures are lacking for NDV prevention (Wang *et al.*, 2018).

For years, NDV outbreaks of various genotypes have been reported in several regions around the globe, mainly China, Southeast Asia, and southern Africa (Abolnik, 2017). However, lack of reporting and data collection have hampered any findings of conclusive NDV trends within these regions. Given the devastating effect of NDV on the poultry industry, it is crucial to understand the various trends underlying NDV transmission so that the virus may be contained. In this study, the geographic and biodiversity trends of NDV were investigated in southern Africa. The blood samples of 418 waterfowl were collected and tested for NDV from four sites of interest: Barberspan, South Africa; Lake Ngami, Botswana; Strandfontein, South Africa; and Lakes Manyame and Chivero, Zimbabwe. A diverse array of species was assessed within these sites, including the Cape shoveler, Cape teal, yellow-billed duck, red-billed duck, white-faced whistling duck, blue-billed teal, spur-winged goose, and Egyptian goose. Two central components of the virus' structure, known as the fusion and matrix genes, were used to screen the samples (Seal *et al.,* 2000). Following amplification of the DNA samples using the polymerase chain reaction (PCR), the samples underwent gel electrophoresis. After analyzing the results across the four locations, the highest rate of species infected by Newcastle disease was found to predominate in Lake Ngami, Botswana. Of the waterfowl species screened within this region, the white-faced whistling ducks held the highest infection rate.

METHODS

Collection of Samples:

The blood samples of 418 wild waterfowl were obtained from four locations in southern Africa: Barberspan, South Africa; Lake Ngami, Botswana; Strandfontein, South Africa, and Lakes Manyame and Chivero, Zimbabwe. These samples were provided by Cummings et al., who had previously screened the samples for avian malaria. In this study, eight different types of waterfowl species were assessed for Newcastle disease: the Cape shoveler, Cape teal, yellow-billed duck, red-billed duck, white-faced whistling duck, blue-billed teal, spur-winged goose, and Egyptian goose. Due to a lack of some species' samples in certain regions, there was a variability in the types of species assessed across the four regions. Moreover, given the lack of sufficient samples, a discrepancy in the number of sample sizes existed for each region. However, equal effort was utilized by Cummings *et al.* (2012) to capture the waterfowl and collect their blood samples. Lake Ngami was a key area of interest in this study, which analyzed species trends in Newcastle disease rates. From this location, 4 Egyptian geese, 11 Hottentot teals, 16 red-billed teals, and 6 white-faced ducks were screened in the laboratory for the Newcastle disease virus. Note that the difference in sample sizes from each species is representative of the relative abundance of the species in Lake Ngami.

DNA Extraction, PCR, and Gel Analysis

To determine if the waterfowl samples were infected with NDV, DNA from the blood samples was extracted, amplified, and visualized using gel electrophoresis. DNA extraction was performed on the samples by Cummings *et al.* (2012), who utilized the protocol listed in the DNeasy tissue kit. A reaction mixture was then prepared following a pre-specified laboratory protocol, with the solution containing 6.25 μM for each of the forward and reverse primers, 0.25 units of Taq polymerase, 25 nM of $MgCl_2$, and 10 μM of dNTP. The master mix was then added to 2 μL of each extracted DNA sample to form a 25 μL solution. This was completed for all 418 samples screened for NDV.

Following the samples' preparation, both PCR and gel electrophoresis were utilized to screen the samples for Newcastle disease. PCR was first used to amplify any potential DNA fragments containing the Fusion or Matrix gene of the Newcastle disease virus. Serving as structural components of the avian paramyxovirus type-1 virus (APMV-1), the presence of the Fusion and Matrix genes were assessed in two *distinct* trials to confirm whether the samples were infected with NDV. A 2% agarose gel containing 1.5 μL of SYBR® Green was prepared for gel electrophoresis, with approximately 6 μL of each sample being used during the analysis. After running the samples from each trial on the gel, the results were analyzed thoroughly under fluorescent lighting. The presence of either the Fusion or Matrix gene indicated a positive infection, which manifested as a distinct band during gel electrophoresis. The apparent absence of a band insinuated that the bird sample lacked the APMV-1 gene and thus was not infected with Newcastle disease. Establishing consistent results using the two different components of the virus reduced the possibility of false negative or positive results. If a discrepancy still existed following both trials, the samples were screened further until a conclusive result was established.

Analysis of the Results

Following gel electrophoresis, both the geographic and biodiversity trends were graphically assessed using Excel software. The NDV rates were first analyzed across the four sites of interest in southern Africa. A chi-square analysis was then conducted to establish any remarkable variation in NDV prevalence across these locations. Due to Lake Ngami having the highest NDV prevalence of the four locations, this site was selected as an area of interest for a secondary investigation of NDV trends across species. The results were ultimately assessed for trends across species using Excel software.

RESULTS

In this study, the NDV infection rates of waterfowl species were assessed across four sites in southern Africa. A total of 418 samples were screened for NDV: 146 samples from Barberspan, South Africa; 37 samples from Lake Ngami, Botswana; 175 samples from Strandfontein, South Africa; and 60 samples from Lakes Manyame and Chivero, Zimbabwe (Table 1). Note that eight different types of waterfowl species were assessed across these sites: the Cape shoveler, Cape teal, yellow-billed duck, red-billed duck, white-faced whistling duck, blue-billed teal, spur-winged goose, and Egyptian goose. However, due to a lack of available samples for *all* species in each region, there was a discrepancy in the types of species assessed across the four sites. PCR and gel electrophoresis were utilized to detect the virus in the samples, with a band during gel electrophoresis indicating a positive result for NDV.

Table 1

NDV Infection Rates for 418 Samples in Four Regions of Southern Africa

Location	Birds Sampled	Positive for NDV	Negative for NDV	% Positive for NDV	% Negative for NDV
Barberspan, South Africa	146	25	121	17.1%	82.9%
Lake Ngami, Botswana	37	15	22	40.5%	59.5%
Strandfontein, South Africa	175	17	158	9.7%	90.3%
Lakes Manyame & Chivero, Zimbabwe	60	11	49	18.3%	81.7%
All Regions (Total)	418	68	350	16.3%	83.7%

Note. See Figure 1 for a visual of the geographic distribution of NDV infection rates. Using the results in Table 1, the distribution of NDV cases among the four sites was deemed statistically significant (P = 0.00072, df = 3).

Based on the results of the study, approximately 16.3% of the 418 samples from the four regions tested positive for NDV. The percentage of positive samples ranged greatly across the four locations, with Strandfontein having the lowest infection rate at 9.7% and Lake Ngami having the highest infection rate at 40.5%. Using these data, a chi-square test was conducted to determine the significance of the NDV infection rates across the four different sites. For all locations, the resultant infection rates were compared with the expected infection rates, given the total percentages of samples positive and negative for NDV. From this statistical analysis, a p-value of 0.00072 (where df = 3) confirmed a significant difference between the resultant and expected NDV occurrences across the four locations.

To further visualize the geographic trends of NDV prevalence across southern Africa, Figure 1 was constructed to display the percentage of samples testing positive versus negative for NDV in each of the four locations. The pie charts were sized according to the total number of bird samples assessed in each region, as indicated by N; however, these charts are not made to scale. An apparent trend can be illustrated as the most southern region of Strandfontein holds the lowest infection rate, while the more northern site of Lake Ngami holds the highest infection rate. Generally, NDV infection rates increase moving northward and more inland. Moreover, despite Strandfontein having the largest sample size among the four regions (N = 175), only a slim number of these samples tested positive for the virus. This starkly contrasts with the results from Lake Ngami, where the region had the lowest number of samples collected for analysis (N = 37) yet the highest NDV infection rate among the four sampling sites.

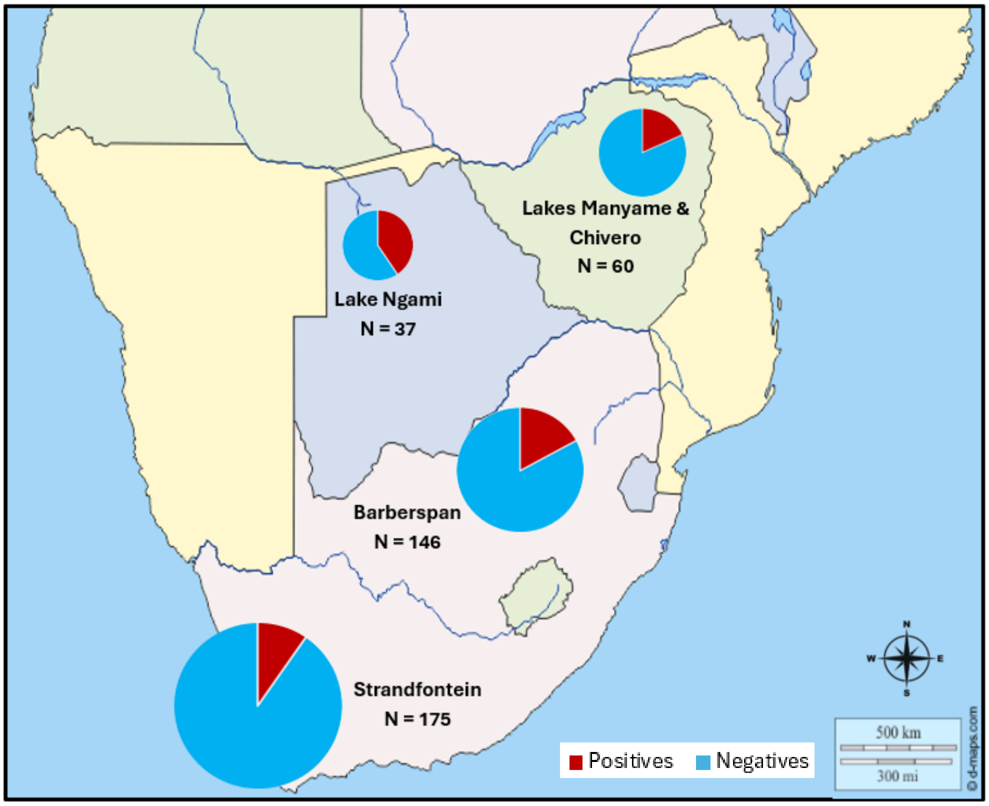

Fig. 1 *Geographic distribution of NDV infection rates in southern Africa. The red portion for each pie chart indicates a positive result for NDV, while the blue portion signifies a negative result for NDV. Samples from four regions in southern Africa were collected and screened for NDV: Barberspan, South Africa; Lake Ngami, Botswana; Strandfontein, South Africa; and Lakes Manyame and Chivero, Zimbabwe. Given the close proximity of Lake Manyame and Lake Chivero, these sampling sites were categorized as a single region. The sample sizes, indicated by N, are shown below each of the defined locations.*

The significantly high infection rate associated with Lake Ngami warranted further investigation of NDV infection rates by waterfowl species. In this study, NDV trends in only four of the eight waterfowl species were assessed in Lake Ngami due to a lack of samples for all species types. Samples from 4 Egyptian geese, 11 Hottentot teals, 16 red-billed teals, and 6 white-faced ducks in the region of Lake Ngami were screened for NDV. Note that a lack of samples provided for each of these species contributed to the variability in sample sizes assessed in Lake Ngami.

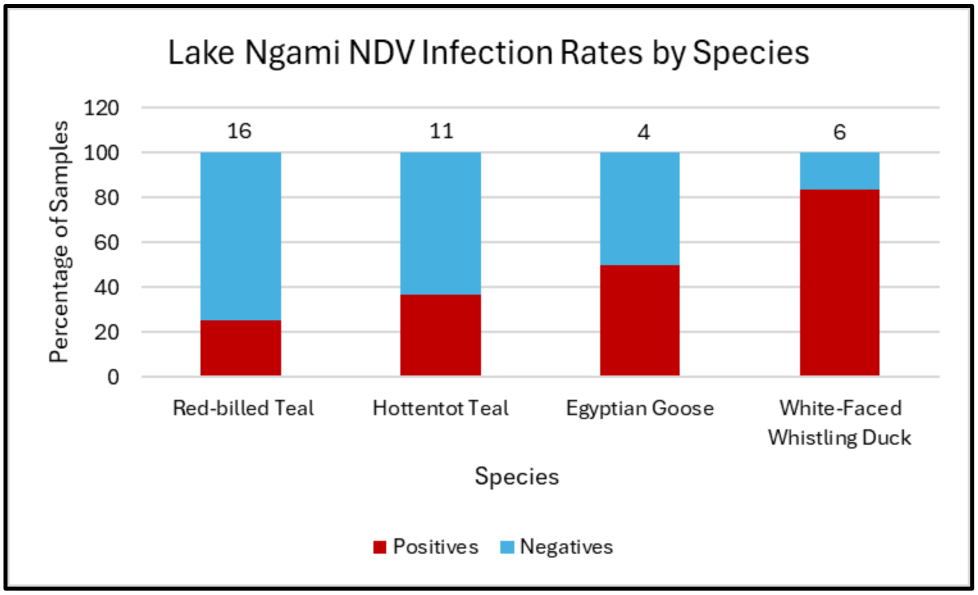

Fig. 2 *NDV infection rates by species in Lake Ngami. The red portion indicates a positive result for NDV, while the blue portion signifies a negative result for NDV. Due to a lack of sufficient samples for all eight species, only four types of waterfowl species were assessed in Lake Ngami.*

As shown by the results in Figure 2, red-billed teals held the lowest infection rate at 25%, while white-faced whistling ducks held the highest infection rate at an astounding 83.3%. Overall, a trend can be found where both species of teals hold lower infection rates compared to geese and ducks. However, given the lack of uniform sample sizes across the four species, additional samples must be screened for NDV to verify this trend.

DISCUSSION

Newcastle disease, derived from the avian paramyxovirus type-1 (APMV-1), is highly infectious and lethal to numerous bird species worldwide (Abolnik, 2017). However, the trends in some regions, namely southern Africa, have not been investigated sufficiently. In this study, 418 blood samples of several waterfowl species were analyzed via PCR and gel electrophoresis across four sites in southern Africa: Barberspan, South Africa; Lake Ngami, Botswana; Strandfontein, South Africa; Lakes Manyame and Chivero, Zimbabwe. A chi-square test deemed the differences across the four sample sites to be statistically significant. This warrants further investigation as the result suggests that NDV cases are not randomly dispersed among these regions. While the samples analyzed in this study were previously screened by *Cumming et al.* (2012) for avian malaria, they have *not* been screened for NDV. Thus, the results and trends established from this study serve as novel findings. Moreover, a comparative analysis can be conducted to gain insight into the relation (if any) between NDV and avian malaria infection rates of southern African waterfowl.

Based on the results of the infection rates across southern Africa, an interesting trend was revealed as the most southern region (South Africa) held the lowest infection rate, while more northern sites (Zimbabwe and Botswana) held the highest infection rates. Hence, infection rates were generally found to decrease moving southward. It is plausible that the natural migratory patterns of birds in southern Africa—or from other regions—contributed to this trend. Past studies have revealed the role of wild birds' seasonal migration patterns in the transmission and clustering of H5N1 along key flyway regions (Si *et al.*, 2009). Given that the samples in this study were collected at various time periods, additional studies are needed to understand how seasonal migration patterns affect the NDV prevalence in southern African waterfowl. Despite the unestablished role of migration on NDV infection rates, the results are nevertheless novel as NDV trends in southern Africa have not been thoroughly and consistently recorded in the past.

It is also important to note that Lake Ngami, which held the highest infection rate in this study, is located farther inland compared to all the other investigated sites. Given that waterways serve as a common mode of transmission for Newcastle disease, it was expected for more coastal regions (near waterways) to have higher NDV infection rates. Consequently, the high infection rate associated with Lake Ngami in this study is particularly confounding. Thus, a more thorough investigation of inland versus coastal NDV infection rates is needed to establish a conclusive trend. Along with temperature and rainfall trends, other factors that should be considered in future research include potential differences in diets and current vaccination measures for waterfowl across the various sites sampled in southern Africa. Although more research is necessary, the results of this study provide pivotal information regarding the general trends for NDV infection rates in southern African waterfowl, which had formerly remained unknown.

Interestingly, some of the results of NDV infection rates from this study contrast with those from Cumming et al., who analyzed the same samples for avian malaria. For instance, past sample screenings for avian malaria conducted by Cumming et al. have indicated Zimbabwe as having a high infection rate and Botswana as having a low infection rate. As shown in this study, the opposite holds true for the prevalence of NDV in these regions. The discrepancy in results between this study and previous literature is a subject of further research; however, it is clear that an inverse relation exists between NDV and avian malaria rates. Perhaps, avian malaria serves as a protective factor against the Newcastle disease virus, or vice versa. Certainly, future research would be beneficial to establish the potential relationship between the two diseases.

The samples collected from Lake Ngami, which held the highest infection rate, were further assessed for trends across waterfowl species. As shown by the results, the red-billed teals held the lowest infection rate, followed by Hottentot teals and Egyptian geese. The white-faced whistling ducks held the highest infection rate among all the four species. Whether these trends across species remain consistent in other regions of southern Africa is a subject of further investigation. The high prevalence of NDV in white-faced whistling ducks may stem from either innate, behavioral, or environmental factors. Specifically, differences in migratory patterns between these ducks and other species could contribute to the discrepancy in NDV infection rates. Further, white-faced whistling ducks are a highly social species that are known to associate in large numbers, even during non-breeding seasons (Sacramento Zoo, n.d.). This unique flocking behavior could place the species at a greater risk of contracting NDV. Other factors, such as dietary differences between the four species, may also contribute to discrepancies in infection rates. Due to a lack of sufficient samples, the results are confounding and may not be representative of all the species present in Lake Ngami. Despite this limitation, the high percentage of white-faced whistling ducks infected at this site relative to other species is significant. As of yet, no conclusive record of NDV patterns across the various species of southern African waterfowl have been provided by other literature. Thus, the results of this study provide much insight into potential differences in the NDV prevalence across waterfowl species in Lake Ngami.

Overall, this study produced novel findings regarding the geographic and biodiversity trends of NDV in southern Africa. The increased NDV prevalence moving northward and inland may be linked to migration paths of wild bird species (Si *et al.*, 2009). Tracking migration patterns alongside disease prevalence would thus serve as a future direction for this study. Moreover, the high prevalence of white-faced whistling ducks in Lake Ngami was especially striking. The uncertainty as to whether this result stems from genetic predispositions to the virus or ecological factors presented a limitation to this study; therefore, these factors must be more effectively isolated in future research. Certainly, the results of this study are confounding, and more research is needed to identify and gain insight into the specific factors contributing to these trends. Namely, the effects of season and migration patterns are among the most important factors that demand further investigation. Increased sample sizes from each species from all the sites are crucial to obtaining a more conclusive understanding of NDV trends in southern African waterfowl. Lastly, sequencing of the samples positive for NDV would be needed to verify the specific strain of the Newcastle disease virus. Ultimately, the insight gained from these future directions will aid in effectively tracking and preventing the transmission of Newcastle disease.

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