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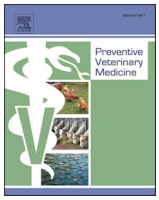

Evaluation of SARS-CoV-2 antibody detection methods for wild Cervidae

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ABSTRACT

Wildlife surveillance programs often use serological data to monitor exposure to pathogens. Diagnostic sensitivity and specificity of a serological assay quantify the true positive and negative rates of the diagnostic assay, respectively. However, an assay's accuracy can be affected by wild animals' pathogen exposure history and quality of the sample collected, requiring separate estimates of an assay's detection ability for wild-sampled animals where an animal's true disease status is unknown (referred to hereafter as sampling sensitivity and specificity). We assessed the sampling sensitivity and specificity of a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) surrogate virus neutralization test (sVNT) and conventional virus neutralization tests (cVNT) to detect antibodies for ancestral and Omicron B.1.1.529 variants of SARS-CoV-2 in wild white-tailed deer (*Odocoileus virginianus*) and mule deer (*Odocoileus hemionus*). We studied the influence of sample collection method using paired blood samples collected in serum separator tubes and on Nobuto strips from the same animal. Mean estimates of sampling sensitivity and specificity ranged from 0.21–0.95 and 0.94–1.00, respectively, varying by sample collection method, host species, and SARS-CoV-2 variant targeted by the assay. Broadly, sampling sensitivity was estimated to be higher for 1) sera collected in tubes, 2) detecting pre-Omicron SARS-CoV-2 variants, and 3) sVNT relative to cVNT assays. Sampling specificity tended to be high for all tests. We augmented our study with SARS-CoV-2 spike protein sequences derived from sampling locations and times coincident with white-tailed deer captures, finding common amino acid mutations relative to the sVNT Omicron antigen variant. The mutations may indicate that the SARS-CoV-2 variants circulating in cervids from 2021

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through 2024 may be better adapted to cervid hosts and more closely related to variants that circulated in humans prior to Omicron variants. We conclude our study with an inter-test comparison of sVNT results, revealing that 40 % inhibition is an optimal threshold for test positivity when testing deer sera for responses to Omicron variant B.1.1.529, compared to the 30 % inhibition recommended for ancestral variants.

1. Introduction

Serosurveillance is the monitoring of specific antibodies in host populations to identify the signature of infection from a target pathogen (s). Serological monitoring affords an extended temporal window for monitoring pathogen exposure because antibodies are often present in hosts longer than pathogens, which can have short infection periods. Thus, serosurveillance is often used to understand the geographic distribution of a pathogen in wild animals (Gilbert et al., 2013) and can provide important information about outbreak dynamics and epidemiology even after an outbreak (Pepin et al., 2019; Wilber et al., 2020). However, interpreting serological data in animal species can be complicated due to several factors. Sample availability and collection methods, host-specific assay sensitivity and specificity, individual-level variation in immune responses, and poor knowledge of animal ecology can all impact serosurveillance data (Gilbert et al., 2013). Diagnostic sensitivity quantifies the probability that an animal with targeted antibodies is correctly identified (i.e., the true positive rate; Jia et al., 2020). Similarly, diagnostic specificity quantifies the probability that an animal without targeted antibodies is correctly identified (i.e., the true negative rate; Jia et al., 2020). However, assay detection rates are affected by additional sources of error not captured by diagnostic sensitivity and specificity when samples are collected from wild animals. For example, field conditions can lead to non-ideal sample collection and storage conditions, and wild animals have more complex pathogen exposure histories than captive animals in controlled experimental conditions where infection histories are usually known (World Organisation for Animal Health, 2018, Chapter 2.2.7). These field-induced complexities require integrated estimates of assay detection rates that incorporate diagnostic sensitivity and specificity and account for sampling methods. One approach is through latent class statistical analyses on paired serosurveillance data, where the animal's true disease status is unknown (World Organisation for Animal Health, 2018; Jia et al., 2020), hereafter referred to as *sampling* sensitivity and specificity. In the case of emerging pathogens in new host species, rapid evolution can also obscure interpretation of serological results through changes in host-specific diagnostic sensitivity and specificity (Cao et al., 2022). In particular, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) exposure in wild animals has been reported for a variety of animal species using serological analysis (Chandler et al., 2021; Robinson et al., 2023; Caballero-Gómez et al., 2024). The most widespread detection of SARS-CoV-2 antibodies in a free-ranging species has been in North American white-tailed deer (*Odocoileus virginianus*, WTD; Bevins et al., 2023). In light of the recent infections of wild cervids with SARS-CoV-2 and concerns regarding their role as reservoir hosts, we estimate influences on sampling sensitivity and specificity for common serological assays used to detect SARS-CoV-2 antibodies in cervids to better interpret SARS-CoV-2 serosurveillance data.

Two common assays for detecting serological responses to SARS-CoV-2 exposure include the conventional virus neutralization test (cVNT) developed and validated at the Cornell Animal Health Diagnostic Center (Cornell AHDC; Palmer et al., 2021) and the commercially available Genscript cPass surrogate virus neutralization test (sVNT; Genscript, 2024). The cVNT uses live SARS-CoV-2 virus, requiring biosafety level 3 containment, and measures the amount of virus neutralized by antibodies in sera samples (Palmer et al., 2021). In contrast, the sVNT does not require live virus and can be completed in biosafety level 2 conditions. The sVNT detects neutralizing antibodies in sera by screening for antibody-mediated blockage of the interaction between the primary host-cell receptor and the receptor-binding domain (RBD) from the SARS-CoV-2 spike protein (Tan et al., 2020). The cVNT quantifies results as antibody titers, whereas sVNT quantifies the "percent inhibition" of a known quantity of antigen at a fixed sample dilution. The cVNT and sVNT assays have also been adapted to better assess exposure from Omicron and other variants. The initial use of sVNT for serological detection of SARS-CoV-2 in humans showed percent inhibitions greater than 30 % were indicative of SARS-CoV-2 antibodies with high sensitivity and specificity (Tan et al., 2020). Reagents used in the sVNT assay are not host species-specific, allowing for application to cervid sera without any further assay optimization (Tan et al., 2020; Chandler et al., 2021). Early research using sVNT in cervids showed sVNT percent inhibitions in white-tailed deer were rarely observed to be greater than 30 % before the pandemic (Chandler et al., 2021).

However, it is unclear how the sampling sensitivities and specificities of cVNT and sVNT assays are influenced by sVNT thresholds, sample type and field sampling conditions, and the variations in natural SARS-CoV-2 infection among wildlife species. Low sVNT percent inhibition thresholds increase sensitivity and high thresholds increase specificity, but the exact relationship must be informed by data. Sampling sensitivity and specificity may also change over time, as pathogens evolve and mutate. Sampling sensitivity and specificity arise from the impact of non-laboratory factors on an assay's diagnostic sensitivity and specificity. Therefore, sampling sensitivity and specificity are difficult to quantify using standardized standard laboratory practices (Jia et al., 2020), such as the World Organisation for Animal Health's (WOAH) assay development pipelines (World Organisation for Animal Health, 2018, Chapter 1.1.6). The WOAH pipelines acknowledge that field-based experiments using latent class statistical analyses can provide useful estimates of sensitivity and specificity when applicable laboratory evaluations of diagnostic assays are not available (World Organisation for Animal Health, 2018, Chapter 2.2.7, Section 1.2.2). Latent class statistical analyses have been applied to many disease systems (cf. Drewe et al., 2010; Wyckoff et al., 2015; Picasso-Risso et al., 2022).

Not knowing the sampling sensitivity and specificity of diagnostic assays as they are applied in different species and populations makes interpreting results from serosurveillance programs challenging. In cervid species, the 30 % sVNT inhibition threshold was used for wild white-tailed deer (*Odocoileus virginianus*), per the manufacturer's instructions (Chandler et al., 2021; Genscript, 2024). Chandler et al. (2021) focused on serological analysis of SARS-CoV-2 during the early phases of the pandemic (e.g., ancestral variants of SARS-CoV-2) before the emergence of Omicron and other variants that may elicit serologically different responses (Liu et al., 2022; Simon-Loriere and Schwartz, 2022). As more variants have emerged and transmission in additional species (e.g., mule deer (*Odocoileus hemionus*)) has been identified (Porter et al., 2024), it is important to continue to evaluate the interpretation of qualitative and quantitative diagnostic assay results to ensure adequate documentation of SARS-CoV-2 exposure in wildlife hosts and understanding of viral transmission dynamics.

To address potential challenges with interpreting serological results from serosurveillance studies of SARS-CoV-2 in deer, we estimated sampling sensitivity and specificity for different assays (sVNT compared to cVNT targeting specific neutralizing antibodies against ancestral and Omicron variants), sample types (sera extracted from serum separator tubes compared to Nobuto strips), and cervid species (white-tailed deer and mule deer). Specifically, we compared: 1) antibody titer data from

sVNT and cVNT assays for ancestral and Omicron variants, and 2) samples that were collected using Nobuto strips compared to venipuncture. We use latent class statistical analyses to compare test results. Our analysis evaluated how much variation in apparent seropositivity is explained by host-specific factors, sampling, and diagnostic procedures, providing knowledge for interpreting true seroprevalence.

2. Materials and methods

2.1. Sample collection

Targeted surveillance of deer provided paired serological samples from wild mule deer from capture efforts in Colorado and Utah and white-tailed deer from capture efforts in Colorado; Illinois; Minnesota; New York; Pennsylvania; Tennessee; and Washington, District of Columbia in the United States from October 2022 through January 2024 (Table 1). Live animal captures were conducted by collaborating universities and state agencies under approved capture protocols at the host university (Supplementary Table 1). Capture methods included helicopter capture, Clover trapping, darting, and drop netting. All appropriate handling procedures were followed to prevent spillover of SARS-CoV-2 into deer from capture crews at each field site. Sample collectors wore disposable gloves and face masks, and all blood collection tools were disposable and switched out between animals to avoid cross-contamination. At some field sites, paired samples were collected post-mortem from white-tailed deer harvested at the site by hunters.

Paired samples were collected from this national targeted surveillance program that longitudinally samples individual locations and acquires SARS-CoV-2 infection status data from live mule deer and white-tailed deer. Each paired sample consists of whole blood 1) collected in a serum separator tube and 2) onto two Nobuto filter strips from one animal (see Supplement Section 1 for additional details regarding Nobuto strip loading and evaluation). Whole blood was centrifuged by field personnel before pipetting out the serum and storing frozen until testing. Nobuto filter strips were air-dried, placed in individual envelopes and stored in a (open, to avoid humidity) bag with desiccant at room temperature. Blood samples were stored for comparative analysis as sera extracted from whole blood (hereafter referred to as *sera*) and sera extracted from Nobuto filter strips (hereafter referred to as *Nobuto*) following procedures described in Bevins et al. (2023).

2.2. Serological testing

Sera and Nobuto strip samples were screened using the Genscript cPass sVNT "wild type" kit and Omicron B.1.1.529 patch kit to test for SARS-CoV-2 neutralizing antibodies (NAbs) to ancestral (hereafter referred to as *wild type* per Genscript nomenclature) and Omicron variants of SARS-CoV-2 (Genscript, 2024). The Genscript sVNT kits were used to evaluate presence or absence of SARS-CoV-2 NAb in sera following procedures described in Tan et al. (2020). The Genscript sVNT kits were used to evaluate Nobuto samples following procedures described in Bevins et al. (2023). Sera samples were additionally evaluated with the cVNT assay developed and validated at the Cornell AHDC (Palmer et al., 2021) using ancestral and Omicron B.1.1.529 strains of SARS-CoV-2 for comparison of sVNT to a secondary assay type. The cVNTs were performed following procedures described in Palmer et al. (2021). In particular, the cVNT was implemented as a 100% plaque reduction neutralization test ($PRNT_{100}$). Nobuto samples were not submitted for cVNT testing.

2.3. Multisequence analysis

Genetic sequences of SARS-CoV-2 were obtained from white-tailed deer sampled in a separate national surveillance program in white-tailed deer (Feng et al., 2023). Sequences that spatially and temporally overlapped the targeted surveillance sampling were retained for analysis. Protein sequences for SARS-Cov 2 were manually aligned to the reference sequence for sVNT RBD provided by the manufacturer (Genscript, 2024) using program Clustal Omega (Sievers et al., 2011).

2.4. Statistical analyses

2.4.1. sVNT consistency between sample types

We studied the impact that sVNT percent inhibition threshold choice, sample type (i.e., sera and Nobuto), and variant (i.e., wild type and Omicron) had on diagnostic interpretation (i.e., positive and negative). For the sVNT assay, a threshold is used to interpret a sample's test result as "positive" when the percent inhibition exceeds the threshold, and "negative" otherwise. Optimal threshold choice can be influenced by sample type and variant because of their effects on sVNT percent inhibitions. We used Cohen's κ for both SARS-CoV-2 variants to help choose thresholds that mitigate sample type and variant effects as much as possible, to reduce the chance that samples analyzed from sera or Nobuto strips have different sVNT test results. Cohen's κ is an exploratory summary statistic that measures inter-rater reliability between two rating systems (i.e., consistency). Cohen's κ is large when two rating systems agree with each other more frequently than chance (McHugh, 2012).

We computed Cohen's κ using sVNT interpretations for sera as the first rating system, and the sVNT interpretations for Nobuto samples as the second rating system. One Cohen's κ value was computed for each combination of cervid species, SARS-CoV-2 variant, sera threshold, and Nobuto threshold. The Cohen's κ values helped choose sVNT thresholds used to subsequently study sensitivity and specificity more formally, using multiple-population statistical methods.

2.4.2. Sampling sensitivity and specificity estimates

We adapted multiple-population methods to estimate sampling sensitivity and specificity of samples collected from wild cervids. Multiple-population methods assume differences in population

Table 1
Summary information about captures and testing for each species, including the total number of times samples were taken from animals across sites (N), the total number of samples tested for different combinations of specimen and assay type (in parentheses), and the proportion of seropositive test results in the data.

		Wild type						Omicron				
	N	sVNT/Sera		sVNT/Nobuto		cVNT		sVNT/Sera		sVNT/Nobuto		cVNT
Mule Deer												
Colorado	38	0 %	(34)	6 %	(16)	0 %	(36)	0 %	(21)	0 %	(15)	0 % (36)
Utah	482	11 %	(472)	5 %	(169)	8 %	(472)	5 %	(419)	9 %	(191)	5 % (472)
White-tailed Deer												
Colorado	28	19 %	(21)	0 %	(10)	17 %	(24)	12 %	(17)	0 %	(8)	8 % (24)
District of Columbia	44		(0)	11 %	(44)		(0)		(0)	7 %	(43)	(0)
Illinois	61	8 %	(38)	4 %	(56)	0 %	(37)	20 %	(30)	0 %	(56)	3 % (37)
Minnesota	281	21 %	(190)	13 %	(204)	18 %	(191)	16 %	(172)	11 %	(204)	12 % (191)
New York	83	25 %	(83)		(0)	15 %	(79)	18 %	(51)		(0)	9 % (79)
Pennsylvania	47	42 %	(24)	11 %	(45)	12 %	(24)	22 %	(23)	0 %	(45)	17 % (24)
Tennessee	132	0 %	(25)	5 %	(107)	0 %	(26)	0 %	(1)	1 %	(107)	0 % (26)

seroprevalence, sensitivity, and specificity are the main factors that create differences in the observed proportion of positive tests within and between populations (Hui and Walter, 1980; Enøe et al., 2000). The methods assume exposure rates to the pathogen varies between populations, but sensitivity and specificity is constant for each test type. The assumptions imply 1) sensitivity and specificity create within-population differences in paired test results, and 2) prevalence creates between-population differences in test results. Importantly, the assumptions imply sampling sensitivity and specificity can only be statistically identified with field data from multiple populations having unequal pathogen exposure.

In our study, a "population" refers to a collection of individuals who have the same, fixed probability for antibody presence (i.e., seroprevalence). We assumed cervids sampled from the same site and 2-week period comprised a distinct population since SARS-CoV-2 prevalence varies over space and time (Hewitt et al., 2024). Samples from Utah were from eight geographically separated locations, which we modeled via eight sites. Samples from the other 7 states (including Washington, D.C.) were from one geographic location each, representing 7 distinct sites. Formally, we specified a hierarchical Bayesian model to estimate sampling sensitivity and specificity, jointly for all tests. Model implementation and estimation used Markov Chain Monte Carlo methods via the R package nimble (de Valpine et al., 2023). We modeled data separately for each species. Full model details and uninformative prior specifications are presented in Supplement Section 2.

3. Results

3.1. Samples collected

Samples were collected from 676 white-tailed deer and 520 mule deer across all sites. There was a wide range of sample sizes and apparent positivity from 0 % to 42 % (i.e., raw proportion of positive tests; Table 1). In particular, there were greater percentages of sVNT-positive wildtype sera samples compared to Nobuto samples in four of the five states having both sera and Nobuto samples for white-tailed deer, and one of the two states for mule deer (Table 1). Similarly, there were greater percentages of sVNT-positive Omicron sera samples compared to Nobuto samples in four of the five states having both sera and Nobuto samples for white-tailed deer, but neither of the two states for mule deer. Across all states where paired sVNT and cVNT testing was conducted, there were greater-or-equal percentages of sVNT-positive sera samples compared to cVNT-positive sera for both wild type and omicron targeted-tests, and for both white-tailed and mule deer.

3.2. Sample collection method impacted sVNT assay performance

The sVNT data were observed to differ between sVNT variants. Among paired samples, 80 % of mule deer and 75 % of white-tailed deer had sVNT wildtype percent inhibitions less than 20 % for both sera and Nobuto samples (Fig. 1). By comparison, 0 % of mule deer and 10 % of white-tailed deer had sVNT Omicron percent inhibitions less than 20 %

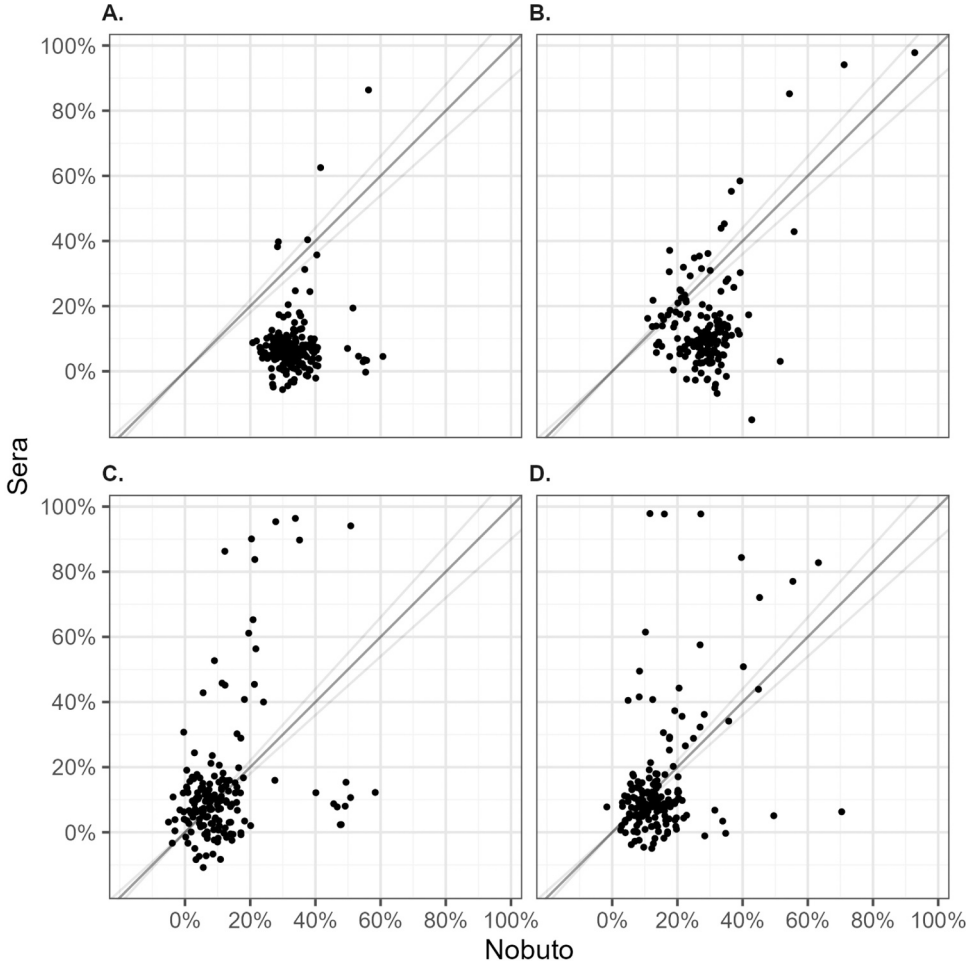

Fig. 1. Comparison of sVNT percent inhibitions in paired samples for A and C) mule deer (*Odocoileus hemionus*) and for B and D) white-tailed deer (*Odocoileus virginianus*), for sera and Nobuto. Paired samples tested for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) antibodies A and B) Omicron variant, and C and D) wild type variant. Plot panels include a dark grey 1:1 reference line with light grey lines indicating ± 10 % higher and lower than the reference line.

for both sera and Nobuto samples. Instead, 91 % of mule deer and 93 % of white-tailed deer had sVNT Omicron percent inhibitions less than 40 % for both sera and Nobuto samples.

The sVNT data were also observed to differ between sample collection methods. Among paired samples with either a sera or Nobuto sVNT wildtype percent inhibition greater than 20 %, we observed 100% of mule deer and 93 % of white-tailed deer had sera percent inhibitions that were at least 10 % larger or smaller than their corresponding Nobuto percent inhibition (Fig. 1). Similarly, among paired samples with either a sera or Nobuto sVNT Omicron percent inhibition greater than 40 %, we observed 94 % of mule deer and 90 % of white-tailed deer had sera percent inhibitions that were at least 10 % larger or smaller than their corresponding Nobuto percent inhibition.

Percent inhibitions were observed to be up to five times greater for sera samples compared to Nobuto (Fig. 1). For Nobuto, sVNT percent inhibitions were also observed to be 10 % points greater for the sVNT Omicron variant compared to the wild type variant. The sVNT percent inhibitions only tended to be large for sera (i.e., greater than 20 %) when corresponding, paired Nobuto samples had wild type sVNT percent inhibitions greater than 20 %, or Omicron sVNT percent inhibitions greater than 40 %.

Differences in sVNT percent inhibitions influenced test interpretations. Cohen's κ values less than one (i.e., perfect agreement) indicated sVNT results for sera would have led to different diagnostic interpretations than Nobuto in some cases (i.e., presence or absence of SARS-CoV-2 antibodies; Fig. 2). Disagreement rates for the sVNT Omicron variant in white-tailed deer samples were minimized (i.e., Cohen's κ maximized) when using percent inhibition thresholds between 40 and 60 % for sera and Nobuto. Disagreement rates for the sVNT wild type variant in white-tailed deer samples were minimized when using percent inhibition thresholds between 30 and 60 % for sera and between 20 and 40 % for Nobuto. Disagreement rates for the sVNT Omicron variant in mule deer were similar when using percent inhibition thresholds between 40 and 60 % for sera and between 40 and 50 % for Nobuto. Disagreement rates for the sVNT wild type variant in mule deer were similar when using percent inhibition thresholds between 40 and 60 % for sera and between 30 and 45 % for Nobuto.

3.3. Relationship between sVNT and cVNT

The sVNT percent inhibitions were observed to increase along with cVNT titers (Fig. 3). Percent inhibitions from the sVNT were observed to span all values between 0 and 100 % for both wild type and omicron variants. Among samples identified via cVNT as containing SARS-CoV-2 antibodies, percent inhibitions for the sVNT wild type variant tended to be much larger than percent inhibitions for the sVNT Omicron variant. The sVNT–cVNT relationship tended to be similar for mule deer and white-tailed deer.

3.4. Antibody cross-reactivity between SARS-CoV-2 variants

The sVNT percent inhibitions for wild type and Omicron variants were largely similar for sera. Roughly half of samples had an sVNT percent inhibition for the Omicron variant that was no more than 60 % different from its sVNT percent inhibition for the wild type variant. However, samples with large sVNT percent inhibition differences overwhelmingly tended to have higher percent inhibitions for the wild type variant (Fig. 4). Genetic sequences of SARS-CoV-2 from 29 white-tailed deer sampled separately via hunter harvest with Nobuto in Pennsylvania were available to explore potential drivers for the relationship in Fig. 4. Sera were not available for the 29 white-tailed deer with genetic sequence data, so they were not included in Fig. 4. However, 27 of the 29 white-tailed deer had from 9 to 12 amino acid mutational differences relative to the sVNT Omicron variant. In particular, 26 of the 29 white-tailed deer had the same 9 amino acid mutations.

3.5. Estimates for sampling sensitivity and specificity

Sampling sensitivity and specificity were estimated for all combinations of assay, variant, and species. Informed by our results in Section 3.2, we interpreted sVNT percent inhibitions as positive for SARS-CoV-2 antibodies when percent inhibition was greater than 30 % for the wild type variant and 40 % for the Omicron variant. Specificity estimates were high for all tests (i.e., greater than 85 %). Sensitivity estimates were more variable (Fig. 5, Table 2). The Bayesian multiple-population methods analysis of the data provided statistically significant evidence via 95 % highest posterior density intervals (HPDI) for pairwise

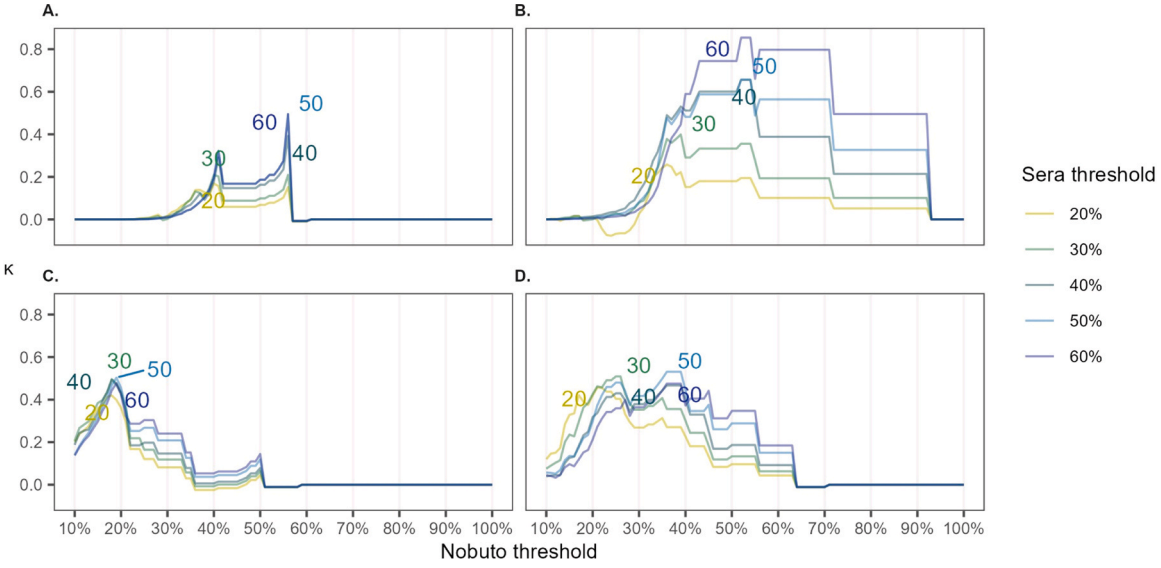

Fig. 2. Cohen's κ values calculated from paired samples for A and C) mule deer (*Odocoileus hemionus*) and for B and D) white-tailed deer (*Odocoileus virginianus*) to explore inter-test reliability when using sVNT percent inhibitions as evidence for presence or absence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) antibodies. Paired samples tested for A and B) Omicron variant, and C and D) wild type variant. Inter-test reliability was explored by using different combinations of thresholds to interpret sVNT percent inhibitions as positive or negative for SARS-CoV-2 antibodies. Cohen's κ values within each curve used the same sVNT percent inhibition threshold to interpret tests that used sera. The sVNT percent inhibition threshold for tests that used Nobuto increases along the x-axis.

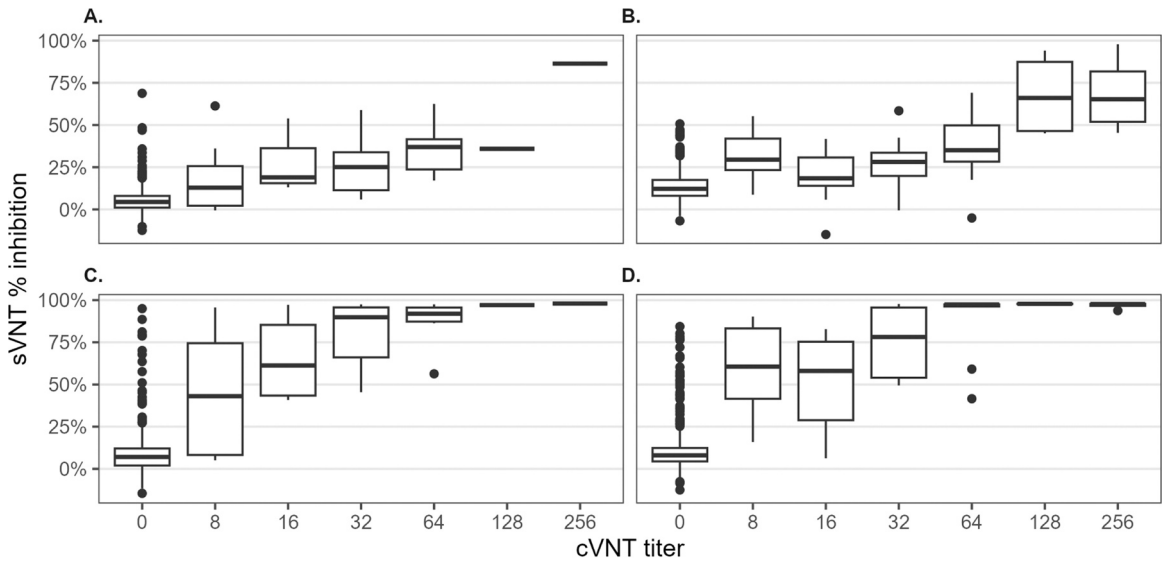

Fig. 3. Comparison of sVNT percent inhibition for sera to cVNT titers (reciprocal serum dilution factor required for complete Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) virus neutralization) for A and C) mule deer (*Odocoileus hemionus*) and for B and D) white-tailed deer (*Odocoileus virginianus*). Paired samples tested for A and B) Omicron variant, and C and D) wild type variant.

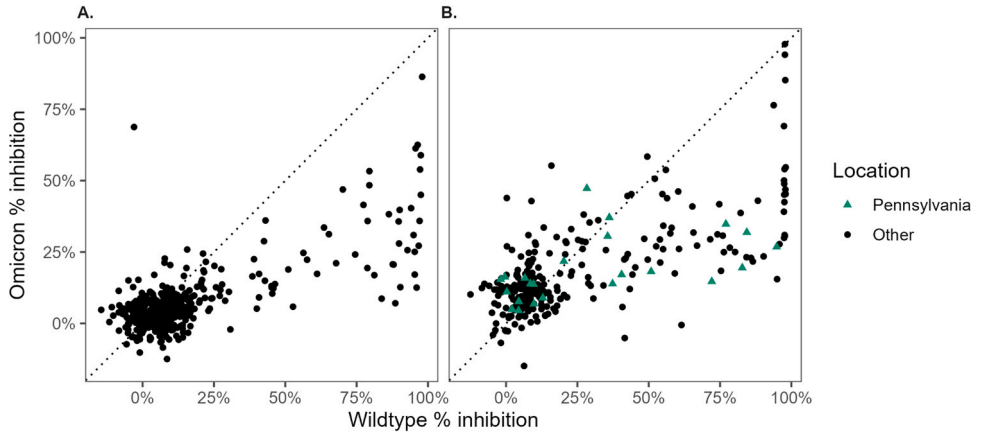

Fig. 4. Comparison of sVNT percent inhibition across variants for sera from A) mule deer (*Odocoileus hemionus*) and B) white-tailed deer (*Odocoileus virginianus*), highlighting the relationship for white-tailed deer sampled in Pennsylvania.

differences that sensitivity and specificity depended on test type. Sensitivity for SARS-CoV-2 Omicron variant in mule deer using sVNT with sera was lower than using cVNT (difference and 95 % HPDI: −0.41, −0.66– −0.14). In contrast, sensitivity for SARS-CoV-2 wild type variant in mule deer using sVNT with sera was higher than using sVNT with Nobuto (difference and 95 % HPDI:.74,.55–.92) or using cVNT (difference and 95 % HPDI:.25,.10–.40). Specificity for SARS-CoV-2 Omicron and wild type variants in mule deer using sVNT with sera was higher than using sVNT with Nobuto (difference and 95 % HPDI for Omicron:.07,.04–.11; wild type:.05,.01–.09). Sensitivity for SARS-CoV-2 wild type variants in white-tailed deer using sVNT with sera was higher than using sVNT with Nobuto (difference and 95 % HPDI:.54,.39–.70) or using cVNT (difference and 95 % HPDI:.28,.13–.42). Sensitivity estimates were higher for SARS-CoV-2 Omicron antibody detection in white-tailed deer than in mule deer. Additionally, for both species, sVNT sensitivity estimates tended to have high uncertainty using Nobuto samples. For both species, sensitivity was highest for sVNT using sera. Sensitivity was lowest for sVNT using Nobuto.

3.6. Net impacts on seroprevalence estimates

Accounting for sampling sensitivity and specificity impacted data interpretation for population-level seroprevalence. Posterior mean seroprevalence estimates, ranging from 4 % to 20 % across species and variants, tended to be higher than the proportion of positive sVNT sera tests (i.e., “apparent” seroprevalence estimates), ranging from 0 % to 42 %, and the proportion of cVNT tests, ranging from 0 % to 18 %, since the posterior seroprevalence estimates accounted for false negative rates in testing (i.e., sensitivity; Fig. 6). Uncertainties for apparent seroprevalence estimates were directly linked to the number of sVNTs and cVNTs conducted at each site, respectively, via frequentist confidence intervals for proportions. Uncertainty for Bayesian posterior seroprevalence estimates depended on the total number of tests conducted at each site in addition to the paired sample results, so more fully reflected all available data and sources of uncertainty in seroprevalence estimates.

4. Discussion

Estimating sampling sensitivity and specificity for antibody

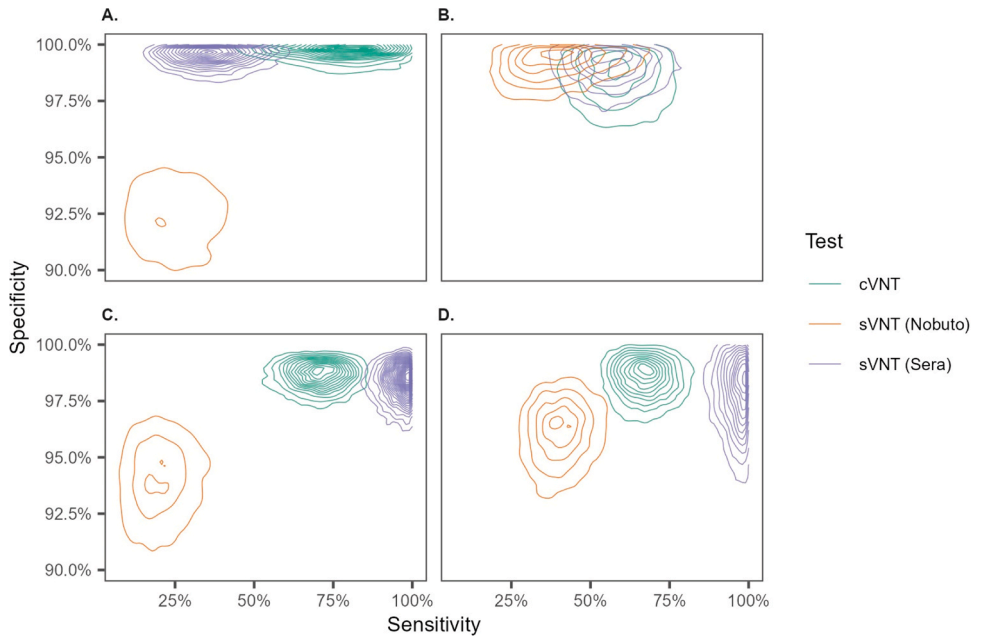

Fig. 5. Posterior density estimates for sampling sensitivity and specificity for A and C) mule deer (*Odocoileus hemionus*) and for B and D) white-tailed deer (*Odocoileus virginianus*), highlighting the regions most likely to contain the true values for sampling sensitivity and specificity. Posterior density estimates grouped by A and B) Omicron variant, and C and D) wild type variant.

Table 2
Posterior mean and 95 % highest posterior density intervals (HPDIs) for diagnostic sensitivities and specificities of tests evaluated.

	Wild type			Omicron		
	sVNT/Sera	sVNT/Nobuto	cVNT	sVNT/Sera	sVNT/Nobuto	cVNT
Mule Deer						
Sensitivity	.95 (.88–1.00)	.21 (.05–.39)	.70 (.56–.84)	.36 (.19–.56)	.29 (.04–.57)	.77 (.54–1.00)
Specificity	.98 (.97–1.00)	.94 (.90–.97)	.99 (.98–1.00)	.99 (.99–1.00)	.92 (.88–.96)	1.00 (.99–1.00)
White-tailed Deer						
Sensitivity	.95 (.88–1.00)	.41 (.27–.56)	.67 (.55–.81)	.58 (.37–.79)	.40 (.19–.66)	.60 (.38–.82)
Specificity	.98 (.95–1.00)	.96 (.93–.99)	.98 (.97–1.00)	.99 (.97–1.00)	.99 (.97–1.00)	.98 (.96–1.00)

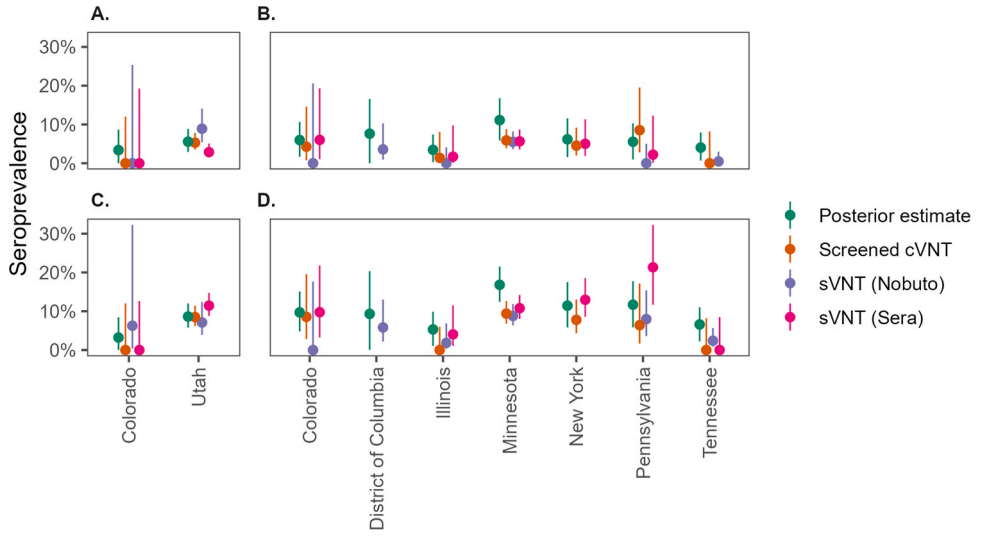

Fig. 6. Apparent seropositivity rates for each type of sample/assay (orange, purple, pink) compared with posterior estimates for time-averaged seroprevalence (green), split by state, species, and variant for A and C) mule deer (*Odocoileus hemionus*) and for B and D) white-tailed deer (*Odocoileus virginianus*). Panels grouped by A and B) Omicron variant, and C and D) wild type variant. The proportion of positive cVNT tests tended to be much higher than seroprevalence estimates due to test screening procedure.

detection in addition to other characteristics of serological tests used in wildlife serosurveillance studies provides critical information for interpreting serosurveillance data. True and false positive rates may differ between sample collection methods and may also lead to underestimation of disease prevalence without informed adjustment. Estimates based on field data may be less precise than estimates based on captive animal studies (World Organisation for Animal Health, 2018, Chapter 2.2.7). However, it can be challenging to coordinate captive animal studies with broad scale serosurveillance programs for emerging diseases, potentially leaving gaps in scientific understanding (Jia et al., 2020).

Our study used targeted serosurveillance of wild deer populations to balance the need to carefully evaluate diagnostic methods in focal species (i.e., by collecting paired serological data) with the need to surveil disease characteristics in a range of wild populations. The disease biology of SARS-CoV-2 in wild deer is complex and not well resolved. Deer live in close proximity to humans and may be a potential reservoir for both novel and existing SARS-CoV-2 variants of concern (Caserta et al., 2023; McBride et al., 2023). The effectiveness of diagnostic methods needs to be routinely monitored as at-risk populations shift and pathogens change in ways that may evade diagnostic tests. We estimated sampling sensitivity and specificity for common serological assays used to detect SARS-CoV-2 exposure in deer, additionally providing several areas for further investigation of detection probability and serosurveillance of variant trends in wild populations.

We showed that the sampled white-tailed deer and mule deer tended to have lower antibody titers to the post-Omicron surrogate sequences and variants. The difference may potentially be explained by a larger evolutionary distance between the SARS-CoV-2 RBD sequences observed in cervids and the post-Omicron sequences used in the sVNT. The sVNT's post-Omicron sequences are developed from SARS-CoV-2 variants sampled from humans, rather than cervids. Other Omicron sequences could potentially be used in sVNT protocols. Pre-Omicron SARS-CoV-2 variants are known to have widely infected white-tailed deer and evolved in white-tailed deer (Caserta et al., 2023; Feng et al., 2023; McBride et al., 2023). Recent SARS-CoV-2 variants circulating in cervids may be better adapted to cervid hosts and more closely related to earlier variants that circulated in humans prior to Omicron variants. Accordingly, it may be important to update diagnostic assays to improve sensitivity in cervids rather than exclusively relying on assays developed for humans.

While sVNT was estimated to have 95–100 % diagnostic sensitivity and 99.93 % specificity for detecting SARS-CoV-2 antibodies in humans (Tan et al., 2020), we evaluated sampling sensitivity and specificity for deer since no laboratory validation studies are available. We found evidence that sampling sensitivity and specificity for field data depends on the sample collection and testing method, and SARS-CoV-2 variant. For Nobuto, sampling sensitivity may also depend on the quality of the Nobuto strip's preparation (Supplement Section 3). We estimated sampling sensitivity to be highest for sVNT using sera (posterior means between .36 and .95 across species and variants), followed by cVNT (posterior means between .60 and .77 across species and variants). Sampling sensitivity was estimated to be lowest for sVNT using Nobuto (posterior means between .21 and .41 across species and variants). Sampling specificity was generally estimated to be high for all test types (posterior means above .97 for both tests). Future studies of SARS-CoV-2 in cervids can use sampling sensitivity and specificity estimates to optimize sample sizes to obtain desired detection probabilities and precision for prevalence estimates (cf. Humphry et al., 2004).

Similar to previous literature, we demonstrated that estimates for sampling sensitivity and specificity can be used to estimate population-level (sero-)prevalence from observed test positivity rates, by accounting for false positive and negative rates (cf. Enøe et al., 2000). SARS-CoV-2 seroprevalence was estimated to be up to 32 % for Omicron variants and 42 % for wild type variants in the sampled populations. Future, laboratory-based studies could be important to study potential cross-reactivity between assays. High seroprevalence estimates are consistent with previous studies of SARS-CoV-2 in deer (Chandler et al., 2021; Hale et al., 2022; Kuchipudi et al., 2022; Pickering et al., 2022; Bevins et al., 2023; Caserta et al., 2023; Feng et al., 2023; Hewitt et al., 2024).

The sensitivity estimates allowed for comparisons despite relatively large estimation uncertainty due to the field sampling methods. Uncertainty could be reduced via captive animal studies, for example. However, the sensitivity for sVNT using sera could potentially be above 95 %. High sensitivity would be consistent with studies of sVNT in humans and some companion animals (Tan et al., 2020; Perera et al., 2021). Sensitivity for sVNT using Nobuto was estimated to be much lower, which would be consistent with other studies that found sampling sensitivity for Nobuto can be lower than sensitivity for sera (Kamps et al., 2015). Nobuto preparation and storage procedures can also, in general, impact sampling sensitivity (Bevins et al., 2016). We found evidence that Nobuto saturation levels potentially impact positivity rates. Further study in laboratory conditions could potentially relate saturation levels to sensitivity more precisely. Sensitivity for cVNT was also estimated to be potentially lower than sVNT using sera. However, the cVNT procedure computed titers for 100 % virus neutralization in samples, which represents a higher standard of evidence than was used for the sVNT procedure.

We documented that sVNT percent inhibitions tended to be larger for wild type than Omicron variants among samples with non-zero cVNT titers. We also documented that sVNT percent inhibitions tended to be smaller for wild type than Omicron variants among samples with no detected antibodies via cVNT. Interpreting sVNT results for the Omicron variant as positive indications for SARS-CoV-2 antibodies when percent inhibitions are greater than 40 % yielded greater consistency across sample collection methods (i.e., sera compared to Nobuto) than the 30 % threshold that was initially developed to detect pre-Omicron variants in humans and later applied to white-tailed deer (cf. Tan et al., 2020; Chandler et al., 2021). The 30 % inhibition threshold still appeared to be appropriate for detecting antibodies to pre-Omicron variants in deer and yield consistent results across sample collection methods. For sVNT, percent inhibitions greater than 30 % and 40 % for sera were associated with non-zero wild type and Omicron cVNT titers, respectively, which used live SARS-CoV-2 virus to detect antibodies.

The greater sVNT percent inhibitions for Omicron than wild type versions of serological tests, overall, could suggest either ecological or biological differences. Wild type SARS-CoV-2 variants may have been more prevalent in sampled deer. Genetic sequences of SARS-CoV-2 from white-tailed deer sampled from sites in Pennsylvania close to locations where serological samples were obtained indicated most white-tailed deer samples had 9 amino acid mutations relative to the sVNT Omicron variant that is based on dominant Omicron variants in humans. This suggests that variants in deer may have been undergoing divergent evolution from human sequences in the receptor binding proteins. Comparison of recent variants isolated from deer to those from humans in the same area using receptor binding studies *in vitro* or *in silico* would be needed to test this hypothesis.

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Declaration of Competing Interest

The authors declare there are no conflicts of interest for this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prevetmed.2025.106522.

Data availability

Data and code to reproduce the figures and tables in the manuscript are available at https://figshare.com/s/ba96d4a5a1809e75c01e.

References

Bevins, S., Pappert, R., Young, J., Schmit, B., Kohler, D., Baeten, L., 2016. Effect of storage time and storage conditions on antibody detection in blood samples collected on filter paper. J. Wildl. Dis. 52 (3), 478–483.

Bevins, S.N., Chandler, J.C., Beckerman, S., Bergman, D.L., Chipman, R.B., Collins, D.T., Eckery, J.P., Ellis, J.W., Gosser, A.L., Heale, J.D., Klemm, J., Lantz, K., Lindera, T.J., Pleszewski, R., Quintanal, C., Ringenberg, J., Weir, K.R., Torchetti, M.K., Lenoch, J. B., DeLiberto, T.J., Shriner, S.A., 2023. SARS-CoV-2 occurrence in white-tailed deer throughout their range in the continental United States. bioRxiv 04 (14), 533542, 2023.

Caballero-Gómez, J., Cano-Terriza, D., Segalés, J., Vergara-Alert, J., Zorrilla, I., Del Rey, T., Paniagua, J., Gonzálvez, M., Fernández-Bastit, L., Nájera, F., Montoya-Oliver, J.I., Salcedo, J., García-Bocanegra, I., 2024. Exposure to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the endangered Iberian lynx (*Lynx pardinus*). Vet. Microbiol. 290, 110001.

Cao, Y., Wang, J., Jian, F., Xiao, T., Song, W., Yisimayi, A., Huang, W., Li, Q., Wang, P., An, R., Wang, J., Wang, Y., Niu, X., Yang, S., Liang, H., Sun, H., Li, T., Yu, Y., Cui, Q., Liu, S., Yang, X., Du, S., Zhang, Z., Hao, X., Shao, F., Jin, R., Wang, X., Xiao, J., Wang, Y., Xie, X.S., 2022. Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies. *Nature,* 602 (7898), 657–663.

Caserta, L.C., Martins, M., Butt, S.L., Hollingshead, N.A., Covaleda, L.M., Everts, M.R.R., Schuler, K.L., Diel, D.G., 2023. White-tailed deer (*Odocoileus virginianus*) may serve as a wildlife reservoir for nearly extinct SARS-CoV-2 variants of concern. Proc. Natl. Acad. Sci. 120 (6), e2215067120.

Chandler, J.C., Bevins, S.N., Ellis, J.W., Linder, T.J., Tell, R.M., Jenkins-Moore, M., Root, J.J., Lenoch, J.B., Robbe-Austerman, S., DeLiberto, T.J., Gidlewski, T., Kim, T. M., Shriner, S.A., 2021. SARS-CoV-2 exposure in wild white-tailed deer (*Odocoileus virginianus*). Proc. Natl. Acad. Sci. 118 (47), e2114828118.

Drewe, J.A., Tomlinson, A.J., Walker, N.J., Delahay, R.J., 2010. Diagnostic accuracy and optimal use of three tests for tuberculosis in live badgers. PloS One 5 (6), e11196.

Enøe, C., Georgiadis, M.P., Johnson, W.O., 2000. Estimation of sensitivity and specificity of diagnostic tests and disease prevalence when the true disease state is unknown. Prev. Vet. Med. 45 (1397 2), 61–81.

Feng, A., Bevins, S.N., Chandler, J.C., DeLiberto, T.J., Ghai, R., Lantz, K., Lenoch, J.B., Retchless, A., Shriner, S.A., Tang, C., Tong, S.S., Torchetti, M., Uehara, A., Wan, X.-F., 2023. Transmission of SARS-CoV-2 in the wild white-tailed deer in the United States. Nat. Commun. 14, 4078.

Genscript (2024). cPass™ SARS-CoV-2 neutralization antibody detection kit (FDA EUA). ⟨https://www.genscript.com/covid-19-detection-fda-eua.html⟩.

Gilbert, A.T., Fooks, A., Hayman, D., Horton, D., Müller, T., Plowright, R., Peel, A., Bowen, R., Wood, J., Mills, J., Cunningham, A., CE, R., 2013. Deciphering serology to understand the ecology of infectious diseases in wildlife. EcoHealth 10, 298–313.

Hale, V.L., Dennis, P.M., McBride, D.S., Nolting, J.M., Madden, C., Huey, D., Ehrlich, M., Grieser, J., Winston, J., Lombardi, D., Gibson, S., Saif, L., Killian, M.L., Lantz, K., Tell, R.M., Torchetti, M., Robbe-Austerman, S., Nelson, M.I., Faith, S.A., Bowman, A. S., 2022. SARS-CoV-2 infection in free-ranging white-tailed deer. Nature 602, 481–486.

Hewitt, J., Wilson-Henjum, G., Collins, D.T., Linder, T.J., Lenoch, J.B., Heale, J.D., Quintanal, C.A., Pleszewski, R., McBride, D.S., Bowman, A.S., Chandler, J.C., Shriner, S.A., Bevins, S.N., Kohler, D.J., Chipman, R.B., Gosser, A.L., Bergman, D.L., DeLiberto, T.J., Pepin, K.M., 2024. Landscape-scale epidemiological dynamics of SARS-CoV-2 in White-tailed deer. Transbound. Emerg. Dis., 7589509

Hui, S.L., Walter, S.D., 1980. Estimating the error rates of diagnostic tests. Biometrics 36 (1), 167–171.

Humphry, R.W., Cameron, A., Gunn, G.J., 2004. A practical approach to calculate sample size for herd prevalence surveys. Prev. Vet. Med. 65 (3-4), 173–188.

Jia, B., Colling, A., Stallknecht, D.E., Blehert, D., Bingham, J., Crossley, B., Eagles, D., Gardner, I.A., 2020. Validation of laboratory tests for infectious diseases in wild mammals: review and recommendations. J. Vet. Diagn. Investig. 32 (6), 776–792.

Kamps, A.J., Dubay, S.A., Langenberg, J., Maes, R.K., 2015. Evaluation of trapper-collected Nobuto filter-paper blood samples for distemper and parvovirus antibody detection in coyotes (*Canis latrans*) and raccoons (*Procyon lotor*). J. Wildl. Dis. 51 (3), 724–728.

Kuchipudi, S.V., Surendran-Nair, M., Ruden, R.M., Yon, M., Nissly, R.H., Vandegrift, K.J., Nelli, R.K., Li, L., Jayarao, B.M., Maranas, C.D., Levine, N., Willgert, K., Conlan, A.J. K., Olsen, R.J., Davis, J.J., Musser, J.M., Hudson, P.J., Kapur, V., 2022. Multiple spillovers from humans and onward transmission of SARS-CoV-2 in white-tailed deer. Proc. Natl. Acad. Sci. 119 (6), e2121644119.

Liu, Y., Liu, J., Shi, P.-Y., 2022. SARS-CoV-2 variants and vaccination. Zoonoses 2 (1), 6.

McBride, D.S., Garushyants, S.K., Franks, J., Magee, A.F., Overend, S.H., Huey, D., Williams, A.M., Faith, S.A., Kandeil, A., Trifkovic, S., Miller, L., Jeevan, T., Patel, A., Nolting, J.M., Tonkovich, M.J., Genders, J.T., Montoney, A.J., Kasnyik, K., Linder, T. J., Bevins, S.N., Lenoch, J.B., Chandler, J.C., DeLiberto, T.J., Koonin, E.V., Suchard, M.A., Lemey, P., Webby, R.J., Nelson, M.I., Bowman, A.S., 2023. Accelerated evolution of SARS-CoV-2 in free-ranging white-tailed deer. Nat. Commun. 14, 5105.

McHugh, M.L., 2012. Interrater reliability: the kappa statistic. Biochem. Med. 22 (3), 276–282.

Palmer, M.V., Martins, M., Falkenberg, S., Buckley, A., Caserta, L.C., Mitchell, P.K., Cassmann, E.D., Rollins, A., Zylich, N.C., Renshaw, R.W., Guarino, C., Wagner, B., Lager, K., Diel, D.G., 2021. Susceptibility of white-tailed deer (*Odocoileus virginianus*) to SARS-CoV-2. J. Virol. 95 (11), e00083–21.

Pepin, K.M., Pedersen, K., Wan, X.-F., Cunningham, F.L., Webb, C.T., Wilber, M.Q., 2019. Individual-level antibody dynamics reveal potential drivers of influenza A seasonality in wild pig populations. Integr. Comp. Biol. 59 (5), 1231–1242.

Perera, R.A., Ko, R., Tsang, O.T., Hui, D.S., Kwan, M.Y., Brackman, C.J., To, E.M., Yen, H.-l, Leung, K., Cheng, S.M., Chan, K.H., Chan, K.C.K., Li, K.-C., Saif, L., Barrs, Vanessa, R., Wu, J.T., Sit, T.H.C., Poon, L.L.M., Peiris, M., 2021. Evaluation of a SARS-CoV-2 surrogate virus neutralization test for detection of antibody in human, canine, cat, and hamster sera. J. Clin. Microbiol. 59 (2), e02504–e02520.

Picasso-Risso, C., Schwabenlander, M.D., Rowden, G., Carstensen, M., Bartz, J.C., Larsen, P.A., Wolf, T.M., 2022. Assessment of real-time quaking-induced conversion (RT-QuIC) assay, immunohisto-chemistry and ELISA for detection of chronic wasting disease under field conditions in white-tailed deer: a Bayesian approach. Pathogens 11 (5), 489.

Pickering, B., Lung, O., Maguire, F., Kruczkiewicz, P., Kotwa, J.D., Buchanan, T., Gagnier, M., Guthrie, J.L., Jardine, C.M., Marchand-Austin, A., Massé, A., McClinchey, H., Nirmalarajah, K., Aftanas, P., Blais-Savoie, J., Chee, H.-Y., Chien, E., Yim, W., Banete, A., Griffin, B.D., Yip, L., Goolia, M., Suderman, M., Pinette, M., Smith, G., Sullivan, D., Rudar, J., Vernygora, O., Adey, E., Nebroski, M., Goyette, G., Finzi, A., Laroche, G., Ariana, A., Vahkal, B., Côté, M., McGeer, A.J., Nituch, L., Mubareka, S., Bowman, J., 2022. Divergent SARS-CoV-2 variant emerges in white-tailed deer with deer-to human transmission. Nat. Microbiol. 7, 2011–2024.

Porter, S.M., Hartwig, A.E., Bielefeldt-Ohmann, H., Marano, J.M., Root, J.J., Bosco-Lauth, A.M., 2024. Experimental SARS-CoV-2 infection of Elk and Mule deer. Emerg. Infect. Dis. 30 (2), 354–257.

Robinson, S.J., Kotwa, J.D., Jeeves, S.P., Himsworth, C.G., Pearl, D.L., Weese, J.S., Lindsay, L.R., Dibernardo, A., Toledo, N.P., Pickering, B.S., Goolia, M., Chee, H.-Y., Blais-Savoie, J., Chien, E., Yim, W., Yip, L., Mubareka, S., Jardine, C.M., 2023. Surveillance for SARS-CoV-2 in Norway rats (*Rattus norvegicus*) from southern Ontario. Transbound. Emerg. Dis. 2023, 7631611.

Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., et al., 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using clustal omega. Mol. Syst. Biol. 7 (1), 539.

Simon-Loriere, E., Schwartz, O., 2022. Towards SARS-CoV-2 serotypes? Nat. Rev. Microbiol. 20 (4), 187–188.

Tan, C.W., Chia, W.N., Qin, X., Liu, P., Chen, M.I.-C., Tiu, C., Hu, Z., Chen, V.C.-W., Young, B.E., Sia, W.R., Tan, Y.-J., Foo, R., Yi, Y., Lye, D.C., Anderson, D.E., Wang, L.-F., 2020. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2–spike protein–protein interaction. Nat. Biotechnol. 38 (9), 1073–1078.

de Valpine, P., Paciorek, C., Turek, D., Michaud, N., Anderson-Bergman, C., Obermeyer, F., Wehrhahn Cortes, C., Rodríguez, A., Temple Lang, D., Zhang, W., Paganin, S., and Hug, J. (2023). NIMBLE: MCMC, particle filtering, and programmable hierarchical modeling. https://cran.r-project.org/package=nimble.

Wilber, M.Q., Webb, C.T., Cunningham, F.L., Pedersen, K., Wan, X.-F., Pepin, K.M., 2020. Inferring seasonal infection risk at population and regional scales from serology samples. Ecology 101 (1), e02882.

World Organisation for Animal Health (2018). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. ⟨https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/⟩, 12th edition.

Wyckoff, A.C., Galloway, N., Meyerett-Reid, C., Powers, J., Spraker, T., Monello, R.J., Pulford, B., Wild, M., Antolin, M., VerCauteren, K., Zabel, M., 2015. Prion amplification and hierarchical Bayesian modeling refine detection of prion infection. Sci. Rep. 5 (1), 8358.