Avian orthoavulavirus 1 (Newcastle Disease virus) antibodies in five penguin species, Antarctic peninsula and Southern Patagonia

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Abstract

Avian orthoavulavirus 1 (AOaV-1) causes Newcastle disease, one of the most important and contagious infections in poultry, where migratory birds can play a key role as a reservoir. Seven hundred and seven serum samples were collected from five penguin species (King, Magellanic, Gentoo, Chinstrap and Adelie penguins) in the Antarctic and Sub-Antarctic zones. Using a competitive ELISA to detect antibodies against AOaV-1, we identified positive individuals in all penguin species. The Magellanic penguin showed the highest seropositivity rate (30.3%),

suggesting it could be a natural reservoir of this virus. At the Antarctic zones, Chinstrap penguin showed the highest occurrence (7.5%). Interesting, positive sera was only obtained in Sub-Antarctic and Northern zones at the Antarctic peninsula, no seroreactivity was observed in Southern locations. Further studies are needed to establish the role of these penguin species in the epidemiology of the AOaV-1 and determine the effects of this virus in these populations.

Keywords

Antarctica; avulavirus; Newcastle disease; orthoavulavirus; penguins

1 | INTRODUCTION

Avian orthoavulavirus 1 (AOaV-1), formerly known as *Avian avulavirus 1* (AAvV-1), *avian paramyxovirus 1* (APMV-1) and *Newcastle disease virus* (NDV), belongs to the family *Paramyxoviridae*, subfamily *Avulavirinae*, genus *Orthoavulavirus* (International Committee on Taxonomy of Viruses (ICTV), 2019). AOaV-1 causes Newcastle disease, one of the most important and contagious infections in poultry, which negatively affects production and trade (Paramyxoviridae & Pneumoviridae, 2017). Wild birds, including aquatic/migratory birds, may act as natural reservoir hosts of AOaV-1 and may play a remarkable role in the spread of the virus in the environment (Rahman et al., 2018).

Although there are studies that have evaluated the presence of avian paramyxoviruses in penguins, only a few reports have confirmed the presence of AOaV-1 in this species (Table S1). To date, there is evidence of AOaV-1 infecting Adélie (*Pygoscelis adeliae*) and Magellanic (*Spheniscus magellanicus*) penguins (Thomazelli et al., 2010; Uhart et al., 2020), but little or no information is available in other penguin species, such as King (*Aptenodytes patagonicus*), Gentoo (*Pygoscelis papua*) and Chinstrap (*Pygoscelis antarcticus*) penguins. Viral-pathogens surveillance in wildlife enables the determination of virus reference values, detection of potential reservoir hosts, and prevention of epidemics in wildlife and domestic animals. Accordingly, we sought to determine the occurrence and circulation of AOaV-1 in penguins from the Antarctic and sub-Antarctic regions.

2 | MATERIALS AND METHODS

A total of 707 blood samples were obtained from five penguin species, which were collected from 14 locations in the Antarctic Peninsula (Antarctic region) and two locations in the continental area of the Magallanes region, Chile (sub-Antarctic region) (Table S1). A total of 636 samples were obtained from Chinstrap (*n* = 292), Gentoo (*n* = 263) and Adelie (*n* = 81) penguins in Antarctica during Chilean Antarctic Scientific Expeditions in 2017 (*n* = 92), 2018 (*n* = 248) and 2019 (*n* = 296), organized by the Chilean Antarctic Institute (INACH). The sampling locations were distributed in three Antarctic regions: Northwest, Midwest and Southwest of the Antarctic Peninsula. The Northwest region (Northwest Peninsula and South Shetland Islands) included eight sampling locations: Ardley Island, Harmony Point on Nelson Island, Barrientos Island, Cape Shirreff on Livingston Island, Hannah Point on Livingston Island, Lions Rump on King George Island, Low Island, and Bernardo O'Higgins General Base on the Antarctic Peninsula. The Midwest region included

three sampling locations: Biscoe Point on Anvers Island, President Gabriel González Videla Base on Paradise Bay, and Yelcho Base on Doumer Island. The Southwest region included three sampling locations: Avian Island, Lagotellerie Island and Emperor Island (Table 1; Figure 1a). The remaining samples were obtained from King penguins ($n = 38$) at Bahía Inútil, Tierra del Fuego, between 2014 and 2018, and from Magellanic penguins ($n = 33$) on Magdalena Island, Strait of Magellan in 2011 (Table 2; Figure 1b).

Blood samples were obtained by venipuncture in the common plantar digital vein. To achieve this, the penguins were caught with a net, the eyes covered with a black cloth, and gently restrained in a prone position to expose the feet. The samples were centrifuged for 10 min at 800 g and the resulting sera were stored at −20°C until use. Antibodies against AOaV-1 nucleoprotein were detected using a multispecies competitive ELISA commercial kit (ID Screen® Newcastle Disease Competition, IDVet), following manufacturer's recommendations. ELISA results were expressed as percent inhibition values (PI), according to manufacturer's instructions, where a PI greater than 40 was considered positive, between 30 and 40 suspect and lower than 30 negative. Suspect samples were considered as negative in the seropositivity calculation. According to a Shapiro–Wilk test, the data do not follow a normal distribution. Overall results of PI (antibody levels) were compared by species and location using a Kruskal–Wallis test followed by Conover's multiple comparisons test. To determine differences between year of collection for each location, Kruskal–Wallis or Mann–Whitney U test were performed. In addition, chi-square and Fisher's exact test were performed to determine the association between region and species, with the purpose of collinearity, confounding factor checking and logistic regression model building approach. Subsequently, a simple logistic regression analysis was performed to assess for the effect of the specie as potential risk factors to NDV positivity (Dohoo et al., 2010). Goodness-of-fit was assessed using the Hosmer and Lemeshow Test (Hosmer et al., 2013). Statistically significant differences were set at p-value < .05. The statistical analyses were performed using R version 4.0.2 (R Core Team, 2020) and GraphPad Prism version 8.0.0 for Mac, GraphPad Software. Finally, the specificity of this kit was evaluated using positive antisera against novel Avian orthoavulaviruses (AOaV-17, AOaV-18, and AOaV-19), which have been widely detected previously in Chinstrap, Gentoo, and Adelie penguins from the Antarctic Peninsula, demonstrating the no cross-reactivity with them (Neira et al., 2017; Olivares et al., 2019; Wille et al., 2019).

3 | RESULTS AND DISCUSSION

Forty-five out of 707 samples (6.4%) were positive for antibodies against AOaV-1; 34 out of 636 (5.3%) from Antarctic locations and 11 out of 71 (15.5%) from sub-Antarctic locations (Figure 2a). All penguin species reported at least one positive sample (Figure 2b). In the Antarctic region, 22 out of 292 (7.5%) Chinstrap penguins were seropositive to AOaV-1, whereas 11 out of 263 (4.2%) Gentoo penguin and 1 out of 81 (1.2%) Adelie penguin showed positive serology. Of the 8 locations sampled, we found positive Chinstrap penguins at 6 locations, Gentoo penguins at 5 locations and only one location with one positive Adelie penguin (Table 1). In the sub-Antarctic region, 10 out of 33 (30.3%) samples were positive in Magellanic penguins from Magdalena Island and 1 sample out of 38 (2.6%) was positive in a King penguin from Bahía Inútil (Table 2).

Among Antarctic and sub-Antarctic regions, the Magellanic penguin and, therefore, Magdalena Island, had the highest antibody levels of all the species ($H = 109.17$, $df = 4$, $p < .05$, $\varepsilon^2 = 0.155$) (Figure 2). In the Antarctic Peninsula, positive samples were only detected in the Northwest region, where the highest antibody levels were found in Low Island. However, there were no significant differences with other locations except for Barrientos Island and Hannah Point, which had the lowest antibody levels ($p < .05$) according to the multiple comparison test as shown in Figure 2a. The AOaV-17, AOaV-18 and AOaV-19 antisera were negative for the AOaV-1-ELISA; thus, there was no cross-reactivity between these viruses. Despite the low or nor detection of AOaV-1 antibodies, statistically significant differences were observed between years of sampling in Bernardo O'Higgins General Base (2018–2019, $p < .05$), Yelcho Base (2017–2018–2019; $p < .05$) and Avian Island (2017–2018; $p < .05$). The chi-square and Fisher's exact tests confirmed the association between region and specie (p-value $< .001$), indicating that simple logistic regression should be performed, avoiding collinearity and the effect of potential confounding factors. The logistic regression evidence that *S. magellanicus* present a higher risk of positivity to NDV when compared to *A. patagonicus* (OR = 16.087; 95% CI = 1.930–134.087; p-value = .010); however, the other species sampled in this study did not show a significant difference ($p > .05$) (Table S3). Goodness-of-fit show a good adjustment between data and the model (p-value = .345).

These results show evidence of AOaV-1 infection in different penguin species from Antarctic and sub-Antarctic zones. We highlight this is the first report of AOaV-1 in the continental area of the Magallanes region of Chile and the first report that shows evidence of AOaV-1 infection in King penguins. A previous study, based on hemagglutination inhibition (HI) assay, screened AOaV-1 in King penguins but they did not detect antibodies against this virus (Gauthier-Clerc et al., 2002). In addition, this study suggests that this virus might circulate in Magellanic, Adelie, Chinstrap and Gentoo penguins in nature. A previous serologic study of Adelie, Chinstrap and Gentoo penguins from King George Island, Antarctica, by HI against AOaV-1, using an NDV B1 strain, a Class II NDV vaccine strain, reported an overall positivity of 33.3%. However, this study does not specify which species were positive, nor the seropositivity rate by species (Thomazelli et al., 2010) and also HI reports cross-reactivity as a limitation (Nayak et al., 2012).

Penguin species, including Antarctic penguins, have been described as potential reservoirs for several avian orthoavulaviruses, which could have the potential to infect other avian hosts (Wille et al., 2019). Given the high seropositivity (30.3%) to AOaV-1 seen in Magellanic penguins, we speculate that this species could be a natural reservoir for this virus. This is supported by a recent report from Argentina, where breeding colonies of this penguin species distributed along the entire coast were positive to AOaV-1, with a seroprevalence of 44% (Uhart et al., 2020). Furthermore, unlike the other sampled penguin species in this study, Magellanic penguin nest on the coasts and islands of southern Argentina and Chile, and migrate north in winter, reaching the coasts of Uruguay and southeastern Brazil (Yamamoto et al., 2019). Additionally, for at least three decades, over 25 rehabilitation centres have received and kept in captivity Magellanic penguins, until recovery, releasing them back into the wild (García-Borboroglu et al., 2006). It has been previously suggested that AOaV-1 may spillover from poultry into the environment (Garcia

et al., 2013). Thus, the extended geographical movement of this species in South America and closer contact with people might result in their exposure to other animal pathogens. The high seroprevalence in this species may be of concern for potential outbreaks that could affect this or other avian species and highlights the need for further long-term surveillance in the region.

AOaV-1-positive penguins were detected in all sampled locations form the Northwest region of the Antarctic Peninsula, suggesting that at least at some point this virus circulated widely in this area. Thomazelli et al. (2010) also reported AOaV-1-positive penguins in this region (King George Island) (Thomazelli et al., 2010). Noteworthy, in our study no positive samples were detected in the Midwest and Southwest zones of the Peninsula. This might be a relevant finding due to the proximity of the Northwest zone of the Peninsula to South America and the possibility of migratory birds reaching this zone during the Southern Hemisphere summer. For example, South Polar Skuas (*Stercorarius maccormicki*), which have been found seropositive for AOaV-1 (Miller et al., 2008), migrate to the northern hemisphere and back in different times of the year. In fact, Yogui and Sericano (2009) suggest that skuas breeding at King George Island may winter in the northwestern Pacific Ocean (Yogui & Sericano, 2009). It would be important to confirm this hypothesis and determine the potential long-distance birds that might serve as carriers and introduce this virus to Antarctica or vice versa. Alternatively, penguins nesting in sub-Antarctic zones have been seen in Antarctica and vice versa, allowing the transmission of AOaV-1 from and to this region. For instance, King penguins have been seen in the north of the Antarctic Peninsula (Gryz et al., 2018; Juáres et al., 2017; Schiavini et al., 2005); as well as colonies of Gentoo penguins have been found on the south coast of Argentina (Schiavini et al., 2005). Finally, some species included in our study breed in mixed-species colonies or in proximity to other penguin species or other seabirds, which could facilitate the spread of the virus.

In general, serology has the advantage of showing the historical record of exposure to certain pathogens, so it is not necessary to detect animals during infection and shedding, which can be limited to a few days. This is especially important when asymptomatic or difficult to detect infections occurs, such as in penguins. Although the dynamics of AOaV-1 infection in penguins remain unknown, in poultry, the virus can be shed for up to 4 weeks, while antibodies can be detected within 6– 10 days of infection and persist for over a year (Paramyxoviridae & Pneumoviridae, 2017). The commercial ELISA kit used in this study is able to detect anti-AOaV-1 nucleoprotein antibodies in domestic and wild avian species. According to the manufacturer, this is a validated commercial with high sensitivity and specificity, earlier detection of seroconversion (7 dpi), detection of all AOaV-1 strains, and no cross-reactions with AOaV-3, which produces almost all the false positives in immunodiagnostic tests (Nayak et al., 2012; Rauw et al., 2020). Alternatively, we determine no cross-reactions with AOaV-17, AOaV-18, and AOaV-19, which have been widely detected in penguins from the Antarctic Peninsula (Neira et al., 2017; Olivares et al., 2019; Wille et al., 2019). However, the ELISA has some limitations, such as that it is not able to distinguish lineages and differentiate between Class I and Class II AOaV-1 strains. Therefore, we are not able to infer the potential virulence of the AOaV-1 strains that are infecting these penguin populations, which could have been achieved by an HI assay, using a specific AOaV-1 strain.

Another limitation is that the commercial ELISA kit has been not previously tested in penguins. However, the manufacturer recommended this kit for both, domestic and wildlife. The cut-off value for commercial ELISA kits may be optimized between species. Shriner et al. (2016), demonstrated different cut-off values in an ELISA Influenza kit for mallard ducks, using experimentally inoculated animals (Shriner et al., 2016). Additional studies aimed at viral isolation and sequencing are necessary to understand viral ecology and fully characterize the AOaV-1 strains circulating in Antarctic and sub-Antarctic penguins.

In conclusion, different penguin species and populations have been infected with AOaV-1. Further studies are necessary to determine the role of these penguin species in the ecology of the AOaV-1, as well as the pathogenic potential of this virus in these animal populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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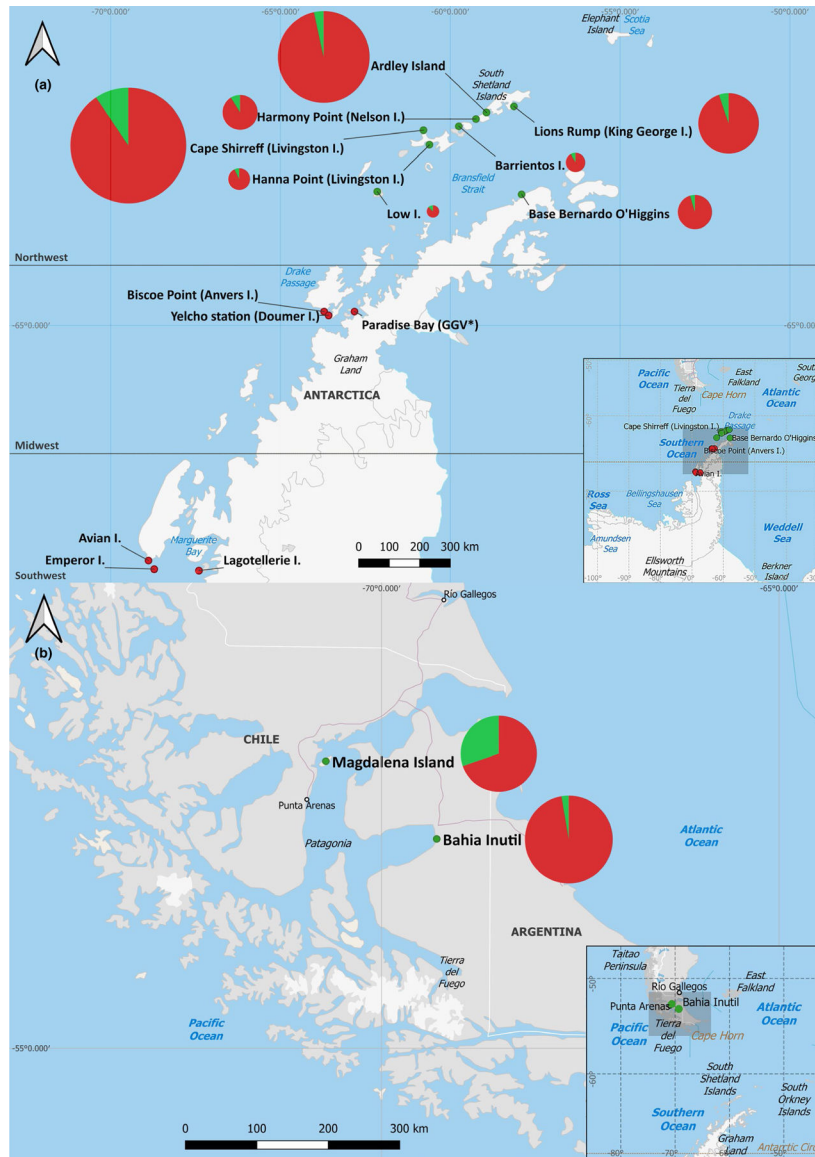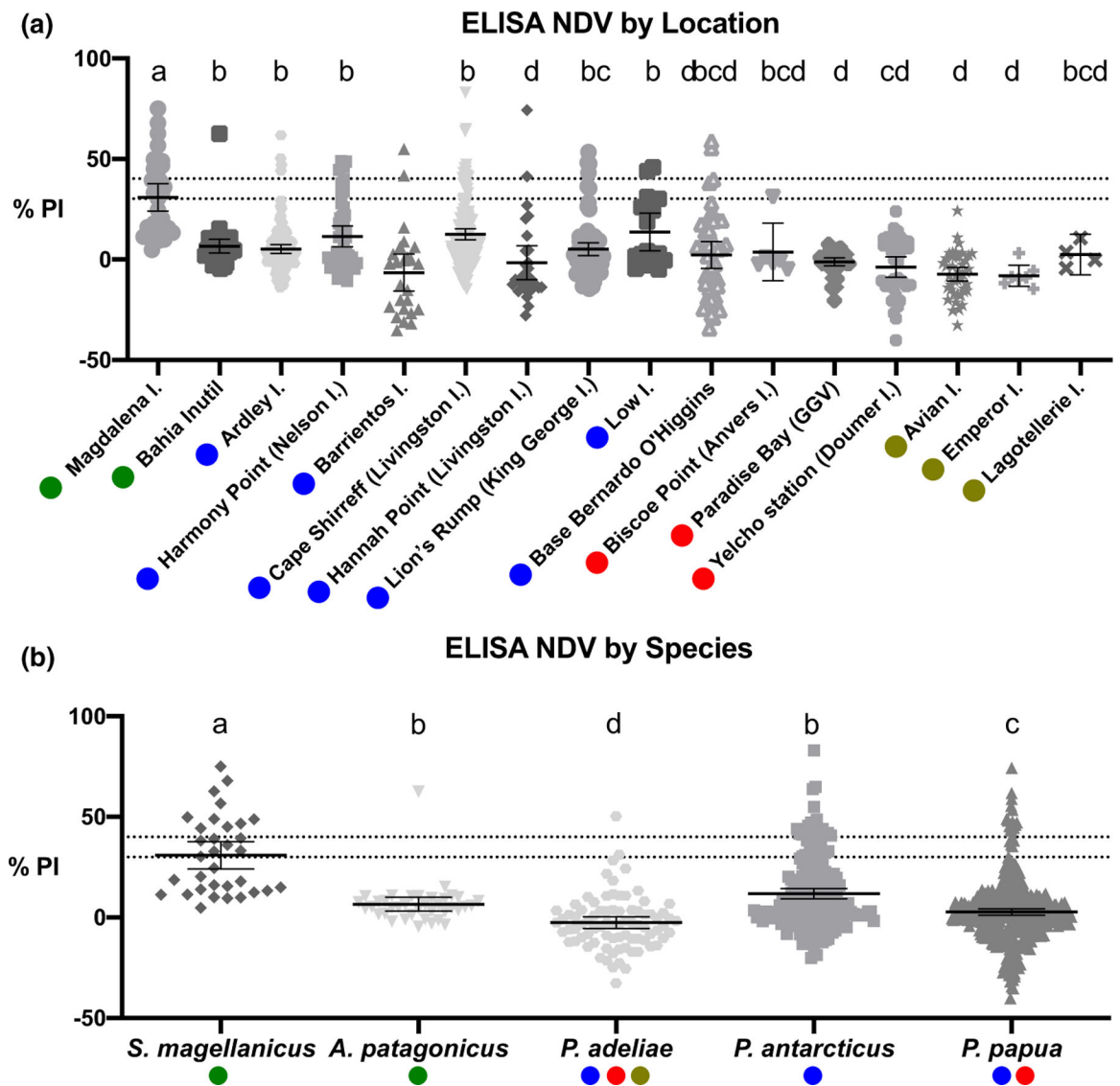
FIGURE 1.

Map of sampling sites on the Antarctic Peninsula (a) and continental area of the Magallanes region (b). Sampling locations are shown in coloured dots, with green dots representing NDV positive sites and in red the negative. Seropositivity pie charts are shown for each location using the same colour code, and their size is proportional to the sample number

(a)

(b)

FIGURE 2.

Identification of NDV seropositive positive penguins from Sub-Antarctic and Antarctic zones. Scatter plot of mean percent inhibition values (PI) with 95% CI, indicating antibody levels of penguin samples by location (a) and by of 30% and 40% (PI value 40: positive; 30–40: suspect; <30: negative). Green circles indicate samples taken in the continental area of the Magallanes region (sub-Antarctic locations); blue circles for samples collected from the Northwest Antartic region; red from Midwest; and brown for Southwest. Different letters above the columns indicate statistical difference ($p < .05$) [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1

Penguin species, sampling locations and serological results against NDV in the Antarctic Peninsula

Region	Location name	*P. adelia* (+)	N	%	*P. antarcticus* (+)	N	%	*P. papua* (+)	N	%	Total (+)	N	%
Northwest	Ardley Island	1	14	7.1	3	104	2.9	-	-		4	118	3.4
	Harmony Point (Nelson Island)	-			1	29	3.4	3	16	18.8	4	45	8.9
	Barrientos Island	-			2	12	16.7	-	13	-	2	25	8.0
	Cape Shirreff (Livingston Island)	-			13	112	11.6	1	37	2.7	14	149	9.4
	Hannah Point (Livingston Island)	-			1	14	0.1	1	14	7.1	2	28	7.1
	Lions Rump (King George Island)	-	5	-	-	4	-	4	69	5.8	4	78	5.1
	Low Island	-			2	16	0.1	-			2	16	12.5
	Bernardo O'Higgins Base	-	1	-	-	1	-	2	42	4.8	2	44	4.5
Midwest	Biscoe Point (Anvers Island)	-	6	-				-			-	6	-
	GGV Base[a] (Paradise Bay)	-						-	37	-	-	37	-
	Yelcho Base (Doumer Island)	-	1	-				-	35	-	-	36	-
Southwest	Avian Island	-	43	-				-			-	43	-
	Emperor Island	-	7	-				-			-	7	-
	Lagotellerie Island	-	4	-				-			-	4	-
Total		1	81	1.2	22	292	7.5	11	263	4.2	34	636	5.4

Note: (+) seropositive samples, *N* samples total, % relative frequency.

[a] Gabriel González Videla Base.

TABLE 2

Penguin species, sampling locations and serological results against NDV in the continental area of the Magallanes region, by penguin species

Region	A. patagonicus			S. magellanicus			Total		
	(+)	N	%	(+)	N	%	(+)	N	%
Continental Magallanes	1	38	2.63	10	33	30.30	11	71	15.49

Note: (+) seropositive samples, *N* samples total, % relative frequency.