RESEARCH

Rapid Detection of West Nile Virus in Birds Using the VecTest™ WNV Antigen Assay

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OBJECTIVE: To determine if the VecTest™ West Nile Virus Antigen Assay (for testing mosquitoes) could be adapted to detect West Nile virus (WNV) rapidly and accurately in birds for screening purposes.

DESIGN: Cloacal swabs and tissue (kidney and spleen) were harvested from 40 fresh dead birds. The VecTest was used for each swab specimen for detection of WNV; PCR was used for each tissue specimen for confirmation of WNV.

SETTING: Mississippi Veterinary Diagnostic Laboratory (MVDL) in Jackson Mississippi and College of Veterinary Medicine-Mississippi State University (CVM-MSU) in Starkville Mississippi.

SPECIMENS/SUBJECTS: Forty birds of the Corvid family (31 blue jays and 9 American crows) were included in the study. Fresh dead birds that died from no obvious cause were submitted for testing.

RESULTS: VecTest results were 35 positives and 5 negatives. PCR results were 35 positives and 5 negatives.

CONCLUSION: The VecTest showed 100% accuracy.

ABBREVIATIONS: CVM-MSU = College of Veterinary Medicine-Mississippi State University; MVDL = Mississippi Veterinary Diagnostic Laboratory; PCR = polymerase chain reaction; PFU = plaque forming unit; RT-PCR = reverse transcription-nested polymerase chain reaction; SLE = St Louis encephalitis; VI = virus isolation; WNV = West Nile virus.

INDEX TERMS: West Nile virus; virus testing.

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West Nile virus (WNV) is an arbovirus that can cause encephalitis in humans and horses, as well as death in birds. In 1937, the first case of WNV was isolated from a febrile adult woman in the West Nile District of Uganda.[1] In the early 1960s, the first noted equine disease was found in Egypt and France.[1] The virus has been described in Africa, Europe, the Middle East, west and central Asia, Oceania, and most recently, North America in 1999.[1] Cases have been reported in humans, horses, and birds.[1] The first outbreak of WNV in humans, horses, and birds in the United States occurred in 1999 in New York.[2] From 1999 through 2003, the virus spread south and west at a rapid rate. As of January 20, 2004, the virus has been documented in 45 states and the District of Columbia with cases in humans, horses, and birds.[1] There have been an estimated 9100 human cases with over 400 deaths.[1]

WNV belongs to the family *Flaviviridae*, genus *Flavivirus*. Several other viruses fall in this genus, such as St. Louis encephalitis, Kunjin, and Japanese encephalitis. They are single-stranded RNA enveloped viruses.

WNV is monitored through the use of sentinel birds such as crows and blue jays.[3] The virus is maintained in nature by birds.[3] Since birds are the principal reservoir hosts, the virus should occur earlier and more frequently in them than in humans and horses. Therefore, the use of sentinel birds helps determine where the virus is present sooner, which in turn, is used for implementing human and veterinary interventions when necessary. Bird species that are most susceptible to the

virus should be used as sentinels. With regard to WNV infection, crows and blue jays seem to be the most susceptible.

STATEMENT OF PROBLEM/RATIONALE FOR STUDY

The primary purpose of this project was to determine if the VecTest (Medical Analysis Systems Inc, Camarillo CA) West Nile Virus Antigen Assay, which is used for detection of WNV in mosquitoes, could be adapted to detect the virus rapidly and accurately in birds for screening purposes. This assay would not replace any current testing protocols for WNV. It would be used only for screening purposes to detect the virus quickly. PCR, virus isolation (VI), and ELISA assays are used for detecting WNV in birds presently. These procedures work well, but they are time consuming. They can take three days to several weeks for results. The VecTest takes 20 to 30 minutes to complete. This quick assay would allow the epidemiologist to know in which areas the virus exists and intervention in the human and veterinary population could take place sooner. Presently all dead birds collected from across the state are sent to Mississippi Veterinary Diagnostic Laboratory (MVDL). Some of these birds are so deteriorated and covered in maggots and ants by the time they arrive at the laboratory that no tissue can be collected to perform PCR testing. Technicians could perform the VecTest assay in the field where the birds are found. By performing the test in the field, the epidemiologist would at least have some data if the birds were too deteriorated to provide tissue for further testing when they arrive at the laboratory. The VecTest would benefit all laboratories and especially those that currently have to send samples out to reference laboratories for results. Reference laboratories with which MVDL was acquainted were backlogged with WNV samples and would only accept ten samples per month from any given laboratory. In this situation, MVDL needed a screening test such as the VecTest.

This screening test would allow the laboratory to have a preliminary result on these birds. The tissues collected from these birds could then be frozen until the reference laboratories would accept them for confirmation testing.

METHOD USED IN RESEARCH

Postmortem specimens were collected from 40 corvids (9 American crows and 31 blue jays) that had died from no obvious cause. Kidneys and other organs were harvested, and postmortem cloacal swabs were collected (using sterile-cotton tipped applicators). The organs harvested were frozen at –20 °C until assayed for WNV with PCR. The cloacal swabs were assayed on the same day they were collected.

The assay of the cloacal swabs was done at MVDL and the PCR on the tissue was done at the College of Veterinary Medicine–Mississippi State University (CVM-MSU). The procedure for the cloacal swab was done using the VecTest. The materials provided in the kit included: VecTest WNV Antigen Assay dipsticks, grinding solution, culture tubes, conical tubes, and tube racks. The materials required but not provided included: timing device, pipette and tips, vortex machine, and sterile cotton-tipped applicators. The following procedure was followed when assaying cloacal swabs and tissue.

1. Dispense 1,000 µL of grinding solution into each plastic culture tube.
2. Place a cloacal swab specimen into each plastic culture tube.
3. With the swab still in the tube, vortex for 30 seconds.
4. Dispense 300 µL of the mixed solution into a conical tube.
5. Insert a test strip into the conical tube with the arrows pointing down.
6. Incubate for 20 minutes at room temperature and determine results.

The presence of only a control line on the dipstick indicates a negative test result. The presence of two lines (control and WNV line) indicates the presence of WNV antigen. If the control line on the dipstick is not present, the test is invalid and needs to be run again.

The tissue samples (kidneys) that were collected from the postmortem birds were used for confirmation testing. These samples were tested for WNV using the RT-PCR assay previously described.[4]

RESULTS/STATISTICS

Cloacal swabs (screening)

Cloacal swab specimens were collected from 40 fresh dead birds (blue jays and crows). These samples were tested for WNV using the VecTest™ WNV Antigen Assay. Of these 40 samples, 35 were positive and 5 were negative.

Tissue samples (confirmation)

Tissue samples (kidneys) were collected from the same 40 birds. These samples were tested for WNV using the RT-PCR assay as previously described.[4] Of these 40 samples, 35 were positive and 5 were negative.

Both methods revealed the same results showing 100% accuracy in the correlation between the two assays.

DISCUSSION

The goal of this research project was to determine if the VecTest™ WNV Antigen Assay for testing mosquitoes could be modified and used as a screening test to detect WNV in fresh dead birds. At the beginning of the project, the laboratory (MVDL) and epidemiologist decided to implement the assay as a screening test if 85% or higher accuracy was achieved between the VecTest and the standard PCR assay. The VecTest assay would not replace any current testing methods for WNV. PCR-based molecular methods are the 'gold standard' for WNV identification. According to the manufacturer, the VecTest assay uses monoclonal antibodies against WNV and the *Flavivirus* group to identify the presence or absence of viral antigen specific to WNV.[5]

Sensitivity/specificity studies were performed during assay development in a laboratory setting.[5] The manufacturer stated the sensitivity of the WNV assay is 10^3 fold dilution of a culture antigen, and 10^5 PFU/mL WNV in laboratory infected mosquito pools when the individual WNV assay was tested.[5] Also, the manufacturer stated there was no cross-reactivity between WNV and SLE antigens during development.[5] The assay is specific for WNV.[5] This assay is a rapid, one step assay, providing rapid results requiring no specialized equipment, and easily stored at 4 °C.

Blue jays and crows, which belong to the Corvid family, were used in this project. The virus seems to be more detrimental in this family of birds. There is a need for a quick and accurate screening test to detect the virus in birds to help the public health departments identify the location of the virus and possibly predict where human cases may occur. Since 1999, surveillance of dead birds has become a standard method for detecting the spread of WNV transmission in the U.S.[6] CDC states that birds with acute WNV infection frequently shed the virus in cloacal or oral cavities.[6]

In this project, cloacal swab and kidney specimens were collected from 40 fresh dead blue jays and crows. The VecTest™ WNV Antigen Assay was used to screen for WNV using cloacal swab specimens. RT-PCR was the method used for confirmation using the kidney specimens collected. The goal was to see if the VecTest would give the same results as PCR, the 'gold standard'. The correlation between the two assays yielded 100% accuracy; therefore, the VecTest could be used as a screening test for WNV in crows and blue jays.

CONCLUSION/SUMMARY

The results of this study indicate that the VecTest can be used as a screening test on cloacal swabs from fresh dead birds (crows and blue jays) with 85% or higher accuracy. This has been and will continue to be very beneficial to Mississippi health departments and epidemiologists by helping to quickly determine where to expect human and animal cases of WNV to occur rather than waiting a few days or weeks for results. Studies need to be done on other species of birds using the VecTest. Other species could carry the virus and never show any clinical signs. Also, the possibility of using the VecTest out in the field should be addressed. This would allow field technicians to perform the assay on birds on the spot and at least give the epidemiologist some data in case the birds are too deteriorated to provide samples when arriving at the laboratory.

MVDL incorporated the VecTest to screen dead birds for WNV into its surveillance program. By using the VecTest as a screening assay for WNV in birds, the laboratory has been able to report preliminary results to the epidemiologist sooner. This has allowed the laboratory and epidemiologist to know the locations where the virus is present. Thus intervention or precautionary measures can be implemented sooner in the human and animal population.

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