Comparison of Different Sampling and Testing Methods to Detect West NileVirus Infection in Dead Birds

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ABSTRACT: Dead bird testing is commonly used to monitor enzootic transmission of West Nile virus (WNV). This paper reports on comparative WNV test results of dead bird tissues (kidney, brain, retina) and oropharyngeal swabs/RNASound™ Card by VectorTest®, RAMP® and RT-qPCR assays. The brain and retina tissues were sampled if a bird carcass was too dry for oropharyngeal swabbing. When using brain and retina samples, there were no significant differences in WNV-positive rates between VectorTest and RT-qPCR assays. Among all sampling/testing methods, the RT-qPCR assay on kidney tissue yielded the highest WNV-positive rate, and the threshold cycle (Ct) values in RT-qPCR using kidney tissue were comparable with using brain tissue samples. VectorTest buffer may adversely impact RT-qPCR test results because of its incompatibility with RT-qPCR reagents.

INTRODUCTION

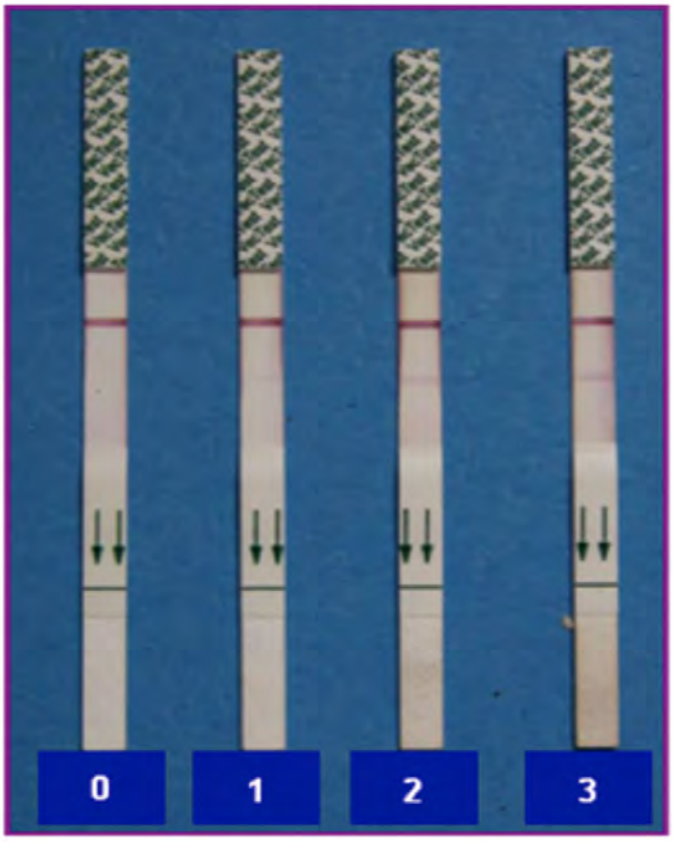
Dead bird testing is a convenient and efficient surveillance tool for monitoring enzootic transmission of West Nile virus (WNV). In this study, various sampling and testing methods were evaluated for their feasibility and reliability. The current paper reports on comparative WNV test results of kidney, brain, retina tissues and oropharyngeal RNASound™ card swabs from dead birds by VecTest® / VectorTest®, Rapid Analyte Measurement Platform (RAMP)® and RT-qPCR assays.

MATERIALS AND METHODS

Sampling. Brain and retina samples were collected from dead bird carcasses acquired by the West Valley Mosquito and Vector Control District (MVCD), Orange County MVCD (OCMVCD), San Gabriel Valley MVCD (SGVMCD) and Northwest MVCD. Carcasses provided by SGVMVCD, NWMVCD and OCMVCD were previously tested by oropharyngeal swab/RAMP test, oropharyngeal swab/RNASound card/RT-qPCR or kidney tissue/RT-qPCR, respectively.

For dead birds without prior test results by oropharyngeal swab/RNASound card/RT-qPCR, oropharyngeal swab samples were collected using dry sterilized cotton swabs and smeared onto RNASound cards (RNA card) according to product instructions (FortiusBio LLC, San Diego , CA. 92130, U.S.A.). Brain and retina samples were collected in 250 µL (brain) or 100 µL (retina) VectorTest buffer or PBS following the guidance described by San Diego County Vector Control Program (SDCVCP).

Testing. RNA card samples from 37 dead birds were tested at UC Davis/CVEC by RT-qPCR. Each brain or retina sample of 66 dead birds was tested by taking a 50 µL aliquot of the sample in either VectorTest buffer or PBS and mixed with 50 µL VectorTest buffer. VectorTest strips were read per manufacturer's instruction (Su and Cheng 2012) (Figure 1). In addition, brain and retina samples in VectorTest buffer or PBS were shipped to San Joaquin County MVCD and tested by qRT-PCR.



0 – No visible band (-); 1 – Visible band (+)
2 – Moderately visible band (++); 3 – Strongly visible band (+++)

Figure 1. Scoring criteria of VecTest® in dead bird test (Su and Cheng 2012).

RESULTS

Results of the various WNV testing methods using different bird tissues were compared among 66 dead bird carcasses (Table 1). Among the initial 10 dead birds collected by WVMVCD, only two of six corvids that were sampled by oropharyngeal swabs with RNA cards tested WNV-positive by RT-qPCR. Brain and retina samples that were held in VectorTest buffer from the first ten dead birds and tested by RT-qPCR showed an overall lower WNV-positive rate compared to VectorTest results (Table 2). This was probably due to the incompatibility of the VectorTest buffer and RT-qPCR reagents. The RT-qPCR results of these ten dead birds were excluded from calculations and analysis of positive rates shown in Table 3. Subsequently, we switched to PBS for collecting brain and retina samples from all remaining dead birds for comparison of sampling/testing methods (Table 4).

Table 1. Dead bird carcasses used in comparative study on sampling and testing methods.

Dead birds	Numbers
American crow	37
Barn owl	2
Calif. towhee	1
House finch	7
Hummingbird	1
Mockingbird	3
Parrot	1
Rock dove	1
Western scrub jay	4
House sparrow	8
Warbler	1
Total	**66**

Table 2. Test results of WNV infection in dead birds by RT-qPCR (Brain and retina samples were collected and kept in VectorTest® buffer).

DBs and samples	Oropharyngeal swab/RNA Card / RT-qPCR (CVEC)	VectorTest® (WVMVCD)	RT-qPCR (SJCMVCD)
Corvids (American crows and western scrub jays)			
Oropharyngeal swab / RNA Card	2/6		
Brain		6/6	2/6
Retina		5/6	2/6
Small Passerines (House finches and house sparrows)			
Oropharyngeal swab / RNA Card			
Brain		1/2	0/2
Retina		0/2	1/2
Others (Hummingbird and mockingbird)			
Oropharyngeal swab / RNA Card			
Brain		0/2	1/2
Retina		0/2	1/2

Table 3. Positive rate ± SE (# of samples) by different sampling and testing methods.

Tests	Oropharyngeal swab	Oropharyngeal swab-RNA card	Kidney	Brain	Retina
RAMP®	54. 5 ± 15.0 (11)				
VectorTest®				57.6 ± 6.1 (66)	57.6 ± 6.1 (66)
RT-qPCR		62.2 ± 8.0 (37)	90.5 ± 6.4* (21)	53.6 ± 6.7 (56)	60.7 ± 6.5 (56)

* Significantly higher than other sampling/testing methods ($X^2 \geq 5.45$, $P < 0.05$). No significant differences were found among other methods.

Table 4. Test results of WNV infection in dead birds by different methods (Brain and retina samples were collected and kept in PBS).

DBs and samples	RAMP test (SGVMVCD)	Oropharyngeal swab/RNA Card / RT-PCR (CVEC)	VectorTest (WVMVCD)	RT-PCR (SJCMVCD)	RT-PCR (OCVCD)
Corvids (American crows and western scrub jays)					
Oropharyngeal swab	6/11				
Oral swab / RNA Card		20/22			
Kidney					17/17
Brain			28/35	27/35	
Retina			30/35	27/35	
Small Passerines (House finches and house sparrows)					
Oropharyngeal swab					
Oral swab / RNA Card		1/7			
Kidney					1/2
Brain			2/13	3/13	
Retina			3/13	6/13	
Others (Barn owls, hummingbird, mockingbird, parrot, rock dove, California towhee, warbler)					
Oropharyngeal swab					
Oral swab / RNA Card		0/2			
Kidney					1/2
Brain			1/8	0/8	
Retina			0/8	1/8	

Further test results of 56 dead birds that were sampled/tested by various methods are summarized in Table 4. Of the eleven corvid oropharyngeal swabs tested by RAMP by SGVMVCD, six were WNV-positive. Twenty of the corvids and one small passerine of the 22 corvids, seven small passerines and two other birds that were sampled by oropharyngeal swab/RNA card and tested by RT-qPCR tested WNV-positive (Table 4). Brain and retina samples were harvested from all 56 dead birds and tested by both VectorTest and RT-qPCR (Table 4). Of 35 corvids, 28 brain and 30 retina tissues tested WNV-positive by VectorTest, whereas 27 brain and retina samples tested WNV-positive by RT-qPCR. Of the 13 brain or retina samples from small passerines, two brain samples and three retina samples tested WNV-positive by VectorTest; however, three brain samples and six retina samples tested WNV-positive by RT-qPCR. Of the eight brain or retina samples from other birds, only one brain sample tested WNV-positive by VectorTest and one retina sample tested WNV-positive by RT-qPCR (Table 4).

Orange County MVCD harvested kidney tissue from 17 corvids, two small passerines and two other birds for RT-qPCR. All 17 corvids, one of two small passerines and one of two other birds tested WNV-positive. The positive rates in kidney tissue by RT-qPCR was significantly higher ($X^2 \geq 5.45$, $P < 0.05$) than other tissue samples/testing methods, such as oropharyngeal swab by RAMP, oropharyngeal swab/RNA card by RT-qPCR, brain and retina tested by VectorTest or RT-qPCR (Table 3). The average threshold cycle (Ct) value in kidney and brain tissues was significantly lower than those of oropharyngeal swab/RNA card, as well as brain and retina tissues ($F = 22.1$, $P = 0$, $df = 3, 102$) (Table 5).

Table 5. Average Ct values ± SE (# of samples) of RT-qPCR by different sampling methods.

Samples	Oropharyngeal swab-RNA card	Kidney	Brain	Retina
Ct values	24 ± 0.9 (23)	16.7 ± 0.6* (19)	18.1 ± 5.6* (30)	26.2 ± 5.8 (34)

*Significantly lower than other sampling methods ($F = 22.1$, $P = 0$, $df = 3, 102$). No significant differences were found among other methods.

SUMMARY

To summarize, all tissue types and detection methods are viable for detection of WNV infection in dead birds, depending on feasibility and practicability. When using brain and retina samples, there were no significant differences in positive rates between VectorTest and RT-qPCR assays. When a bird carcass was too dry for oropharyngeal swabbing, brain and retina were still available for sampling; no differences were found in test sensitivity between brain and retina tissues when using the VectorTest. The RT-qPCR assay on kidney tissue was shown to be the most sensitive for detection of WNV in dead birds. Higher sensitivity on kidney tissue for WNV detection was also noticed by Krueger et al. (2012); in their study, the positive rate on kidney tissue was higher and the Ct values in RT-qPCR were lower than those in the rapid bilateral intraocular cocktail (BIC) method. When comparing Ct values, sensitivity of the RT-qPCR for detecting WNV in kidney tissue was comparable with brain tissue, while both tissues tested positive more often than oropharyngeal swabs and retina tissue. Results were comparable for the latter two via RT-qPCR. VectorTest buffer may adversely impact RT-qPCR test results because of its incompatibility with RT-qPCR reagents.

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