

ARIZONA GAME AND FISH DEPARTMENT

The Prevalence of Pigeon Paramyxovirus 1 and *Trichomonas gallinae* in Band-tailed Pigeons (*Patagioenas fasciata*), Mourning Doves (*Zenaida macroura*), and White-winged Doves (*Zenaida asiatica*)

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SUMMARY

Pigeon Paramyxovirus 1 (PPMV1) is an emerging disease of concern to native wild bird species in Arizona. This disease is often associated with the Eurasian collared dove (*Streptopelia decaocto*, ECDO), an invasive species with a high level of human commensalism. First identified in the state in the 2001 Christmas bird count, ECDO range has extended to include most of the state and now overlaps with that of the band-tailed pigeon (BTPI). This study set forth to determine the prevalence of PPMV1 in band-tailed pigeons, mourning doves (MODO), white-winged doves (WWDO), and Eurasian collared doves across Arizona using serologic and molecular methods. *Trichomonas gallinae* has caused several epizootics in dove and raptor populations in Arizona. Co-infection with *T. gallinae* and PPMV1 could increase the severity of a mortality event. A second objective was to determine if co-infection with PPMV 1 and *T. gallinae* occurred and if infection with PPMV 1 increased the likelihood of co-infection. We evaluated birds for *T. gallinae* infection by culture and microscopic examination of liquid media.

Band-tailed pigeons (n = 25), MODO (n = 143), WWDO (n = 45), ECDO (n = 59), and rock doves (*Columbia livia*, RODO, n = 1) were sampled in 2012 and 2013. Samples for PPMV1 testing were collected from 273 individuals. Mourning doves had a seroprevalence of 1.4% and ECDO had a prevalence of 44.1%. Prevalence in ECDO was significantly higher than MODO, (OR = 55.5455 P < 0.0001). The majority of birds positive for PPMV1 (75.0%) were found in the Phoenix metropolitan area. All of the positive samples were associated with large scale mortality events during 2012 and 2013 with an estimated total mortality of 1,500 birds. The overall prevalence for *T. gallinae* for 2012 and 2013 in the birds examined was 19.8% (n = 197). Eurasian collared doves had a prevalence of 42.9% (n = 14), MODO had a prevalence of 16.1% (n = 118), and WWDO had a prevalence of 31.8% (n = 44). *Trichomonas gallinae* was found in significantly more ECDO and WWDO than in MODO (OR_{ECDO} = 3.908, P = 0.0220; OR_{WWDO} = 2.432 P = 0.0299). Only 2 live birds tested positive for PPMV1 antibodies suggesting an acute course of infection with little opportunity for the development of immunity.

INTRODUCTION

Pigeon paramyxovirus 1 has recently been identified in ECDO in Arizona and Montana (Schuler et al. 2012). This RNA virus is one of several avian paramyxoviruses 1 (APMV1). Virulent APMV 1, also known as Newcastle disease, is considered to be one of the 2 most important avian diseases in the world. Four panzootics of PPMV1 have occurred since 1927 including the ongoing panzootic that began in 1982 in racing pigeons in Europe (Kaleta et al. 1985). Newcastle disease is listed as reportable by World Organization for Animal Health (OIE), and is considered a foreign animal disease in the United States. Identification of a virulent strain would

result in trade embargos and possibly depopulation of affected aviaries (Aldous et al. 2010). Mortalities caused by PPMV 1 have occurred in racing and feral pigeons, doves, and grey partridges (Kim et al. 2008, Aldous et al. 2010). Since the first confirmed diagnosis in Buckeye, Arizona in December of 2009, there have been several additional avian mortality investigations in Arizona for which PPMV1 has been identified as the cause of death. In one of these *T. gallinae* was also identified. The identification of PPMV1 in association with one of the most successful invasive terrestrial species in North America could have profound implications for native wild bird species such as MODO and other game birds (Fujisaki et al. 2010). The close association of ECDO with human development as well as the species' preference for warmer, dryer climates means that a warming climate could lead to further increases in the spread of this invader and the exposure of additional native game birds, such as sage grouse to PPMV1 (Fujisaki et al. 2010).

Trichomonas gallinae has been implicated as a contributing cause of mortality and morbidity in disease outbreaks in columbiformes and urban raptors in Arizona (AJ-A, unpublished data; USGS-NWHC, http://www.nwhc.usgs.gov/publications/quarterly_reports). Because PPMV1 causes lymphoid depletion, simultaneous infection with PPMV1 and *T. gallinae* could increase mortality or facilitate the development of an epizootic (Komers et al. 2003).

Objectives

1. Determine the prevalence of PPMV 1 in BTPI, MODO, and WWDO in Arizona by PCR of RNA extracted from cloacal and oropharyngeal swabs.
2. Determine seroprevalence of PPMV 1 in BTPI, MODO, and WWDO in Arizona by hemagglutination inhibition (HI).
3. Determine prevalence of *Trichomonas gallinae* BTPI, MODO, and WWDO in Arizona by in-pouch culture and light microscopy.
4. Compare the prevalence rates for PPMV 1 and *T. gallinae* in the 3 species and evaluate the potential for negative impacts to the populations and associations with the density of ECDO, an invasive urban species.

Management Actions

This information could be used to 1) guide future monitoring for potential outbreaks, 2) suggest timing of hunting seasons, 3) suggest other species that could be susceptible to disease outbreaks based on the distribution of current disease prevalence, and 4) suggest surveillance programs for additional species of game birds or special concern.

RELEVANCE TO STRATEGIC PLAN

This study directly addresses the identification of an emerging avian health disease issue, the viral disease caused by PPMV1. In addition, it could identify a linkage between a susceptible host, and the environment and factors that could enhance disease expression in the occurrence of an additional disease agent (*T. gallinae*) and a sympatric species (Eurasian collared doves). Because the distribution of the species being investigated varies with the level of urbanization, the project may identify an increased disease risk associated with human activity. Eurasian collared dove distribution is continuing to expand and is associated with warm, dry climates

(Fujisaki et al. 2010). The results of this project could inform agency response to invasive species response and climate change.

METHODS

Study Sites

Band-tailed pigeons were sampled in conjunction with Webless Migratory Bird Program project across the Mogollon Rim in the Southern Rockies/Colorado Plateau Bird Conservation Region (Figure 1) in 2012. Mourning doves, WWDO, and ECDO samples were collected from doves trapped in bird-banding events at various locations around the state in 2012 and 2013 (Figure 1). Additional samples were collected from ECDO and MODO at a dairy farm to evaluate the occurrence of the diseases at a site that was representative of previous mortality events. Sample collection was stratified across the 3 Bird Conservation Regions (BCR) 34 (Sierra Madre Occidental) and 33 (Sonoran and Mohave Deserts) and three species (BTPI, MODO and WWDO). Additional samples were collected during mortality investigations.

Sample Collection

Band-tailed pigeons (n = 25), MODO (n = 143), WWDO (n = 45), ECDO (n = 59), and RODO (n = 1) were sampled from 18 March 2012 to 16 October 2013. A total of 273 individuals were sampled in BCR 33, 34, and 16 (Table 1). Of the total birds sampled 68.1% came from BCR 33, 29.3% came from BCR 34, and 2.6% came from BCR 16 (Table 1).

Samples from BTPI (n = 25) were collected in cooperation with the Webless Migratory Bird Program project from 17 May to 3 August 2012 (Navajo and Gila counties, Figure 1). Swabs for PPMV1 testing were collected from all individuals sampled (n = 25) and blood was collected from 10 individuals. Twenty-one individuals were tested for *T. gallinae* using InPouch TF[®] (Biomed Diagnostics).

White-winged doves and MODO were sampled by AGFD regional personnel in BCR 33 and 34 in conjunction with annual dove banding. During 2012, 115 MODO and 43 WWDO were sampled. In 2013, an additional 25 MODO and 1 additional WWDO were sampled. Seven ECDO were incidentally captured during the banding efforts in 2012 and 2013 and sampled before release. Swabs for PPMV1 testing were collected from all WWDO (n = 44) and ECDO (n = 7), and all but one MODO (n = 139). Sufficient quantities of blood for testing were collected from 5 ECDO, 89 MODO, and 34 WWDO. All individuals sampled during banding efforts in 2012 were tested for *T. gallinae* using InPouch TF[®].

Samples were obtained from individuals collected during mortality investigations during 2012 (n = 17) and 2013 (n = 15) in BCR 33 and 34 (Table 1). Fifteen ECDO were sampled in 2012 and 12 in 2013. Swabs for PPMV1 testing were collected from 4 of these individuals (with no other sample types collected) and the remaining 23 were tested using tissue samples (a blood sample was also collected from 1 of the individuals). Tissues from 3 MODO were collected (one of these individuals was also sampled via swabs for PPMV1). One WWDO was sampled via swabs for PPMV1 and 1 RODO was sampled via tissue. Thirteen of the mortalities were examined for *T. gallinae*.

In 2013, 25 ECDO carcasses were obtained for sampling from a dairy farm in BCR 33. Twenty-three swabs, and 20 blood samples were collected. Fresh tissue samples were collected from all of the birds. None of the ECDO were tested for *T. gallinae*.

Cloacal and pharyngeal swabs were placed in viral media and stored on ice until they were delivered to the Arizona Game and Fish Department (AGFD) laboratory in Phoenix. Samples were then frozen at -70 °C until the end of the sampling period. Pharyngeal swabs for *T. gallinae* were used to inoculate InPouch TF[®] (Biomed). Pouches were held on gel ice and were delivered to the AGFD laboratory within 48 hours with the exception of samples from BTPI which were delivered up to 5 days after collection. Inoculated pouches were then incubated at 37 °C until growth was detected or for 5 days. Pouches were examined daily for trichomonad growth. Blood was collected from the jugular. Serum was removed after allowing the blood to clot for at least 2 hours. Serum samples were delivered to the AGFD laboratory in Phoenix on gel ice. Samples were frozen at -70 °C until the end of the sampling period.

At the end of the sampling period, samples were shipped overnight to Texas A & M Veterinary Medical Diagnostic Laboratories (TVMDL) on gel ice.

Sample Testing

RNA was extracted and evaluated with RT-PCR using the National Veterinary Services Laboratories (NVSL) protocol at TVMDL with standard molecular and virology procedures. Samples positive for APMV 1 RNA were further evaluated by sequencing for virulence and RT-PCR for the fusion gene. Additionally, carcasses from mortality investigations were examined by U.S. Geological Survey National Wildlife Health Center (USGS-NWHC) when large numbers of birds were involved.

Hemagglutination inhibition was performed using the standard protocol for PPMV 1 at TVMDL.

Analysis

We used odds ratios to compare results between species and BCR.

RESULTS

PPMV1 Testing

We collected blood, cloacal and oropharyngeal swabs, and tissues from 273 individuals for PPMV 1 testing (Table 2). All blood, tissues, and swabs from WWDO, BTPI, and the one RODO tested negative for PPMV1 (Table 2).

No viral RNA was found in either tissues or swabs collected from MODO (n = 143). Two blood samples from MODO were positive (n = 89) for a seroprevalence of 2.2 %. Oropharyngeal and cloacal swabs were also collected from these two MODO and tested negative. These two birds were sampled during normal banding activities in Yuma, Arizona.

Viral RNA was found in the tissues of 23 ECDO (n = 25) and in swab samples of 3 birds (n = 34) for a cumulative prevalence of 44.1% (Table 2). The positive swabs were obtained through mortality investigations in 2012 from moribund or recently deceased birds. One ECDO was collected as a single mortality event in Safford (Graham County) on August 24th. The other two ECDO were collected August 1st in Glendale (Maricopa County) during a mortality investigation that involved over 1,000 individuals. No other sample types were tested from these 3 individuals. None of the blood samples collected from ECDO were positive (n = 27).

Tissue samples from 29 individuals were tested following harvest (n = 2) or mortality investigations (n = 27). Tissue samples from 11 individuals were tested at TVDL and carcasses from 18 individuals were examined by USGS-NWHC. Three MODO, 1 RODO, and 25 ECDO were tested. The RODO and MODO all tested negative. Twenty-three of the 25 ECDO tested positive, 12 in 2013 and 11 in 2012. All 23 positive ECDO were obtained during mortality investigations (the two harvested ECDO tested negative). Only two of these individuals were tested by another means; the blood samples tested negative.

PPMV1 was identified in Yuma (n = 6), Maricopa (n = 21), and Graham (n = 1) counties (Figure 2). The ECDO that tested positive in Graham County (BCR 34), Safford, was a single mortality with no known associated large scale bird die-offs. The 27 PPMV1 positive samples in Yuma and Maricopa County (BCR 33) occurred during 5 mortality investigations and 1 trapping event. Two MODO were seropositive in Yuma in 2012 during banding efforts. In 2013, 4 ECDO tested positive for PPMV1 during a mortality investigation in Yuma that involved over 100 birds.

The majority of birds positive for PPMV1 (75.0%) were found in Maricopa County. The 21 ECDO were collected during mortality investigation in 2012 and 2013 in the Phoenix metropolitan area. Three mortality investigations occurred in 2012. Eight ECDO were collected during a mass mortality event in Glendale that lasted for several months and involved approximately 1,000 birds. Five ECDO were obtained in Mesa from separate locations during 2 different mortality investigations involving several hundred known deaths. In 2013, 2 more mass die-offs occurred in the Phoenix metropolitan area. Three ECDO were collected in Phoenix during a mortality investigation involving around 50 individuals. The other mortality event involved several hundred birds and occurred in Mesa were 5 ECDO tested positive.

Trichomonas Testing

We tested a subset of individuals for *T. gallinae* via InPouch TF[®] with light microscopic examination. Overall prevalence for *T. gallinae* for 2012 and 2013 in the birds examined was 19.8% (n = 197). Eurasian collared doves had a prevalence of 42.9% (n = 14), mourning doves had a prevalence of 16.1% (n = 118), and white-winged doves had a prevalence of 31.8% (n = 44, Table 3). Twenty-one band-tailed pigeons were tested but no trichomonad growth was observed in any of the 21 inoculated InPouch TF[®]. White-winged doves and ECDO were significantly more likely to be infected than MODO (OR_{WWDO} = 2.4, P = 0.0299; OR_{ECDO} = 3.1, P = 0.047).

Sixteen ECDO were examined for *T. gallinae*. Trichomonad growth was observed in 3 of the 5 tested via inoculated InPouch TF[®]. An additional 8 ECDO were examined by microscopic

examination alone and no *T. gallinae* was observed. Three ECDO had visual lesions, consistent with *T. gallinae* infection and were presumed infected.

Trichomonad growth was observed in 16 of 115 InPouch TF[®] from MODO and 3 were positive via microscopic examination. White-winged doves (n = 44) were examined by InPouch TF[®] and 14 showed trichomonad growth.

Trichomonas was found in 6 of the 8 counties sampled; 6 in Cochise, 1 in Coconino, 6 in Graham, 11 in Maricopa, 12 in Mohave, and 3 in Yuma County (Figure 2). Of the 39 positive samples, 33.3% came from BCR 34 (n = 13) and 66.6% came from BCR 33 (n = 26). Doves from Mohave County were significantly more likely to be infected than doves from Maricopa County (OR = 2.9, P = 0.0329). Doves from Navajo County were less likely to be infected than doves from Maricopa County although not significantly so (OR = 0.08, P = 0.084). There were no significant differences for *Trichomonas* prevalence between the 3 BCR.

DISCUSSION

Since PPMV 1 was first identified in a mortality of ECDO in 2009, we have observed mortality events annually in the Phoenix metro area. While the numbers reported dead, the date the first report each year, and the duration of each event varies, we have observed that mortalities usually begin in the summer and continue through the fall. Mortality events are rare in the winter and early spring. Five mortality events were observed in the Phoenix metro area during our sampling period. In 2013, we documented a small epizootic in Yuma. That event reportedly occurred in MODO and ECDO, however no carcasses of MODO were recovered.

With the exception of two blood samples collected from MODO, all of the tests for antibodies were negative. The hemagglutination inhibition (HI) test protocol used has not been validated for native dove species. The test was validated in the rock dove (domestic pigeon) and is specific for PPMV 1. Because the HI test is an antigen-based test that mainly identifies the presence of antibodies directed at the antigen, it is less sensitive to species variability in immunoglobulins and would be expected to effectively detect antibodies from native doves towards PPMV 1. Hemagglutination inhibition has been used in several wild bird surveillance studies for APMV 1 and PPMV 1 (Gough and Alexander 1983, Dortmans et al. 2011, Fornells et al. 2013). The additional caveat is that the test antigen could differ significantly from the viruses circulating in Arizona and the antibodies may not cross-react.

Viral RNA was detected in the tissues of 23 ECDO but only 3 swabs from ECDO tested positive. No viral RNA was detected in the other species of doves. Virus was detected in cell cultures of cloacal and oral swabs up to 10 days post-infection in an infection study of APMV 1 and PPMV 1 in pigeons (Dortmans et al. 2011). Generally, RT-PCR has been found to be as sensitive as tissue culture for the detection of viruses (Dortmans et al. 2011). However, viral shedding has been found to vary with viral strain and animal species infected. For example HPAI H5 N1 was initially believed to be carried in the gastrointestinal tract of wild birds but later research determined that oropharyngeal sampling in combination with cloacal swabs was a more sensitive method for detection of low pathogenicity avian influenza viruses.

The first year of our study, focused on collecting a stratified set of samples from across the state. When only two blood samples from birds not involved in mortality events were positive, we decided to focus on doves located near mortality events the following year. Additionally, we recognized the sensitivity of cloacal and oral swabs could be less than expected. Therefore in the second year of the study, we collected samples from 1 WWDO, 20 MODO, and 28 ECDO from a dairy farm on the outskirts of the Phoenix metro area. Both swabs and tissues were collected from lethally removed ECDO. None of the serum or swabs tested positive. Unfortunately, the tissues were not tested and were discarded by TVMDL without contacting the principal investigator. Two months after the samples were collected a mortality event caused by PPMV 1 was documented 1.2 miles from the dairy farm.

Trichomonas infection was found in all 3 dove species in several areas around the state. Previous reports have implicated the organism as a cause of mortality in MODO and raptors in urban areas of Arizona (Ostrand et al. 1995, Boal et al. 1998, Schulz et al. 2005). We found that WWDO were more likely to be infected than MODO which is consistent with the findings of Boal et al. in Tucson. It is possible that *Trichomonas* is not as pathogenic for this species or mortalities are less likely to be observed because WWDO are less common in urban areas. Co-infection with *Trichomonas* and PPMV 1 was detected in only 2 ECDO and does not appear to occur frequently.

These 3 dove species tend to inhabit different but overlapping niches and possess different social structures that likely influence the rate of transmission within and between species. Eurasian collared doves form very large flocks in close association with livestock and crop agriculture. They are primarily an urban species and are seldom observed in desert. Mourning doves are found throughout southern Arizona in upland Sonoran Desert especially in tree lined washes and have increased densities in urban and suburban areas. They generally do not form large flocks but will roost in groups in various types of trees. White-winged doves are also found throughout southern Arizona but, unlike the other 2 species, tend not to associate with urban or suburban areas. They will occasionally feed in large flocks in grain fields and nests may have a relatively high density in thickets of salt cedar and tamarisk. We would expect to see MODO affected in mortality events near urban areas while WWDO mortalities would be more likely to occur around grain fields. In 2012 a mortality of ECDO and WWDO occurred in Texas.

We were not able to detect PPMV 1 virus in healthy doves and only 2 MODO were positive for prior exposure. The prevalence of *Trichomonas* found in Arizona doves in this study is consistent with previous reports. PPMV 1 continues to be associated with mortality events in Arizona and the question of transmission from the invasive ECDO to native dove populations has not been definitively answered nor were we able to identify a source for the outbreaks. The lack of detection of virus in samples collected from healthy doves suggests that another species may be the reservoir. An experimental infection study could answer many questions regarding transmission and susceptibility. Additionally, viral culture and sequencing would be useful for characterizing the strain responsible for ECDO mortalities.

IMPLICATIONS

Eurasian collared doves represent a hazard to native dove populations by competing for resources and as a reservoir for disease. We need to continue to monitor the occurrence of diseases in ECDO and native MODO and WWDO. Disease agents of all types should be completely characterized with culture and genetic sequencing in order to identify changes in strains that could result in increased pathogenicity or infection of new hosts.

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Table 1: Number of mourning doves, white-winged doves, band-tailed pigeons, and Eurasian collared doves sampled from 18 March 2012 to 16 October 2013 in Bird Conservation Regions 33 (Sonoran and Mojave Deserts), 34 (Sierra Madre Occidental), and 16 (Southern Rockies/Colorado Plateau) in Arizona.

Bird Conservation Region	Species					Total	Percent of Total
	Mourning Doves	White-winged Doves	Band-tailed Pigeons	Eurasian Collared Doves	Rock Pigeon		
33	101	26	0	58	1	186	68.1 %
34	35	19	25	1	0	80	29.3 %
16	7	0	0	0	0	7	2.6 %
Total	143	45	25	59	1	273	

Table 2: Samples from mourning doves, white-winged doves, band-tailed pigeons, Eurasian collared doves, and rock pigeons tested for PPMV1 in Arizona in 2012 and 2013.

	Mourning Dove	White-winged Dove	Band-tailed Pigeon	Eurasian Collared Dove	Rock Pigeon
Swab Samples (RT-PCR)					
Tested	140	45	25	34	0
Positive	0	0	0	3	0
Prevalence	0.0%	0.0%	0.0%	8.8%	—
Blood Samples (antibody detection)					
Tested	89	34	10	27	0
Positive	2	0	0	0	0
Prevalence	2.2%	0.0%	0.0%	0.0%	—
Tissue Samples/Carcasses (RT-PCR)					
Tested	3	0	0	25	1
Positive	0	0	0	23	0
Prevalence	0.0%	—	—	92.0%	0.0%
Total Individuals Tested	143	45	25	59	1
Total Positive	2	0	0	26	0
Prevalence	1.4%	0.0%	0.0%	44.1%	0.0%

Table 3: Number of mourning, white-winged and Eurasian collared doves, and band-tailed pigeons tested for *Trichomonas* in Arizona in 2012 and 2013.

Species	<i>Trichomonas</i>		
	Total Tested	Total Positive	Prevalence
Mourning Doves	118	19	16.1%
White-winged Doves	44	14	31.8%
Band-tailed Pigeons	21	0	0.0%
Eurasian Collared Doves	16	6*	42.9%

*Includes three suspected positive by USGS-NWHC due to oral lesions but not examined microscopically or by InPouch TF[®].

Table 4: Numbers of mourning, white-winged and Eurasian collared doves, and band-tailed pigeons tested for *Trichomonas* in each county.

County	MODO		WWDO		BTPI		ECDO		Total Tested	Total Positive
	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive		
Cochise	9	2	17	4	0	0	0	0	26	6
Coconino	5	1	0	0	0	0	0	0	5	1
Gila	0	0	0	0	2	0	0	0	2	0
Graham	21	5	2	1	0	0	1	0	24	6
Maricopa	43	4	3	2	0	0	13	5*	59	11
Mohave	9	5	21	7	0	0	0	0	30	12
Navajo	7	0	0	0	19	0	0	0	26	0
Yuma	24	2	1	0	0	0	2	1	27	3
Total	118	19	44	14	21	0	16	6	199	39

*3 presumptive

Table 5: Numbers of mourning, white-winged and Eurasian collared doves, and band-tailed pigeons tested for *Trichomonas* in each BCR.

BCR*	MODO		WWDO		BTPI		ECDO		Total Tested	Total Positive
	Tested	Positive	Tested	Positive	Tested	Positive	Tested	Positive		
16	7	0	0	0	0	0	0	0	7	0
33	76	11	25	9	0	0	15	6**	116	26
34	35	8	19	5	21	0	1	0	76	13
Total	118	19	44	14	21	0	16	6	199	39

*Bird Conservation Regions 33 (Sonoran and Mojave Deserts), 34 (Sierra Madre Occidental), and 16 (Southern Rockies/Colorado Plateau)

**3 presumptive

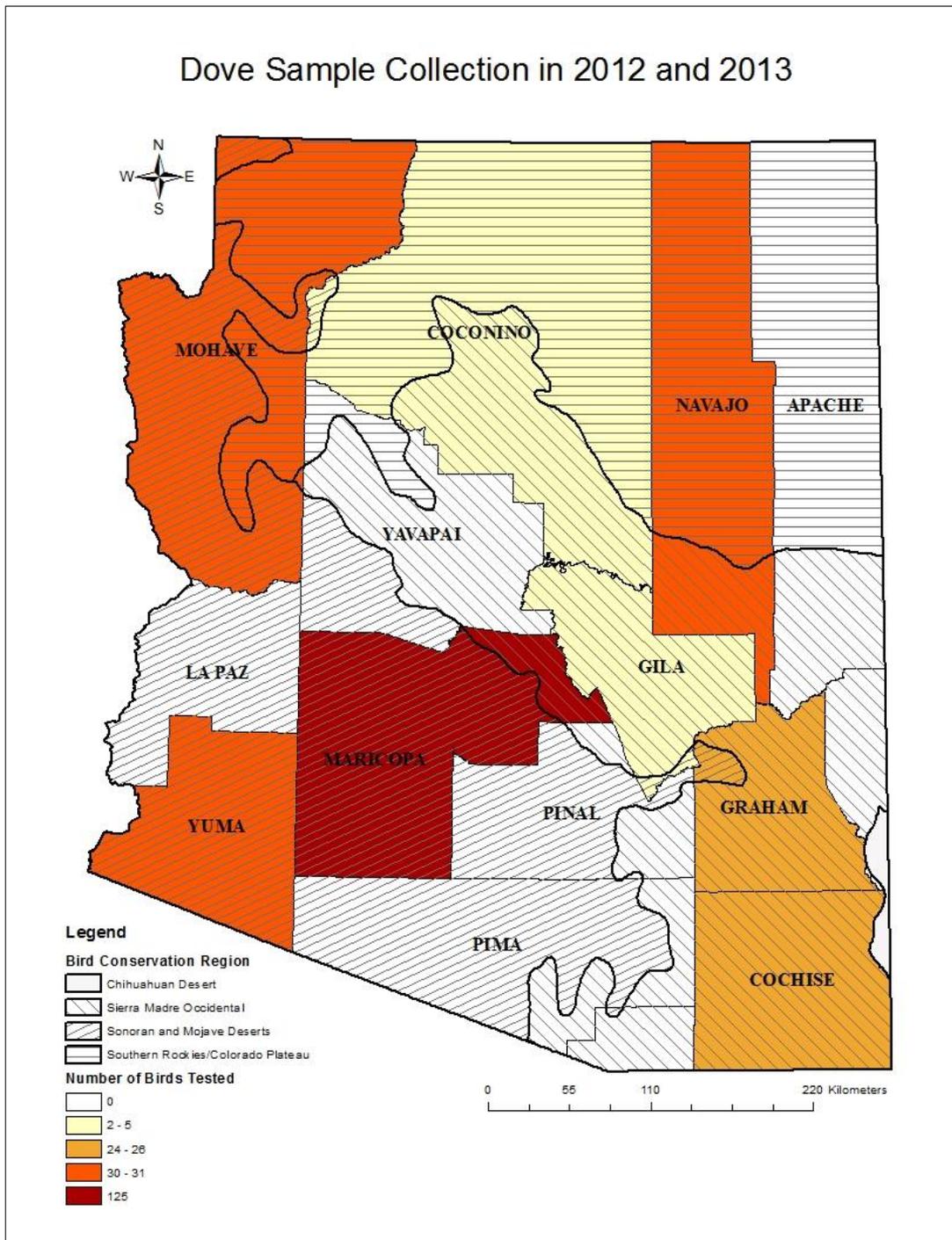
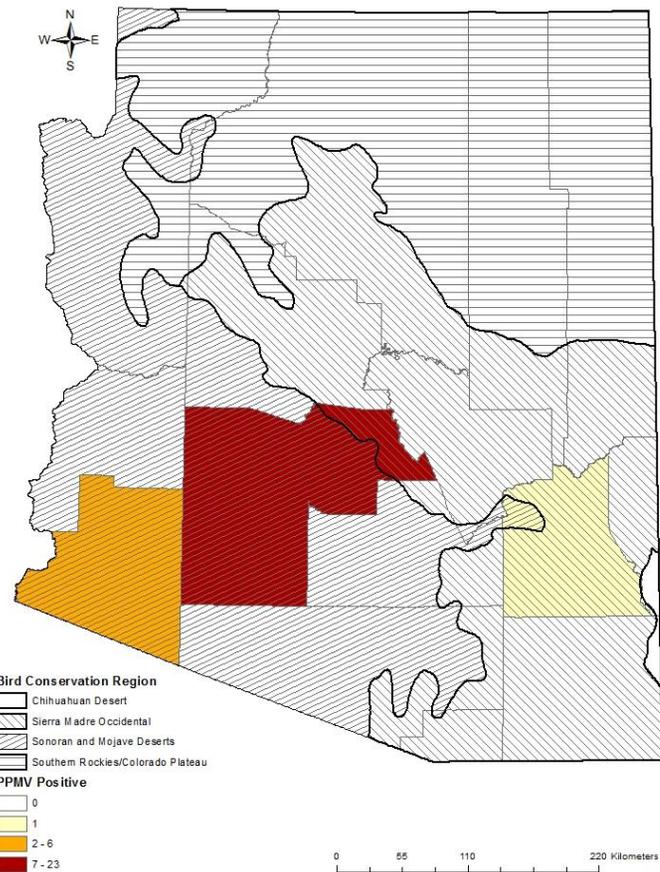


Figure 1: Map showing the location of sample collection from white-winged doves, mourning doves, band-tailed pigeons, and Eurasian collared doves 18 March 2012 to 16 October 2013 in Arizona.

Dove Samples Positive for PPMV1 in 2012 and 2013



Dove Samples Positive for *Trichomonas* in 2012 and 2013

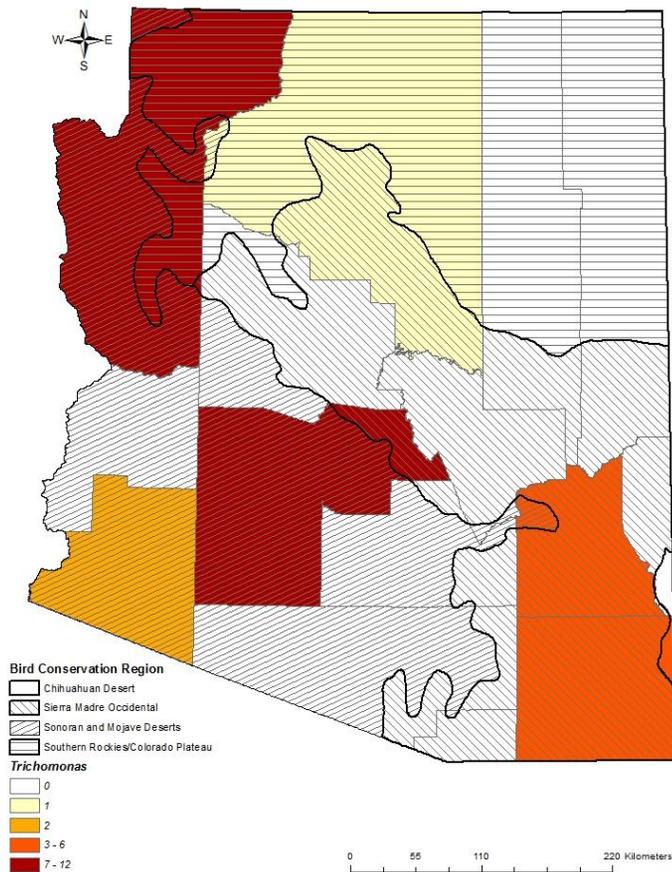


Figure 2: Maps showing the location of white-winged doves, mourning doves, and Eurasian collared doves sampled in 2012 and 2013 in Arizona that were positive for PPMV and *Trichomonas*.