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Serosurvey for Detection of Equine's West Nile Infection in Albania

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Abstract

West Nile fever is a mosquito-borne infection affecting mainly wild birds, mammals, horses, and humans. The disease is listed by the WOAH requiring member countries to report its occurrence. In Albania, WNV infections in humans were first reported in 2010 -2011, and in 2013 a serological survey revealed the presence of this infection in horses, in different areas of the country. The aim of the study was to detect the presence of this infection in healthy equine population in order to check WNV activity. During 2018-2019, in spring and autumn seasons, were collected 245 serum samples from no vaccinated horses in 17 districts. Samples were tested by using ELISA IgM – ID Screen West Nile Virus IgM Capture (MAC) for detection of prE IgM, and ELISA IgG - Ingezim West Nile Compac (blocking ELISA) for detection of specific antibodies against WNV. 47 out of 179 samples (26.2%) resulted positive for the IgG presence, and 1 sample out of 66 (1.5%) was found positive for IgM, which is related with recent infection.These results clearly revealed that the virus is widespread within the country. Compared with previous report, the prevalence is higher and the infection is expanded in new other areas. In areas where the disease is common, vaccination of horses should be considered as an effective control measure.

Keywords: equine, serosurvey, IgG, IgM, WNV, blocking ELISA

1. Introduction

West Nile virus (WNV) is 1 of more than 70 viruses of the family Flaviviridae of the genus Flavivirus. Serologically, West Nile virus is a member of the Japanese encephalitis serocomplex, which includes Japanese encephalitis virus and an endemic North American flavivirus, St Louis encephalitis virus. WNV is an enveloped single-stranded RNA virus. It causes West Nile Fever (WNF) that is a zoonotic disease. Other than humans, usually only horses become ill after natural infection. It can cause a severe neuro-invasive disease in 1–10% of infected horses. WNF cases in horses have to be reported to international organizations, OIE and the EC for European countries. Acting as incidental hosts, mammals can also become infected when bitten by an infected mosquito. Generally, humans and horses are considered as dead-end hosts owing to the low-level viraemia [10]. Birds represent the vertebrate reservoir. Viral transmission takes place through mosquitoes, and has so far been discovered in more than 40 different mosquito species mainly *Culex spp* and *Aedes spp* and in several tick species. Humans are mainly infected through the bite of an infected mosquito but transmission through blood transfusion, organ transplants, or breast feeding also occurs.

It has been discovered in 1937 in West Nile District, Uganda [13]. To date, WNV circulates on many continents, including Africa, Americas, Australia and Europe [12]. Human infections of WNV were mainly associated with sporadic cases up to the mid-1990s [1]. During the last few decades, Europe has experienced an upsurge in the recurrence, a geographic expansion of the outbreak areas, and a higher incidence associated

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with WNV-outbreaks [9], particularly in 2010 and 2018 [7].

Te first human case in Albania in 2010, (reported in 2011) was detected in a 14 year-old child in Korça prefecture (South East, bordering Greece). In 2011, 15 out of 49 cases were confirmed for the presence of WNV, Lineage 2. The 2010 and 2011 cases were the 1 st clinical WNV infections reported in Albania [8].

In 2011, a seroprevalence survey in horses found 20.3% of positive cases [3]. Due to the continuous introduction of the virus through infected wild migratory birds, the natural carriers of WNV, and the presence of competent vectors (female Culex spp. mosquitoes), seroprevalence in horses has been reported in several districts including central and western part of the country.

As the degree of antigenic similarity within the Flavivirus genus is high, antibody cross reactions can occur. Therefore, positive results were confirmed using virus neutralisation test, and it confirmed the circulation of strain WNV NY 99, Lineage 1.

Table 1. Virus Neutralisation Test, WNV-NY99 (lineage 1)

No	District	Samples	VNT WNV-NY99 (lineage 1)	Positive %
1	Tirane	4	0	0,0%
2	Kavaje	20	3	15,0%
3	Lushnje	20	9	45,0%
4	Fier	20	12	60,0%
5	Gjirokaster	8	0	0,0%
6	Lezhe	29	4	13,8%
7	Shkoder	10	1	10,0%
8	Elbasan	13	1	7,7%
9	Berat	13	1	7,7%
10	Burrel	5	0	0,0%
11	Pogradec	11	0	0,0%
	TOTAL	**153**	**31**	**20,3%**

WNV surveillance varies among European countries, ranging from clinical surveillance of horses or humans to active surveillance of birds or other infected species through regular serological screening and/or active WNV detection in trapped mosquitoes. Nevertheless, due to its clinical sensitivity to WNV infection, the horse is a sentinel whatever the surveillance system used. Cases in horses can usually be diagnosed before human cases. In such a context, the improved detection of WNV infection in this species would be extremely helpful. After an incubation period of 3-15 days, most horses show a short period of viraemia with low virus titers [2].Because of that, direct detection of the virus in living animals is often unsuccessful, so the primary tools used to diagnose WNV are serological tests [11]. The virus can only be detected post mortem in the brain or spinal cord of horses, e.g. using RT-PCR. For these reasons, the serological detection of specific antibodies by means of ELISA is of major importance [4]

2. Material and Methods

Between 2018 and 2019, 245 horses were conveniently sampled from 17 districts, exposed areas were WNV circulation has been detected before and from areas no included in previous surveys. All animals were subjected to blood sampling according to the best veterinary practices. Sera were collected in vacutainer dry tubes with a serum separator by centrifuging for 10 min at 3,500 rpm. Harvested sera were stored at −20° C until analysis.

Specific IgM antibodies can be detected in equine serum after 7 -10 days and generally persist for 1-2 months, sometimes even for much longer. IgM ELISA assays were highly specific and did not detect antibodies against related flaviviruses [5]. Investigating low-avidity antibodies of class IgG is offering an additional parameter that significantly enhances serological analyses of fresh WNV infections. IgG antibodies can be detected for at least 15 m after infection [6].

2.1. Detection of IgM antibodies

IDVET has developed the ID Screen® West Nile IgM Capture ELISA. This test allows the detection of anti pr E IgM antibodies in horse sera. Equine anti-WNV IgMs present in horse sera bind to anti-horse IgM antiserum coated on ELISA plates. This binding is revealed by the addition of Ag, that require an overnight incubation, and after that a Mab conjugated to HRP directed against WNV antigen is added to conclude with chromogenic substrate. There are 3 levels of S/P values for test interpretation, negative, doubtful and pozitive.

2.2. Detection of IgG antibodies

These competitive ELISA kits detect virtually every Ig isotype, but are mainly classified among the IgG detection tools. The detection of IgG by ELISA is widely used in Europe. Comparison of serological diagnostic methods highlighted the higher sensitivity of IgG ELISA compared to WNV VNTs. They also revealed that the low specificity of IgG ELISA kits meant that it could detect animals infected with other flaviviruses.

Ingezim WN Compaq kit (Ingenasa)- The kit is able to detect a very low titter of antibodies in sera of different infected animal species (birds, horses, humans, etc). This test is based on a blocking ELISA model. The plates are coated with inactivated viral Ag. After adding the sample to the well, if it contains specific Ab against WNV, they will bind to the Ag absorbed on plate. If we add a specific Mab (conjugated with peroxidase) against the ectodomain of glycoprotein E of WNV, it will compete with the Ab of the serum. If the serum samples contain specific Ab, they will not permit the binding of the labelled Mab to the Ag, whereas if it does not contain specific Ab, the Mab will bind to the Ag on the plate. After washing the plate to eliminate all non-fixed material, we can detect the presence or absence of labelled Mab by adding the substrate (TMB) that in presence of the peroxidase will develop a colorimetric reaction.

3. Results and Discussion

66 blood samples, from 6 districts (10 communes) were tested with MAC ELISAs to detect acute infections in horses. The numbers in Table 2 show that only 1 sample, from Shushica, Elbasan resulted pozitive for IgM antibodies. No doubtful results were detected. The short duration of anti-flavivirus IgM response in horses, associated with the high specificity, indicates that MAC ELISAs can be used to confirm recent WNV infection in horses.

Table 2. Results from MAC ELISAs

Country	District	Blood samples/serum	WNV ELISA IgM		
			Positive	Doubtful	Negative
Tirana	**Farka**	5	0	0	5
	Berzhita	24	0	0	24
Vlora	**Vlora**	3	0	0	3
Lushnja	**Dushk**	2	0	0	2
Lezha	**Torovica, Balldre, Dragosiq**	8	0	0	8
Elbasan	**Labinot fusha**	2	0	0	2
	Selita	11	0	0	11
	Shushica	11	1	0	10
	TOTAL	**66**	**1**	**0**	**65**

The situation is different while using IgG ELISA. 47 out of 179 samples resulted positive (Table 3). A large no of positive results was registered in Fier, Tirana, Vlora, Lezha and Elbasan. Other important data are doubtful results, 45 out of 179 samples, or 25.1 % in total.

In other studies when considering horses infected with flaviviruses belonging to the same serocomplex as WNV i.e. USUV (S13) and JEV (S14), horses were found positive or doubtful even in larger numbers, whatever the ELISA kit used. False positive reactions with related flaviviruses are clearly mentioned on the leaflet of the kit. VNT is the gold standard serological tool for confirming WNV diagnosis, nevertheless, cross-neutralisation by antibodies directed against

viruses within the same serocomplex can still be observed in the field.

Table 3. Results from IgG ELISA

Country	District	Blood samples/serum	WNV ELISA IgG		
			Positive	Doubtful	Negative
Fier	**Asturkoj**	5	5	0	0
	Baltëz	5	4	0	1
	Ndërmenas	5	5	0	0
	Mucoj	5	5	0	0
Tirana	**Vora**	12	7	0	5
	Farka	10	3	5	2
	Berzhita	30	3	9	18
Vlora	**Vlora**	29	3	3	23
Lushnja	**Dushk**	3	0	2	1
Lezha	**Torovica Balldre, Dragosiq**	16	6	8	2
Elbasan	**Labinot fusha**	7	0	2	5
	Gjinar	3	0	0	3
	Selita	29	1	9	16
	Shushica	20	5	7	4
	TOTAL	**179**	**47 (26.2%)**	**45 (25.1 %)**	**80 (44.6 %)**

This analysis aims at comparing 2011 and 2018-19 WNV seasons in terms seroprevalence and geographic distribution. Investigation is promoted in endemic areas where horses have been previously infected WNV. We optained serological evidence for IgG antibodies in almost all districts with a very high percentage of positive and doubtful results, respectively 26.2% and 25.1%. These results on Table 3 confirm that the virus is largely circuating although clinical manifestation in horses have not been reported.

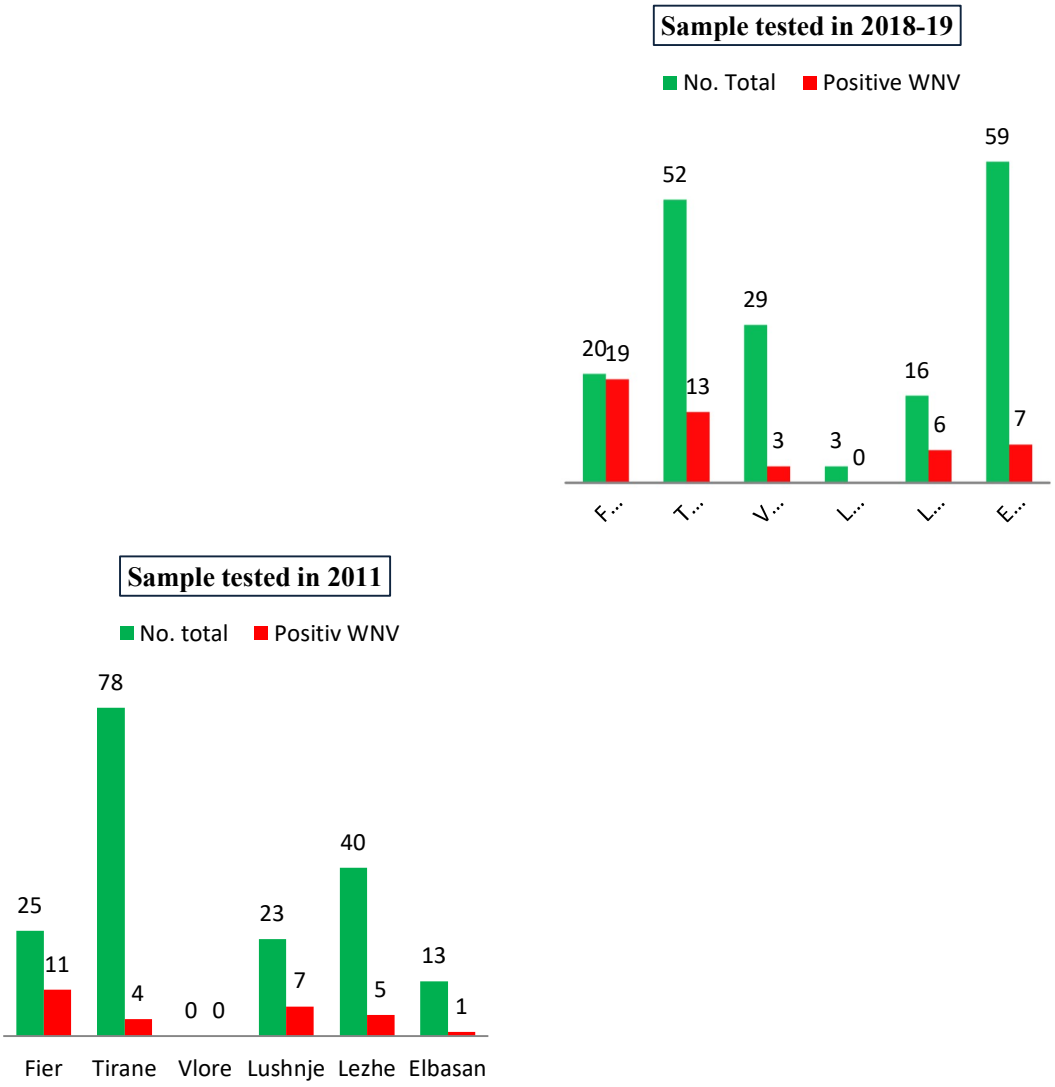

Figure 1. Comparison of WNV seroprevalence and geographic distribution in 2011 and 2018-2019

If we make a comparison between two charts in Figure 1, seroprevalence detected in Fier (95%), Tirana (25%), Lezha (37.5%) and Elbasan (11.8%) in 2018 was clearly higher than that reported in 2011. Viral circulation in Lushnja, in 2018-19 is not statistically significant, also because of sample quantity. Meanwhile, a new trend for WNV activity was observed in Vlora (10.3% positivity and 10.3% doubtful).

4. Conclusions

WNV, already endemic in many districts of Albania, will likely continue to disperse to naive areas, as conditions for its vectors become more favorable due to the changing climate. The result of this study confirmed an increase WNV activity and dynamic. Sero-prevalence in the same areas is higher in 2018-19, and additional affected areas were registered. These differences cannot be due to the methodology of analysis or the living conditions, because they were the same. They are most likely due to differences in the present ecosystems (mosquitoes and migratory birds).

The development of new commercial ELISAs ensuring the early detection of infected horses. The detection of WNV-specific IgM in blood provides good evidence of recent infection in horses. WNV IgG antibodies generally are detected shortly after IgM antibodies and persist for many years following a symptomatic or asymptomatic infection.

SN test should be considered to check the distribution of other flaviviruses, and to exclude cross reactivity,

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also because percentage of doubtful results (25.1 %) is high.

Veterinary surveillance could be essential for estimating the risk for humans. Concrete and well-coordinated efforts are needed given the increasing significance of WNV for public health.

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