Surveillance of avian influenza and Newcastle disease viruses in backyard poultry raised near migratory bird sites in Mato Grosso state, Brazil

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Summary

The Pantanal and Cerrado biomes in the state of Mato Grosso contain migratory bird sites in the municipalities of Cáceres and Araguaiana, respectively. The levels of avian influenza (AI) and Newcastle disease (ND) viral activity in backyard poultry at these sites are unknown owing to a lack of studies. Considering the risk of introduction of AI and ND to Brazil from migratory birds, as well as the importance of active surveillance in the detection and prevention of diseases for official control, monitoring in these poultry populations is faster, more practical and cheaper for official service veterinarians. The objective of this study was to verify the presence of AI and ND viral activity in backyard poultry reared near these migratory bird sites in the years 2016 and 2019. Serum samples and cloacal and tracheal swab samples collected from chickens, turkeys, quails, ducks and geese were evaluated by indirect diagnostic methods including enzyme-linked immunosorbent assay and haemagglutination inhibition tests and direct detection of viral sequences using quantitative real-time reverse transcription polymerase chain reaction (qRT–PCR). No positive samples were detected by qRT–PCR. The frequencies of birds seropositive for AI and ND were 0.7% and 19.6% in 2016 and 0.5% and 17.2% in 2019, respectively, in Araguaiana and 0.8% and 32.3% in 2016 and 7.0% and 38.1% in 2019, respectively, in Cáceres. Antibodies belonging to AI subtypes H1, H4, H6 and H14 were identified in Cáceres in 2019. Spatial analysis showed an agglomeration of farms with seropositive poultry within the urban area of Cáceres, suggesting AI and ND virus activity in this area. This study showed no circulation of the notifiable AI subtypes H5 and H7 or the ND virus in backyard poultry raised around migratory bird sites in the state of Mato Grosso. The results of the present study support evidence indicating that the circulation of strains with low pathogenicity in urban areas enables backyard poultry to serve as a source of infection for other birds; thus, increased surveillance is necessary in this population.

Keywords

Avian influenza – Backyard poultry – Brazil – Newcastle disease – Surveillance – Virus.

Introduction

The impact and epidemiology of avian influenza virus (AIV) and Newcastle disease virus (NDV) differ substantially in different regions of the world, and providing specific recommendations for surveillance for all situations is therefore impossible. Therefore, surveillance strategies intended to demonstrate control of avian influenza (AI) and Newcastle disease (ND) at an acceptable level of confidence should be adapted to the local context (1).

Contact between poultry and wild birds has contributed to the emergence, dissemination and maintenance of new strains of AIV and NDV (2, 3). Backyard poultry are usually raised on farms in small flocks of multiple species and ages primarily to provide meat and eggs for household consumption (4). These farms typically lack biosecurity and thus expose the poultry to pathogenic agents from wild and migratory birds, resulting in the potential for flocks to serve as amplifiers, reservoirs and disseminators of these viruses (5, 6).

The Pantanal and other wetland regions, such as along the Araguaia River in Mato Grosso state, Brazil, are among the places noted as wintering sites for several migratory bird species (7). Therefore, these regions are important sites of entry for pathogens that can affect the poultry industry across the whole country. The Brazilian poultry industry is recognised in the international meat market and is one of the global market leaders in chicken meat exports, ranking as the world's second largest producer in this market sector (8). Veterinary authorities from the state of Mato Grosso (Agricultural Defence Institute of Mato Grosso [*Instituto de Defesa Agropecuária de Mato Grosso*, INDEA–MT]) have identified areas along the Paraguay River in the municipality of Cáceres and along the Araguaia River in Araguaiana as sites with significantly frequent contact between

migratory birds and backyard poultry in production systems with no biosecurity measures in place (9).

Considering the interaction between wild birds and poultry and the need for surveillance to provide scientific data that can explain the epidemiology of AI and ND in the region, the aim of the study was to carry out epidemiological surveillance of AI and ND in backyard poultry raised near migratory bird sites in the municipalities of Cáceres and Araguaiana in Mato Grosso state, Brazil.

Materials and methods

Epidemiological surveillance was coordinated and conducted by veterinarians from the INDEA–MT. Samples were collected in December 2016 and 2019 from backyard poultry farms located within a 10-km radius along the Paraguay River in the municipality of Cáceres and along the left shore of the Araguaia River in the municipality of Araguaiana. Sampling procedures were conducted according to the guidelines of the Brazilian Ministry of Agriculture, Livestock and Food Supply (*Ministério da Agricultura, Pecuária e Abastecimento do Brasil*, MAPA) and were approved by the Ethics Committee on Animal Use of the Federal University of Mato Grosso (*Universidade Federal de Mato Grosso*, UFMT), Protocol No. 23108.223675/2017-89.

The numbers of sampled farms and birds were consistent with the technical guidelines of MAPA (10). The number of properties with poultry sampled was chosen to ensure the identification of at least one infected farm, even if the prevalence was less than 5%, with a confidence interval (CI) of 95% and a sensitivity of 99% for properties with chickens, turkeys and quails and a CI and sensitivity of 99% for properties with *Anseriformes*. The number of birds to be sampled from each property was determined to ensure a 95% probability of identifying at least one seropositive bird considering a prevalence of seropositive birds equal to or greater than 30%. Thus, for each farm with chickens, turkeys and quails, samples were collected from a maximum of ten birds, whereas samples from a maximum of 20 birds were collected from farms with *Anseriformes*.

Blood samples and cloacal and tracheal swabs were taken from chickens, quails, turkeys, ducks and geese aged three months and older (Table I). All backyard farmers reported not vaccinating their poultry for ND. Assays to diagnose AI and ND were conducted in the Federal Laboratory for Agricultural Defence (LFDA–SP) located at Campinas, São Paulo, which is the reference laboratory of the World Organisation for Animal Health (OIE) in Brazil.

Table I

Numbers of properties and poultry species sampled in the vicinity of migratory bird sites in Araguaiana and Cáceres in Mato Grosso state, Brazil, in 2016 and 2019

Migratory site	Year	Sampled properties	Chicken	Quail	Turkey	Duck	Goose	Total
Araguaiana	2016	12	127	2	0	19	0	**148**
	2019	18	181	3	1	23	1	**209**
Cáceres	2016	53	675	2	11	153	82	**923**
	2019	53	632	6	10	80	33	**761**

For statistical evaluation, the Statistical Package for Social Sciences (SPSS) for Windows, version 21.0 (©SPSS Inc., Chicago, IL, United States of America [USA]) was used (11). Descriptive statistics with general and relative frequencies for AI and ND were calculated to evaluate risk factors according to location (Cáceres and Araguaiana) and year (2016 and 2019).

Variables such as the presence of domestic waterfowl, free-range poultry rearing, co-mingling of different susceptible species and marketing of poultry and eggs were analysed. Associations were evaluated with the chi-square or Fisher exact test, and odds ratios (ORs) were calculated with a CI of 95%. Tests were considered significant when $p < 0.05$.

Laboratory analysis

All procedures were conducted according to the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (OIE *Terrestrial Manual*) (12). A combination of two assays was used for the serological diagnosis of AI: the competitive enzyme-linked immunosorbent assay (cELISA) as a screening test and haemagglutination inhibition (HI) as a confirmatory test. The ELISA was used for screening samples owing to its high sensitivity and high specificity. The HI assay was performed in order to identify the viral haemagglutinin subtype responsible for the serological reaction in the ELISA. In addition to the use of antibodies against H5 and H7 subtypes as described in the OIE *Terrestrial Manual*, the investigation was extended to all other AI subtypes. At the time of the analysis there was no ELISA available to detect antibodies against NDV for all bird species, so a specific protocol for chickens was used, while for other species the HI test was adopted for screening.

Competitive enzyme-linked immunosorbent assay

The 2016 and 2019 serum samples were tested with the IDEXX Influenza A Antibody (Ab) Test kit (IDEXX Laboratories, Inc., Westbrook, ME, USA) and Multispecies Influenza A Antibody Test kit (BioChek, Smart Veterinary Diagnostics, Scarborough, ME, USA) to assess the presence of anti-influenza A antibodies (Abs). According to the manufacturers, the sensitivity and specificity values are 95.4% and 99.7% for the IDEXX Influenza A Ab Test kit and 95.0% and 99.0%, respectively, for the Multispecies Influenza A Antibody Test kit. Both kits allow the detection of antibodies to any subtype of the influenza A virus and are officially validated by MAPA, Brazil. The presence of antibodies to ND was assessed only in chicken sera with the IDEXX NDV Ab Test for chickens (IDEXX Laboratories, Inc., Westbrook) in 2016 and with the NDV Antibody Test kit (BioChek, Smart Veterinary Diagnostics, Scarborough) in 2019. According to the manufacturers, the sensitivity and specificity values are 91.5% and 99.0% for the IDEXX NDV Ab Test kit and > 95% and 100%, respectively, for the NDV Antibody Test kit. Both kits allow the

detection of antibodies to NDV and are officially validated by MAPA, Brazil.

Haemagglutination inhibition assay

Samples that were seropositive for AI in the ELISA were subsequently subjected to a haemagglutination inhibition (HI) assay to assess the presence of Abs to the H1 to H16 subtypes of the AIV. Samples from quails, turkeys, ducks and geese were subjected to HI tests to detect antibodies against NDV. Viral antigens representing the 16 AIV subtypes and the NDV strain used for HI tests are provided in Table II. Inactivated homologous positive controls were used with antigens from OIE reference laboratories, including the Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) and the National Veterinary Services Laboratories (NVSL), Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA).

Table II

Viral antigens representing the 16 avian influenza virus subtypes and the Newcastle disease virus strain used for haemagglutination inhibition tests

Antigen	AIV subtype/NDV strain	Provider
H1N1	A/duck/Italy/1447/05 (H1N1)	IZSVe
H1N7	A/NJ/8/76-equine-1 (H1N7)	NVSL/APHIS/USDA
H2N3	A/duck/Germany/1215/73 (H2N3)	IZSVe
H2N9	A/pintail/Alberta/293/77 (H2N9)	NVSL/APHIS/USDA
H3N8	A/pass/It/6000/V00 (H3N8)	IZSVe
H3N8	A/duck/Ukraine/1/63 (H3N8)	NVSL/APHIS/USDA
H4N8	A/cockatoo/England/72 (H4N8)	IZSVe
H5N9	A/chicken/Italy/22A/98 (H5N9)	IZSVe
H5N9	A/turkey/Wisconsin/68 (H5N9)	NVSL/APHIS/USDA
H6N2	A/turkey/Canada/65 (H6N2)	IZSVe
H6N8	A/turkey/Ontario/63 (H6N8)	NVSL/APHIS/USDA

H7N3	A/turkey/Italy/9289/V02 (H7N3)	IZSVe
H8N4	A/turkey/Ontario/6118/68 (H8N4)	IZSVe
H9N2	A/turkey/Wisconsin/66 (H9N2)	IZSVe
H10N1	A/ostrich/South Africa/01 (H10N1)	IZSVe
H10N7	A/chicken/Germany/N/49 (H10N7)	NVSL/APHIS/USDA
H11N9	A/duck/Memphis/546/174 (H11N9)	IZSVe
H12N5	A/duck/Alberta/60/76 (H12N5)	IZSVe
H13N6	A/gull/Maryland/704/77 (H13N6)	IZSVe
H14N5	A/mallard/Gurjev/263/82 (H14N5)	IZSVe
H15N9	A/shearwater/Western Australia/2576/79 (H15N9)	IZSVe
H16N3	A/gull/Denmark/68110/02 (H16N3)	IZSVe
NDV	ND Ulster 2C	IZSVe
NDV	LaSota	NVSL/APHIS/USDA

AIV: Avian influenza virus
APHIS: Animal and Plant Health Inspection Service
IZSVe: Istituto Zooprofilattico Sperimentale delle Venezie
NDV: Newcastle disease virus
NVSL: National Veterinary Services Laboratories
USDA: United States Department of Agriculture

Quantitative real-time reverse transcription polymerase chain reaction

Viral ribonucleic acid (RNA) was extracted following procedures for the commercial MagNA Pure LC Total Nucleic Acid Isolation kit (Roche, Rotkreuz, Switzerland) in 2016 and with the MagMAX™-96 AI/ND Viral RNA Isolation kit (Applied Biosystems, Thermo Fisher Scientific, Inc., Waltham, MA, USA) in 2019, according to the manufacturers' instructions.

Quantitative real-time reverse transcription polymerase chain reaction (qRT–PCR) for direct detection of AIV and NDV sequences followed procedures outlined in the OIE *Terrestrial Manual* (12) and was conducted using the VetMAX™ Gold AIV detection kit (Life Technologies Corporation, Austin, TX, USA) and the AgPath-ID™ One-Step RT–PCR Reagents kit for NDV (Life Technologies Corporation, Austin) according to the manufacturers' instructions.

Spatial analysis

Thematic maps with the distribution of positive points for each study site were produced with ArcGIS 10.0 software (13). Spatial distributions were calculated according to kernel density analysis, a non-parametric method of identifying a function that explains a set of data, as proposed by Wickham (14), using the free software R (15). The dataset included two-dimensional points. The function generates an image with variations in heat, in which greater intensity and influence of neighbours correspond to warmer colour gradations.

Results

Estimates of frequencies of farms and poultry seropositive for AI and ND at Araguaiana and Cáceres migratory sites in 2016 and 2019 are shown in Tables III and IV; HIAbs against subtypes H1, H4, H6 and H14 of AIV were detected in chickens from Cáceres in 2019. All cloacal and tracheal samples subjected to qRT-PCR were negative in 2016 and 2019. No statistically significant associations were identified between any of the variables, diseases, periods or locations assessed. The frequencies of independent variables adopted from the questionnaire completed by property owners are given in Table V.

Table III

Estimates of frequencies of properties and poultry seropositive for avian influenza and Newcastle disease at Araguaiana and Cáceres migratory sites in 2016 and 2019

Migratory site		Properties			Poultry		
Araguaiana	**Year**	**Positive/total**	**(%)**	**CI 95%**	**Positive/total**	**(%)**	**CI 95%**
Avian influenza	2016	1/12	(8.3)	0.0–25.0	1/148	(0.7)	0.0–2.0
	2019	1/18	(5.6)	0.0–16.5	1/209	(0.5)	0.0–1.4
Newcastle disease	2016	9/12	(75.0)	42.2–100.0	29/148	(19.6)	13.2–26.0
	2019	14/18	(77.8)	55.6–94.4	36/209	(17.2)	12.1–22.3
Cáceres							
Avian influenza	2016	5/53	(9.4)	3.8–18.1	7/923	(0.8)	0.2–1.3
	2019	23/53	(43.4)	25.9–57.1	53/761	(7.0)	5.2–8.8
Newcastle disease	2016	52/53	(98.1)	92.5–100.0	298/923	(32.3)	29.3–35.3
	2019	49/53	(92.5)	84.4–98.1	290/761	(38.1)	34.7–41.6

CI: confidence interval

Table IV

Estimates of frequencies of birds of several species seropositive for avian influenza and Newcastle disease at Araguaiana and Cáceres migratory sites in 2016 and 2019

Migratory site		Chicken	Quail	Turkey	Duck	Goose
Araguaiana	**Year**			**Positive/total**		
Avian influenza	2016	1/127	0/2	0/0	0/19	0/0
	2019	1/181	0/3	0/1	0/23	0/1
Newcastle disease	2016	29/127	0/2	0/0	0/19	0/0
	2019	36/181	0/3	0/1	0/23	0/1
Cáceres						
Avian influenza	2016	5/675	0/2	0/11	2/153	0/82
	2019	53/632	0/6	0/10	0/80	0/33
Newcastle disease	2016	322/675	0/2	0/11	1/153	0/82
	2019	289/632	0/6	0/10	1/80	0/33

Table V

Independent variables adopted from the questionnaire completed by property owners at migratory bird sites in Araguaiana and Cáceres in 2016 and 2019

Independent variables	Migratory sites							
	Araguaiana				Cáceres			
	Number of holdings (%)							
	2016		2019		2016		2019	
Presence of domestic waterfowl	3	(25.0)	5	(27.7)	31	(58.4)	21	(39.6)
Free-range poultry rearing	9	(75.0)	14	(77.7)	51	(96.2)	51	(96.2)
Co-mingling of different susceptible species	7	(58.3)	9	(50.0)	38	(71.6)	36	(67.9)
Marketing of poultry and eggs	4	(30.7)	7	(38.8)	20	(37.7)	17	(32.0)

Spatial analysis

Figure 1 shows a thematic map with distributions of the farms positive for AI in Cáceres and Araguaiana. Figure 2 shows the analysis for AI in Araguaiana. Notably, few points were observed to be seropositive in both years evaluated, and an alteration in the denser clusters of seronegative points was observed between 2016 and 2019, with a transition from the southern portion to the northern perimeter of the studied region. Figure 3 shows the analysis for AI in Cáceres. Notably, few seropositive points were observed in 2016, and an intense change was noted in 2019, with centralisation of seropositive points in the study area. Among the seronegative points, no significant changes were observed between the years, and the highest numbers of seronegative points were located near the southern perimeter of the studied area.

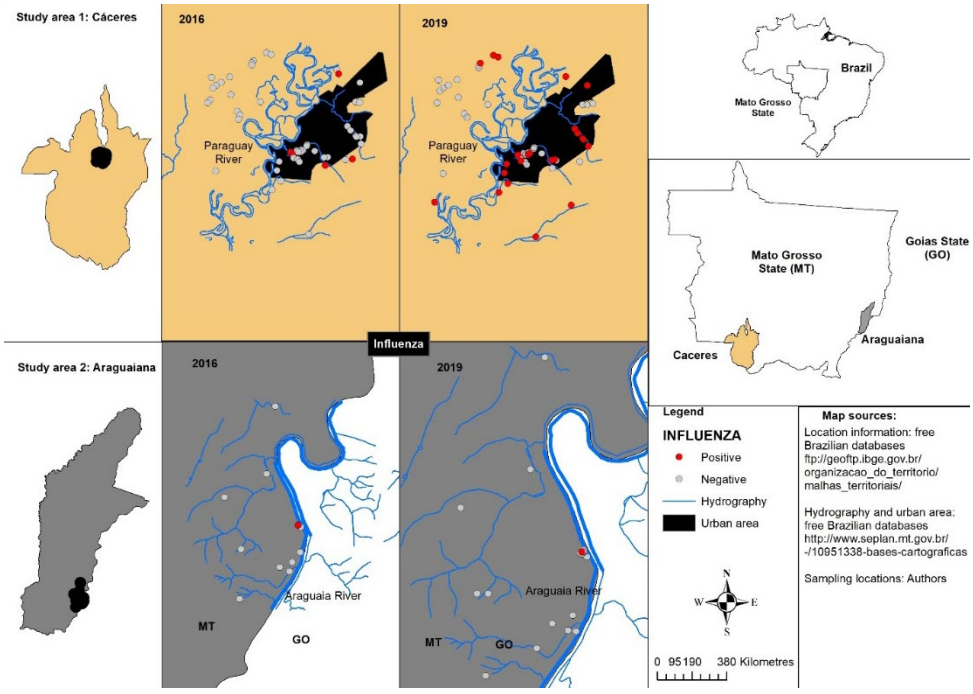

Fig. 1

Thematic map with distributions of the properties positive for avian influenza in Cáceres and Araguaiana in 2016 and 2019

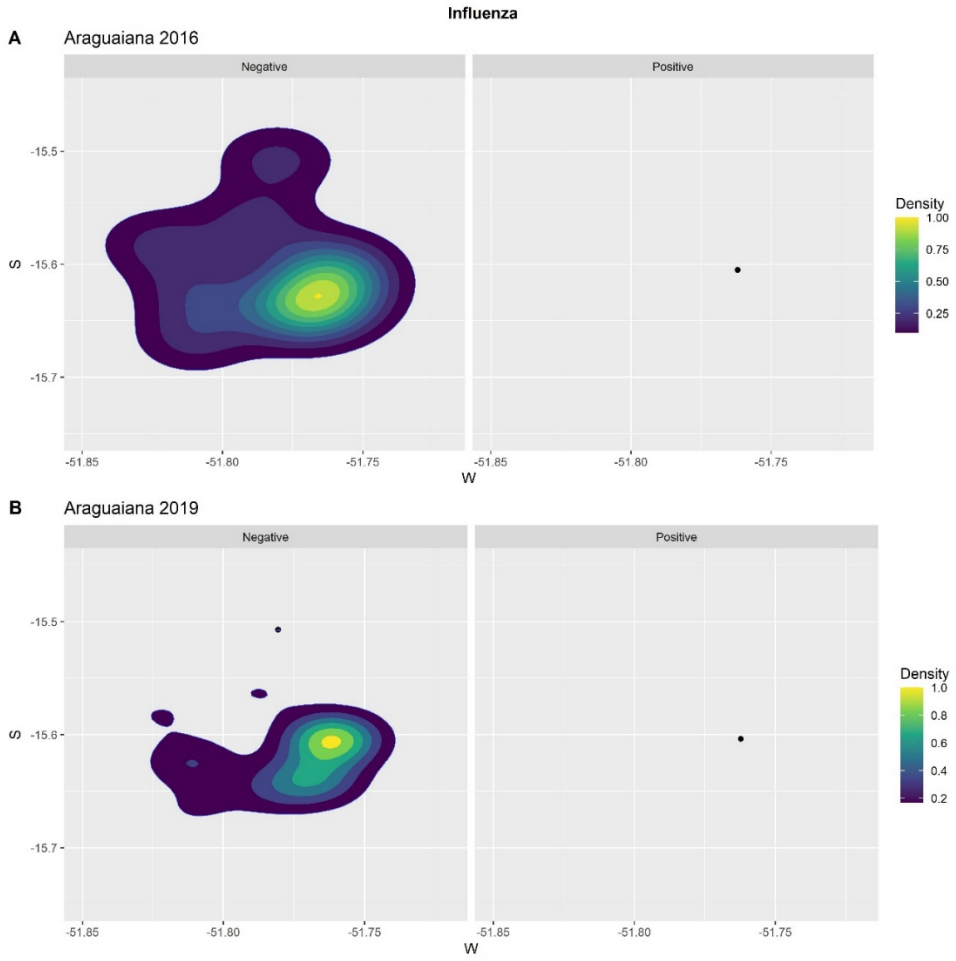

Fig. 2

Kernel analysis for avian influenza in Araguaiana

Notably, few points were observed to be seropositive in both years evaluated, and an alteration in the location of the densest cluster of seronegative points was observed from 2016 to 2019, with a transition from the southern portion to the northern perimeter of the area studied.

The latitudes on the *x*-axis and the longitudes on the *y*-axis are presented in decimal degrees, and as the number of points in a location varies, the density varies by a coefficient ranging from 0 to 1, with 1 representing higher density and 0 representing lower density.

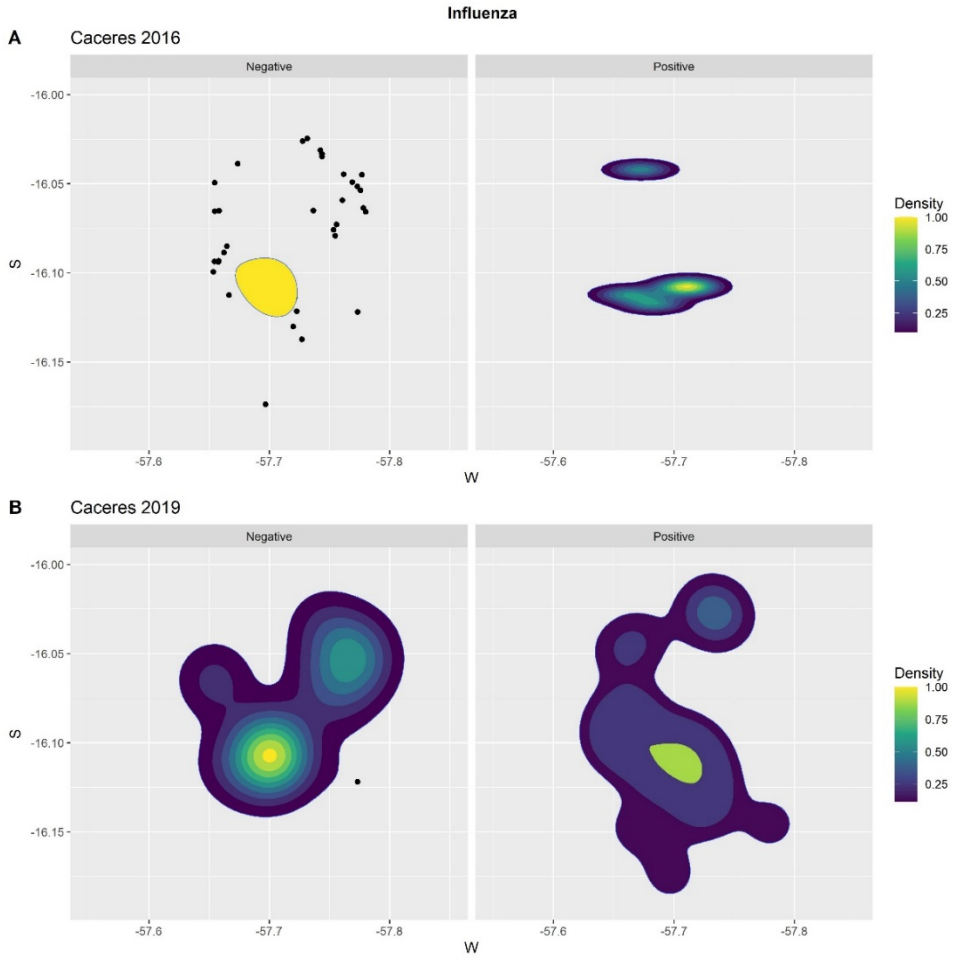

Fig. 3

Kernel analysis for avian influenza in Cáceres

Notably, few seropositive points were observed in 2016, and an intense change was noted in 2019, with centralisation of seropositive points in the study area. Among the seronegative points, no significant changes were observed between the years, and the highest number of seronegative points was located near the southern perimeter of the area studied.

The latitudes on the *x*-axis and the longitudes on the *y*-axis are presented in decimal degrees, and as the number of points in a location varies, the density varies by a coefficient ranging from 0 to 1, with 1 representing higher density and 0 representing lower density.

Figure 4 shows a thematic map with distributions of the farms positive for ND in Cáceres and Araguaiana. Figure 5 shows the analysis for ND in Cáceres. The positivity profile remained practically unchanged between 2016 and 2019, with the densest area corresponding to the urbanised region of the municipality. Figure 6 shows the analysis for ND in Araguaiana. Notably, intense clusters of seropositive and seronegative points were observed in both years, without significant changes in their locations in the study area between study years, and these locations were more centralised in the study area.

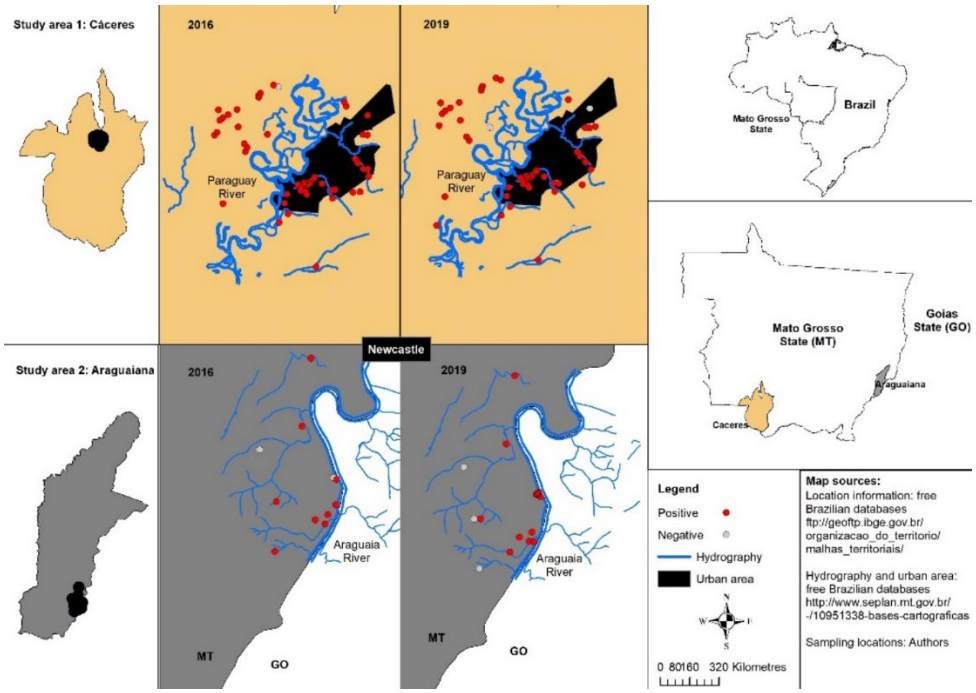

Fig. 4

Thematic map with distributions of the properties positive for Newcastle disease in Cáceres and Araguaiana in 2016 and 2019

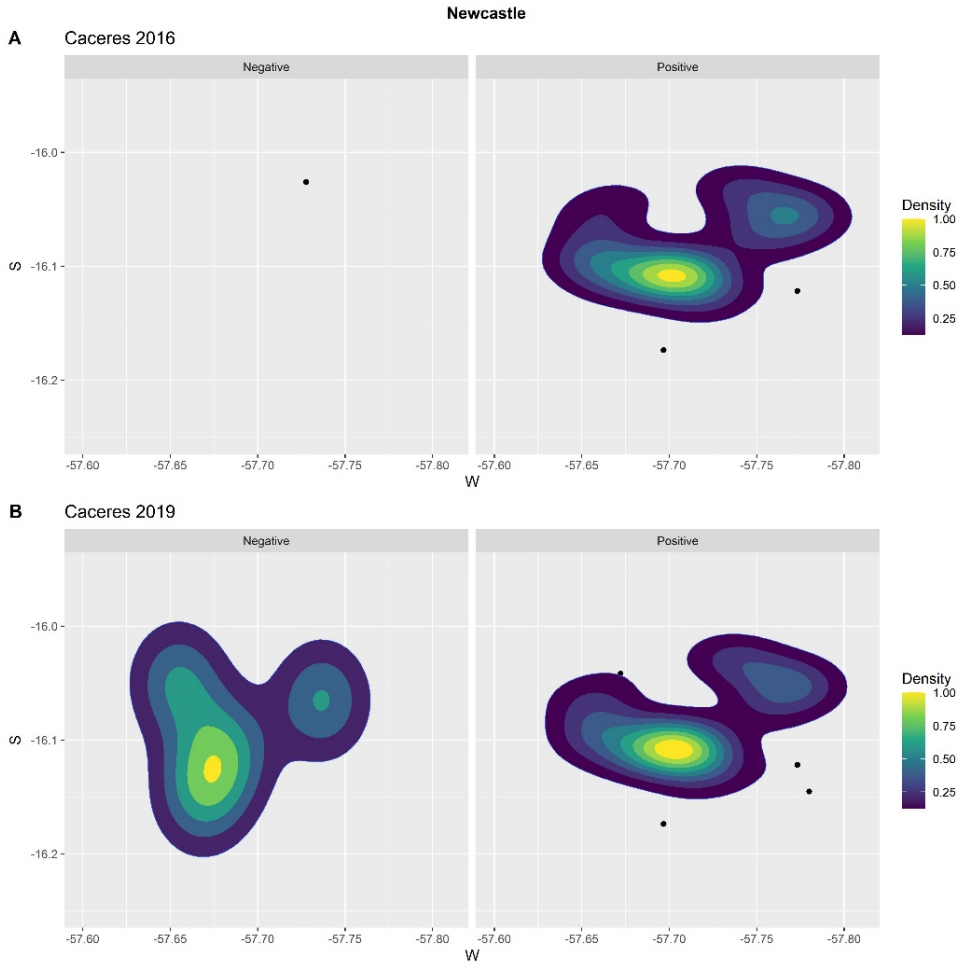

Fig. 5

Kernel analysis for Newcastle disease in Cáceres

The positivity profile remained practically unchanged between 2016 and 2019, with the same dense area corresponding to the urbanised region of the municipality.

The latitudes on the *x*-axis and the longitudes on the *y*-axis are presented in decimal degrees, and as the number of points in a location varies, the density varies by a coefficient ranging from 0 to 1, with 1 representing higher density and 0 representing lower density.

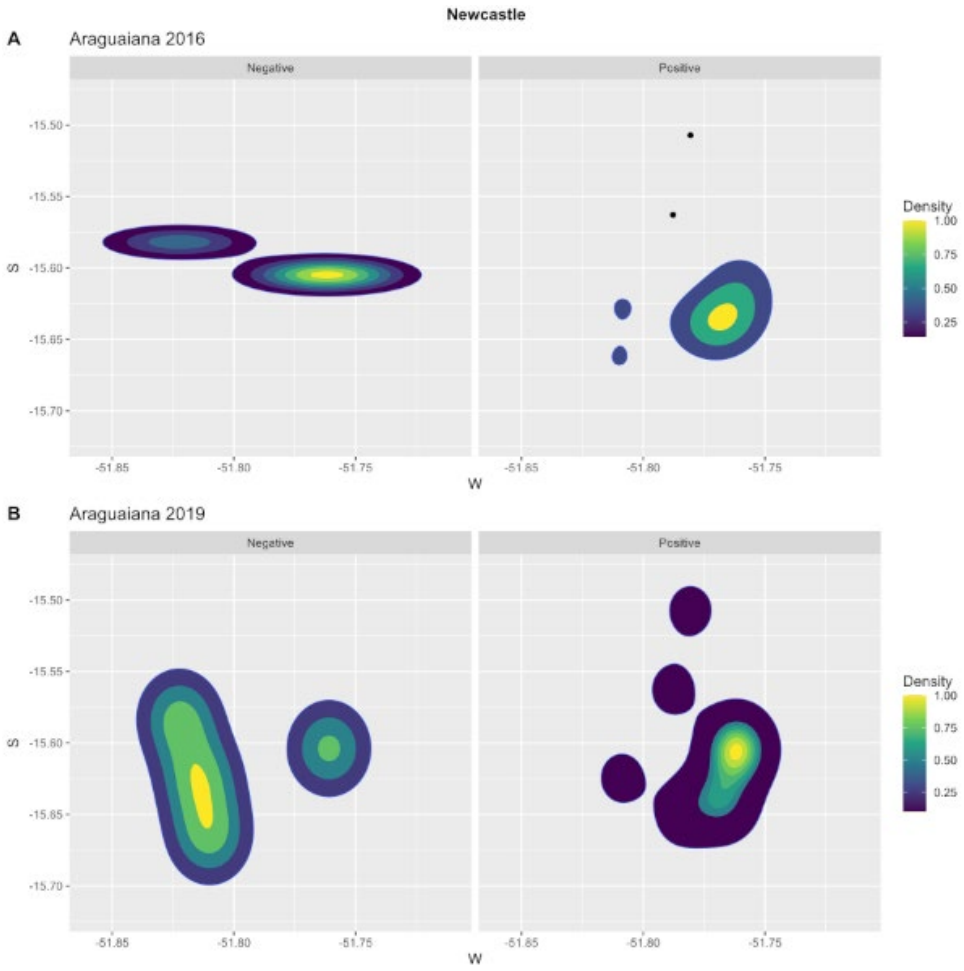

Fig. 6

Kernel analysis for Newcastle disease in Araguaiana

Notably, intense locations of seropositive and seronegative points were observed in both years, without significant changes in their locations in the study area from one year to the next, and these locations were more centralised in the study area.

The latitudes on the *x*-axis and the longitudes on the *y*-axis are presented in decimal degrees, and as the number of points in a location varies, the density varies by a coefficient ranging from 0 to 1, with 1 representing higher density and 0 representing lower density.

Discussion

The survey of AI and ND reported herein was conducted by veterinarians and technicians from the State Animal Defence Service and adopted all official measures imposed by the Department of Sanitary Defence of MAPA of Brazil; therefore, the investigation was considered an official survey of regions with wintering sites for migratory birds. In this context, active surveillance of backyard birds is included in animal health control activities conducted by INDEA–MT and aims to monitor the possible entry and circulation of AI and ND in these regions that are important sites for migratory birds. It should be emphasised that these activities require interventions with practical procedures and must be frequent and carried out in a short period of time. On the other hand, monitoring AI and ND in migratory birds requires greater financial resources, permissions from wildlife agencies, and substantial allocation of time and methodologies specific to each environment, considerations that are not always practicable in active surveillance by governmental offices. In addition, domestic birds reared in the backyard are more likely to come into contact with migratory and wild species owing to the absence of biosecurity measures, so they can serve as AI and ND sentinels. For this reason, the present study was limited to evaluating domestic backyard poultry.

Notably, the viruses were not detected in the birds sampled during the study period because all cloacal and tracheal samples subjected to qRT–PCR were negative for AI and ND in 2016 and 2019. On the other hand, the presence of antibodies indicates that backyard poultry had been previously exposed to the agents, and the agents probably continue to circulate in these locations. Strains with low pathogenicity can induce seroconversion in the absence of clinical signs with rapid excretion of low viral levels, which may explain the absence of symptomatology in the birds evaluated in the present study. Low viral excretion can also be related to the species, the immune status of the host, the viral strain involved, and co-infection with other pathogens (16, 17, 18, 19, 20). In general, most infected birds excrete AI for a

period of only seven to ten days (21). Viral shedding of low pathogenicity AIVs from chickens showed a peak at one to two days post-inoculation followed by rapid decline to low viral titres between three to seven days post-inoculation (16). A previous survey carried out on wild birds in the Pantanal region of Mato Grosso reported no detection of AI or ND (22).

A high frequency of seropositive chickens was observed for both agents, to the exclusion of other avian species in both areas. Chickens are known to be more susceptible to infection, and antibodies can persist for a long time in adults, while ducks show limited and transient production of antibodies and an inability to produce haemagglutinating antibodies (23, 24, 25).

In the Araguaiana site, low frequencies with no significant changes in AI seropositivity of poultry were observed in 2016 and 2019 (Fig. 2). As the ELISA has high sensitivity and the sample size was large, the possibility of false positive results should be considered, especially in the face of inconclusive results in the HI assays (26). Highly pathogenic AI is rare in Brazil; thus, vaccination is prohibited throughout the country (27). Depending on the strain, no viral shedding may occur, and contact transmission between birds may consequently be restricted (16). It is important to note that to meet the bureaucratic procedures of the Department of Sanitary Defence in Brazil for the acquisition of commercial kits, different brands of serological kits were used to diagnose AI and ND between investigations. However, the protocols are similar in format and performance and are validated and authorised for serological diagnosis of AI and ND.

Figure 3 displays the spatial analysis of AI seropositive farms in Cáceres, with lower positivity in 2016 than in 2019. Although birds with antibodies for subtypes H5 and H7 were not detected, antibodies against subtypes of interest to public health were detected, such as those for subtypes H1, H4, H6 and H14. Similar investigations in other Brazilian states have also found seropositivity against H1 in Rio Grande do Sul, against H4 in Santa Catarina and against H6 in Bahia,

Mato Grosso do Sul, Pernambuco, Rio Grande do Sul, Santa Catarina and São Paulo (28). Some of these subtypes are among the most common found in wild birds through AI surveillance (29, 30, 31). The H6 subtype has been reported in outbreaks of low pathogenicity avian influenza in the USA (32) and has also been recognised as one of the influenza subtypes infecting humans in Taiwan, for which viral isolation demonstrated close similarity to a chicken H6N1 isolate (33). The subtype H14 has rarely been detected. Historically, H14 was limited to isolates from the former Soviet Union in the 1980s, North America in 2010 and Central America in 2013 (34, 35). Annually, several species of wild birds migrate to the southern hemisphere, and birds recovered from the Brazilian coast, Amazon region and southeast area were found to originate mostly from North and Central America (36); thus, the introduction of this subtype to Brazil is quite possible.

The frequency of seropositivity for ND was markedly higher in Cáceres than in Araguaiana (Figs 5 and 6). Backyard poultry can often present antibodies to different infections, as these flocks are characterised by the absence of biosecurity measures and frequent contact with various animal species, including humans and wild birds, which often exposes them to different types of virus (5, 37). A previous survey for ND in backyard poultry around a migratory bird site in southern Brazil showed that 87.5% of the properties contained seropositive birds with no detection of viral excretion in these birds (38).

The potential spillover of ND vaccine-related viruses has been observed, probably due to extensive use of live-attenuated vaccines in commercial poultry and close interactions between domesticated and wild bird populations (39). Newcastle disease was detected in backyard avian species raised without simple biosecurity measures located less than 6 km from commercial poultry facilities in the state of California, USA (40). In the present study, the nearest commercial poultry farm (in a straight line) is approximately 300 km from Araguaiana and 75 km from Cáceres (41) (data not shown); therefore, backyard poultry from these regions seem unlikely to have been

infected as a result of proximity to commercial poultry farms. Nevertheless, it is necessary to consider the possibility of illegal sales of birds from commercial poultry flocks to other producers, which could also favour the infection of flocks by vaccine strains (42).

The urban area of the municipality of Cáceres is within the migratory bird site and, interestingly, the central urban area of the municipality showed notably higher positivity for AI (Fig. 3) and ND (Fig. 4). Urbanisation increasingly affects the epidemiological characteristics of infectious diseases. Depending on the timing, dynamics and environment, the urbanisation of a region can either promote or hinder the spread of pathogens (43). The presence of wild birds also may have contributed to the introduction of AI and ND to urban areas in the Netherlands, where the prevalence of AI in these animals varied according to levels of urbanisation (44). Interactions between wild birds and backyard poultry have clearly increased the seroprevalence of ND (40). Birds highly adapted to urban areas, such as pigeons (*Columba livia*) and other members of the family Columbidae, can also act as potential carriers and transmitters of diseases (45, 46). Nevertheless, a high challenge dose of NDV relative to that normally sustained by wild birds is necessary to infect chickens, without some adaptation of the virus to chickens (47).

In the present study, the risk factors related to the presence of *Anseriformes*, free-range poultry rearing and contact with other animal species, as well as the commercialisation of birds and eggs, were investigated. Despite the verification of factors in some designs in both regions and in both periods (Table V), no statistically significant associations were found. Variables associated with the transmission of infectious diseases to backyard poultry raised in urban areas, including lack of hygienic disposal of dead birds and local marketing of birds, were found to be characterised as risk factors in Iran for AI and in the People's Republic of China for AI and ND (37, 48). However, it should be noted that the data presented herein were obtained through a questionnaire submitted to poultry producers, and the authors were limited to reporting their responses. It must be considered that the poultry and egg trades are regulated by federal and often municipal

laws. In other words, there is no permission to trade in poultry products or derivatives without sanitary inspection, and there would be no possibility of this type of activity owing to the characteristics presented by the properties studied. Based on the authors' observations, all the properties studied produce poultry for their own consumption.

Conclusion

The results of this study show no circulation of the notifiable AI subtypes H5 or H7 or ND pathogenic virus in backyard poultry reared near migratory bird sites in the state of Mato Grosso, Brazil. However, the presence of seropositive samples, probably due to low pathogenicity strains, reveals the capacity of backyard poultry to serve as a source of infection for other birds. The data presented in the present study suggest that the migratory bird site in the urban area of Cáceres may have an influence on the activity of AIV and NDV in domestic and wild birds.

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