

## Serologic Investigation for West Nile Virus Infection in Commercial Domestic Chickens (*Gallus gallus domesticus*)

<sup>1</sup>Orhan Yapici, <sup>2</sup>Mehmet Kale, <sup>3</sup>Sibel Gur, <sup>4</sup>Nuri Mamak, <sup>5</sup>Sibel Yavru,  
<sup>2</sup>Sibel Hasircioglu and <sup>5</sup>Oya Bulut

<sup>1</sup>Department of Preclinical Sciences, Faculty of Veterinary Medicine, Kyrgyzstan,  
Turkey Manas University, Bishkek, Kyrgyzstan

<sup>2</sup>Department of Virology, Faculty of Veterinary Medicine,  
Mehmet Akif Ersoy University, Burdur, Turkey

<sup>3</sup>Department of Virology, Faculty of Veterinary Medicine,  
Afyon Kocatepe University, Afyon, Turkey

<sup>4</sup>Department of Internal Diseases, Faculty of Veterinary Medicine,  
Mehmet Akif Ersoy University, Burdur, Turkey

<sup>5</sup>Department of Virology, Faculty of Veterinary Medicine, Selcuk University, Konya, Turkey

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**Abstract:** In this study, West Nile Virus antibody presence (WNV) in white leghorn chickens located in various commercial domestic chicken establishments in Konya was studied serologically. Blood sampling from 380 white leghorn chickens within the age range of 20-40 weeks was carried out. Blood serum samples were studied by West Nile Competition ELISA kit. All samples were found seronegative.

**Key words:** West Nile virus, domestic chicken, serology, competitive ELISA, blood sampling, Turkey

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### INTRODUCTION

West Nile Virus (WNV) is a virus of the family Flaviviridae. The genetic material of WNV is a positive-sense, single strand of RNA which is between 11.000 and 12.000 nucleotides long. The RNA strand is held within a nucleocapsid formed from 12 kDa protein blocks and the capsid is contained within a host-derived membrane altered by two viral glycoproteins (Surhone *et al.*, 2010). The virus mainly infects birds but is known to infect humans, horses, dogs, cats, bats, chipmunks, skunks, squirrels and domestic rabbits as well (Surhone *et al.*, 2010). The virus is transmitted through mosquito vectors which bite and infect birds. The birds are amplifying hosts, developing sufficient viral levels to transmit the infection to other biting mosquitoes which go on to infect other birds (Hayes *et al.*, 2005). Mosquitoes inoculate their saliva into the skin while obtaining blood. Recently, the potential for mosquito saliva to impact the course of WNV disease was demonstrated (Styer *et al.*, 2006; Schneider *et al.*, 2006). It has been determined that WNV infection can be detected either by antibody or virus isolation for many wild and domestic bird species in nature and clinical symptoms and death events are obtained in infected animals (Buckley *et al.*, 2003;

Farfan-Ale *et al.*, 2004; Marra *et al.*, 2004). Virus transmission in domestic chickens, adult poultry and turkeys could only happen by mosquitoes and antibody development was seen, however infected animals showed no clinical symptoms. Because chickens develop antibodies against the virus, they are being used as sentinels to monitor the presence of infected mosquitoes in high-risk areas (Langevin *et al.*, 2001; Haller, 2006). This study was carried out in order to study WNV infection serologically for the 1st time in commercially raised white leghorn chickens in the country and to detect the risk level of the infection on both human and animal populations.

### MATERIALS AND METHODS

**Animals and establishment features:** Konya where the sampling was done is geographically located between 36°22' and 39°08' Northern latitudes and 31°14' and 34°27' Eastern longitudes. The altitude of Konya is 1027 m. Samples were collected in various commercial domestic chicken establishments from 380 healthy looking white leghorn chickens within the age range of 20-40 weeks and showing no clinical WNV symptoms by regarding casual sampling method. Sample collecting was applied between

July and October. Establishments where samplings were done had a capacity of 10000 with a cage system lined in 4 with 3 floors and were 5 in group size curtained as 100 m long and 10 m wide with North-South axis directions and chain type feeders and drip bowl systems.

**Sample collection:** Blood (5 mL) was collected from the brachial vein using non-heparinized syringes. A portion of the blood was placed into serum separator tubes, allowed to clot for approximately 60 min at room temperature, centrifuged at 2×g for 20 min and frozen to -40°C until assayed for antibodies.

**Competitive ELISA:** ID screen West Nile Competition ELISA (ID-VET, Montpellier, France) commercial kit was used for WNV antibody test. This diagnostic kit is designed to detect horse and avian antibodies directed against the WNV Envelope protein (pr-E) containing an epitope common to Japanese Encephalitis by competitive ELISA. The test was carried out according to the procedure from the company. Results and test validation were evaluated in consideration of formulations within the kit prospectus of the firm.

## RESULTS AND DISCUSSION

Blood serum samples from 380 white leghorn chickens within the age range of 20-40 weeks were studied by ID Screen® West Nile Competition ELISA (ID-VET, Montpellier, France). All samples were detected seronegative (S/N>50%) by means of WNV. According to the result evaluation calculations found in test kit procedure, no serum sample showed a suspicious (40%<S/N = 50%) result.

WNV infection was reported to have been seen in Africa, Europe, Middle East, Western and Middle Asia, Oceania and North America (Surhone *et al.*, 2010).

The natural transmission cycle of the WNV occurs between birds and mosquitoes. The bird serves as the reservoir of the virus and the mosquito serves as the vector. The winged (birds) were reported to have WNV as primary reservoir. When they were 1st infected by WNV, viremia was formed within 1-4 days and mosquitoes sucking the blood of infected birds met the virus during this period. Besides, after meeting the virus for the 1st time (around 4 days), immune system of infected birds either developed antibody against the virus or the virus was eliminated from the body or the birds were dead. It has been stated that if immune systems of the birds are adapted to WNV, life-long immunity might be provided. Many winged animals was not die due to WNV infection,

however immunity against the virus is formed after the infection. Therefore, birds are a host reservoir of WNV (Sfakianos and Hecht, 2009).

Immunohistochemistry (IHC), Enzyme Linked Immunosorbent Assay (ELISA), Plaque Reduction Neutralization Test (PRNT), Hemagglutination-Inhibition Test (HIT) and micro-Virus-Neutralization Test (micro-VNT) methods are commonly used to detect antibody in domestic chickens and wild birds dependent on WNV infection (Jozan *et al.*, 2003; Buckley *et al.*, 2003; Farfan-Ale *et al.*, 2004; Figuerola *et al.*, 2008). Weingartl *et al.* (2003) were carried out to detect antibody developed depending on WNV infection in domestic chickens they found that ELISA applications were more sensitive, simpler and faster compared to HIT and PRNT during their studies on various testing methods. In this study, researchers preferred ELISA method to detect antibody in domestic chickens, especially dependent on WNV infection.

During the studies on WNV antibody presence in domestic chickens, 5% (Akov and Golswasser, 1966), 41% (Tsai *et al.*, 1998), 37% (Savage *et al.*, 1999), 0.63% (Komar *et al.*, 2001), 69% (Jozan *et al.*, 2003), 10.9% (Stark, 2005), 1.7 and 0.6% (Lefrancois *et al.*, 2006), 18.18% (Savuta *et al.*, 2008) and various seropositivity rates (%) were detected. No seropositivity was found in 380 white leghorn chickens within the age range of 20-40 weeks. Likewise, some researchers (Kolman *et al.*, 1975; Godsey *et al.*, 2005; Hubalek *et al.*, 2008) also reported that they could not detect seropositivity in domestic chicken serum as in this study. WNV has not affected commercial poultry (*Gallus gallus domesticus*) which are predominantly raised indoors with low potential for exposure to mosquito vectors (OIE, 2000). Also, experimental studies in chickens and turkeys inoculated subcutaneously with WNV isolate had low viremia titres and no clinical disease (Swayne *et al.*, 2000; Senne *et al.*, 2000). While all land surface shapes are seen within Konya province area, bottom lands are mostly dominant. River system in Konya is usually rare because of little rain in bottom lands and the fact that waterpass slopes like limestones in mountainous areas cover too much space. The altitude of Konya is 1027 m. For little rain is seen around Konya area, moorland view is existent. In the establishments where sampling is done, protections with window and door screens against mosquitoes insects and pests can be seen. There are no water bodies, natural rivers, streams, swamps, junkyards and waste plants near the establishments. During Spring, Autumn and Summer, larvacide spraying procedures are carried out against mosquito population in this region where mosquito presence and flights are rare.

## CONCLUSION

As a result, antibody presence against WNV infection in domestic chickens located in commercial establishments in Konya province could not be detected. Researchers have the assumption that this could be due to protective precautions-applications by establishments and advantageous natural geographical structure of the area where these establishments are located. Besides, it is especially important as it has been the 1st WNV infection research in domestic chickens (dependent on the majority of sampled animals) in Turkey.

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## REFERENCES

- Akov, Y. and R. Golswasser, 1966. Prevalence of antibodies to Arboviruses in various animals in Israel. *Bull. World Health Org.*, 34: 901-909.
- Buckley, A., A. Dawson, S.R. Moss, S.A. Hinsley, P.E. Bellamy and E.A. Gould, 2003. Serological evidence of West Nile virus, Usutu virus and Sindbis virus infection of birds in the UK. *J. Gen. Virol.*, 84: 2807-2817.
- Farfan-Ale, J.A., B.J. Blitvich, M.A. Lorono-Pino, N.L. Marlenee and E.P. Rosado-Paredes *et al.*, 2004. Longitudinal studies of West Nile virus infection in avians, Yucatan State, Mexico. *Vector Borne Zoonotic Dis.*, 4: 3-14.
- Figuerola, J., M.A. Jimenez-Clavero, G. Lopez, C. Rubio, R. Soriguer, C. Gomez-Tejedor and A. Tenorio, 2008. Size matters: West Nile virus neutralizing antibodies in resident and migratory birds in Spain. *Vet. Microbiol.*, 132: 39-46.
- Godsey, M.S., M.S. Blackmore, N.A. Panella, K. Burkhalter and K. Gottfried *et al.*, 2005. West Nile virus epizootiology in the southeastern United States, 2001. *Vector Borne Zoonotic Dis.*, 5: 82-89.
- Haller, L.C., 2006. Development of a microsphere-based immunoassay for the detection of IgM antibodies to West Nile Virus and St. Louis Encephalitis Virus in sentinel chicken sera. M.Sc. Thesis, University of South Florida.
- Hayes, E.B., N. Komar, R.S. Nasci, S.P. Montgomery, D.R. O'Leary and G.L. Campbell, 2005. Epidemiology and transmission dynamics of West Nile virus disease. *Emerg. Infect. Dis.*, 11: 1167-1173.
- Hubalek, Z., E. Wegner, J. Halouzka, P. Tryjanowski and L. Jerzak *et al.*, 2008. Serologic survey of potential vertebrate hosts for West Nile Virus in Poland. *Viral. Immunol.*, 21: 247-253.
- Jozan, M., R. Evans, R. McLean, R. Hall, B. Tangredi, L. Reed and J. Scott, 2003. Detection of West Nile virus infection in birds in the United States by blocking ELISA and immunohistochemistry. *Vector Borne Zoonotic Dis.*, 3: 99-110.
- Kolman, J.M., J. Minar and Horak, 1975. Serologic examination of birds from the area of Southern Moravia for the presence of antibodies against arboviruses of the groups alfa, flavo, Bunyamwera supergroup and the virus Yaba 1-Lednice 110. I. Domestic fowls. *Zentralbl. Bakteriolog. Orig. A.*, 233: 279-287.
- Komar, N., A.N. Panella, J.E. Burns, S.W. Duszka, T.M. Mascarenhas and T.O. Talbot, 2001. Serologic evidence for West Nile virus infection in birds in the New York city vicinity during an outbreak in 1999. *Emerg. Infect. Dis.*, 7: 621-625.
- Langevin, S.A., M. Bunning, B. Davis and N. Komar, 2001. Experimental infection of chickens as candidate sentinels for West Nile virus. *Emerg. Infect. Dis.*, 7: 726-729.
- Lefrancois, T., B.J. Blitvich, J. Pradel, S. Molia, N. Vachier and D. Martinez, 2006. West Nile virus in guadeloupe: Introduction, spread and decrease in circulation level: 2002-2005. *Ann. N.Y. Acad. Sci.*, 1081: 206-215.
- Marra, P.P., S. Griffing, C. Caffrey, A.M. Kilpatrick and R. McLean *et al.*, 2004. West Nile virus and wildlife. *Bioscience*, 54: 393-402.
- OIE, 2000. West Nile fever in the United States of America: In horses. *Dis. Inform.*, 13: 150-151.
- Savage, H.M., C. Ceianu, G. Nicolescu, N. Karabatsos and R. Lanciotti *et al.*, 1999. Entomologic and avian investigations of an epidemic of West Nile fever in Romania in 1996, with serologic and molecular characterization of a virus isolate from mosquitoes. *Am. J. Trop. Med. Hyg.*, 61: 600-611.
- Savuta, G.H., L. Ludu, A. Anita, D. Anita and A. Ionescu, 2008. West Nile virus infections in Romania-Past, present and perspective. *Lucrari Stiintifice Med. Vet. Timisoara*, 41: 301-308.
- Schneider, B.S., L. Soong, Y.A. Girard, G. Campbell, P. Mason and S. Higgs, 2006. Potentiation of West Nile encephalitis by mosquito feeding. *Viral. Immunol.*, 19: 74-82.
- Senne, D.A., J.C. Pedersen, D.L. Hutto, W.D. Taylor, B.J. Schmitt and B. Panigrahy, 2000. Pathogenicity of West Nile virus for chickens. *Avian Dis.*, 44: 642-649.

- Sfakianos, J.N. and A. Hecht, 2009. West Nile Virus. Chelsea House Publishers, New York, pp: 42-75.
- Stark, L.M., 2005. Arbovirus surveillance: Annual summary report. Florida Department of Health.
- Styer, L.M., K.A. Bernard and L.D. Kramer, 2006. Enhanced early West Nile virus infection in young chickens infected by mosquito bite: Effect of viral dose. *Am. J. Trop. Med. Hyg.*, 75: 337-345.
- Surhone, L.M., M.T. Tennoe and S.F. Henssonow, 2010. West Nile Virus. âetascript Publishing, Berlin, pp: 1-9.
- Swayne, D.E., J.R. Beck and S.R. Zaki, 2000. Pathogenicity of West Nile virus for Turkeys. *Avian Dis.*, 44: 932-937.
- Tsai, T.F., F. Popovici, C. Cernescu, G.L. Campbell and N.I. Nedelcu, 1998. West Nile encephalitis epidemic in southeastern Romania. *The Lancet*, 352: 767-771.
- Weingartl, H.M., M.A. Drebot, Z. Hubalek, J. Halouzka and M. Andonova *et al.*, 2003. Comparison of assays for the detection of West Nile virus antibodies in chicken serum. *Can. J. Vet. Res.*, 67: 128-132.