

EVALUATION OF THE EFFECT OF HYDRATED LIME ON THE SCAVENGING OF FERAL SWINE (SUS SCROFA) CARCASSES AND IMPLICATIONS FOR MANAGING CARCASS-BASED TRANSMISSION OF AFRICAN SWINE FEVER VIRUS

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EVALUATION OF THE EFFECT OF HYDRATED LIME ON THE SCAVENGING OF FERAL SWINE (*SUS SCROFA*) CARCASSES AND IMPLICATIONS FOR MANAGING CARCASS-BASED TRANSMISSION OF AFRICAN SWINE FEVER VIRUS

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ABSTRACT: African swine fever (ASF) is a devastating hemorrhagic disease marked by extensive morbidity and mortality in infected swine. The recent global movement of African swine fever virus (ASFV) in domestic and wild swine (*Sus scrofa*) populations has initiated preparedness and response planning activities within many ASF-free countries. Within the US, feral swine are of utmost concern because they are susceptible to infection, are wide-spread, and are known to interact with domestic swine populations. African swine fever virus is particularly hardy and can remain viable in contaminated carcasses for weeks to months; therefore, carcass-based transmission plays an important role in the epidemiology of ASF. Proper disposal of ASF-infected carcasses has been demonstrated to be paramount to curbing an ASF outbreak in wild boar in Europe; preparedness efforts in the US anticipate carcass management being an essential component of control if an introduction were to occur. Due to environmental conditions, geographic features, or limited personnel, immediately removing every carcass from the landscape may not be viable. Hydrated lime converts to calcium carbonate, forming a sterile crust that may be used to minimize pathogen amplification. Any disturbance by scavenging animals to the sterile crust would nullify the effect of the hydrated lime; therefore, this pilot project aimed to evaluate the behavior of scavenging animals relative to hydrated lime-covered feral swine carcasses on the landscape. At two of the three study sites, hydrated lime-treated carcasses were scavenged less frequently compared to the control carcasses. Additionally, the median time to scavenging was 1 d and 6 d for control versus hydrated lime-treated carcasses, respectively. While results of this study are preliminary, hydrated lime may be used to deter carcass disruption via scavenging in the event that the carcass cannot be immediately removed from the landscape.

Key words: African swine fever virus, carcass management, feral swine, foreign animal disease, hydrated lime, scavenging.

INTRODUCTION

African swine fever (ASF) is one of the most deleterious diseases at the domestic livestock-wildlife interface (Sánchez-Cordón et al. 2018; Brown et al. 2021); it has a case fatality rate nearing 100% (European Food Safety Authority et al. 2021; Hakizimana et al. 2021) and resulted in countries losing international trade access (Costard et al. 2009). African swine fever virus (ASFV) is a large, double-

stranded DNA virus and the only virus in the genus *Asfivirus* within the family *Asfarviridae*. ASFV genotype II was introduced into Georgia within the Caucasus region of Europe in 2007 and subsequently spread into Eastern Europe (Revilla et al. 2018; Sánchez-Cordón et al. 2018). In 2018, ASFV began to spread through central and western Europe, Asia, and the Caribbean (Zhou et al. 2018; Le et al. 2019; Taylor et al. 2020; United States

Department of Agriculture Animal and Plant Health Inspection Service [USDA APHIS] 2021a, 2021b), which has led to dramatic production losses and mortality events. Prior to the ongoing outbreak, the virus had been limited to the African continent and Eastern Europe (Penrith 2009).

African swine fever virus infects domestic and wild members of the Suidae family, including Eurasian wild boar (*Sus scrofa*) distributed throughout Europe and Asia, which serve as an important source for spillover-spillback disease dynamics (McGregor et al. 2015; Miller et al. 2017; Ruiz-Fons 2017). Although the US does not have native wild boar, populations of invasive feral swine (which are generally hybrids of heritage breeds and wild boar imported from Europe; Smyser et al. 2020) are distributed in the majority of states, with numbers estimated to exceed 6,000,000 animals (USDA APHIS 2020). Feral swine are a highly gregarious species with complex social structure within their family groups (referred to as sounders) and are known to interact with domestic livestock, including swine (Wyckoff et al. 2009). This presents an opportunity for disease transmission between feral and domestic swine.

African swine fever virus is highly stable in a proteinaceous environment (Mazur-Panasiuk et al. 2019), and ASFV-contaminated pork products consumed by swine have been known to initiate outbreak events (Rowlands et al. 2008; Wang et al. 2019). The illegal importation of swine products could serve as route of viral introduction into the US (Brown and Bevins 2018) and feral swine feeding at landfills, which has been observed by field biologists (Mayer et al. 2021), could serve to introduce ASFV to this susceptible host (Herrera-Ibatá et al. 2018). Managing disease outbreaks in feral or wild species presents a substantial and unique challenge (Gortazar et al. 2015) and often results in a failure to eradicate the disease and the assignment of endemic disease status (Pepin and VerCauteren 2016; Croft et al. 2019).

Much of what is known relative to ASF in wild and feral populations is based on

knowledge ascertained from Europe during ASF outbreaks in wild boar. Carcass-based ASFV transmission is known to be an important source for new infections, and removing ASFV-infected carcasses from the landscape is paramount to control and management activities (European Food Safety Authority [EFSA] 2015; Pepin et al. 2020). The USDA's APHIS has worked with state, industry, and other federal partners on emergency preparedness activities related to the threat of an ASFV introduction event. While feral swine carcass removal is the preferred management strategy to reduce the amount of virus on the landscape, carcass removal may not always be feasible immediately after death for several reasons (e.g., landscape geography, personnel limitations, carcass size). Therefore, research evaluating alternative strategies to minimize pathogen transmission from carcasses is warranted.

For centuries, agriculturalists have used lime for disinfection (Blancou 1995) because it creates a dry and alkaline ($\text{pH} \geq 12$) environment unfavorable for pathogen amplification (Krach et al. 2008; Matsuzaki et al. 2021) as well as infestation by insects (Watson et al. 2003; National Lime Association 2022). In addition, hydrated lime (calcium hydroxide) can be used to create a sterile "crust" on the surface of remains or carcasses because, when exposed to air it absorbs carbon dioxide and releases water, hardening to form a solid-like crust of calcium carbonate (Schotsmans et al. 2014a, 2014b). As such, lime has been applied to human remains, animal carcasses, and abattoir waste to reduce pathogen load and dispersal (Sánchez et al. 2008; Avery et al. 2009; Schotsmans et al. 2012). It has also been suggested that treating human remains or animal carcasses with lime may reduce putrefactive odors and deter scavenging (National Agricultural Biosecurity Center Consortium 2004; Schotsmans et al. 2014a, 2014b).

Lime has been applied to pig carcasses to evaluate decomposition rate and inform human forensics (Schotsmans et al. 2012, 2014a, 2014b; Schultz and Martin 2012) as well as added to burial pits during disease outbreaks

among pig herds (Hseu and Chen, 2017). However, the effect of lime treatment on the dispersal of pathogens, such as ASF, from feral swine carcasses that have not been removed or protected is unknown. Any disturbance by scavenging animals to the sterile crust on the surface of the carcass would nullify the effect of the hydrated lime; therefore, this pilot project aims to evaluate the behavior of scavenging animals relative to hydrated lime-covered feral swine carcasses on the landscape.

MATERIALS AND METHODS

This pilot project was conducted in the spring of 2020 and 2021 at three study sites located in Louisiana, Missouri, and Texas, US. These states were chosen because they represent three distinct environments, allowing us to evaluate the efficacy of hydrated lime across a multitude of climatic conditions. Each study site included two treatment groups: hydrated lime-treated carcasses and control carcasses (no hydrated lime), with five feral swine carcasses per group. Carcasses weighing at least 36.3 kg (80 lb) were obtained from normal Wildlife Services operational control activities and randomly assigned to a treatment group. Each carcass in the hydrated lime group was paired with a carcass in the control group and paired carcasses were placed a minimum of 175 m from one another on the landscape. Carcasses were identified using standard nomenclature, which included state abbreviation, C 1-5 for the control group, and L 1-5 for the hydrated lime group (e.g., TX C1). Data collected for each carcass included global positioning system (GPS) coordinates, approximate weight, feral swine sex, age class based on tooth eruption, and trial start and end date. Study site centroids were calculated using GPS coordinates for each carcass and climatologic data were obtained from the nearest National Oceanic and Atmospheric Administration (NOAA) weather station. Commercially available hydrated lime, calcium hydroxide, was purchased and poured on the ground at each application site. The carcass was rolled onto the hydrated lime to ensure complete coverage, with no exposed tissue following application. Personnel applying hydrated lime followed all safety guidelines listed by the manufacturer to prevent exposure.

Carcass monitoring

At each carcass location, trail cameras (Browning Strike Force HD Pro X; Morgan,

Utah, USA) were deployed to monitor carcass scavenging for 10 d. The trail camera was placed 4.6–7.6 m (15–25 ft) from the carcass and affixed to a natural landscape feature whenever possible. In the absence of a natural landscape feature, trail cameras were fastened to an anthropogenic object (e.g., a fence post). The camera settings were programmed for a ≥ 2 -photo burst with a 10-min latent period between motion activations. The cameras were also programmed to provide a time and date stamp for each photo. The study sites were visited on day 1 (initial camera set up), day 5 (to check memory card storage and camera placement), and on day 10 (camera removal). Memory cards were sent to the National Feral Swine Damage Management Program (Fort Collins, Colorado, USA) for review and reporting.

The images and corresponding metadata from the memory cards were uploaded to the Colorado Parks and Wildlife Photo Warehouse version 3.0 (Ivan and Newkirk 2015). Each photo was manually viewed by two independent observers to characterize species presence and carcass interaction. If no animals were present in the photo, the species was listed as “none” for that image. If an animal was present in the frame, but the photo quality or environmental conditions prevented it from being accurately identified, the species was listed as “unknown.” Each individual animal was considered scavenging if the images clearly showed the animal scavenging on the carcass or contacting the carcass. Any discrepancies between the two original observers were resolved between them.

Statistical analyses

For each carcass, photographs taken within a photo burst were considered as one event. Given that image counts within photo bursts varied across cameras, scavenging detection rate for a given event was calculated for each study site. Control and hydrated lime-treated carcasses were paired both geographically and temporally. Accordingly, to evaluate the influence of treatment on scavenging frequency, a one-tailed paired sample *t*-test was performed to determine if there was no difference in the frequency in which paired control and hydrated lime-treated carcasses were scavenged ($H_0: \mu_d=0$) or if the frequency in which control carcasses were scavenged was greater than the frequency in which lime carcasses were scavenged ($H_a: \mu_d>0$; R package “stats”; R Core Team 2022). Specifically, a raw count of the number of events depicting scavenging was obtained for each carcass. Given that the sample size for each study site was <30 , a Shapiro-Wilk test of normality was implemented (R package “stats”; R Core Team 2022) to evaluate whether

TABLE 1. Number of days elapsed between the placement of feral swine (*Sus scrofa*) carcasses on the landscape and first contact by scavengers during a pilot study in the spring of 2020 and 2021 that sought to evaluate the behavior of scavenging animals relative to hydrated lime-treated carcasses partitioned by study site (Louisiana, Missouri, and Texas, USA) and individual carcass.

Study site	Carcass identifier ^a									
	C1	L1	C2	L2	C3	L3	C4	L4	C5	L5
Louisiana	— ^b	—	—	7	7	8	5	6	4	8
Missouri	0	6	1	5	0	—	3	—	1	3
Texas	0	7	0	3	1	1	1	4	1	4

^a Control carcass (C) and hydrated lime-treated carcass (L).

^b Dashes (—) indicate carcasses that were not scavenged during the 10-d study period.

the differences in scavenging frequency between paired carcasses were normally distributed. For the Shapiro-Wilk test, alpha was set to 0.05.

To determine if the application of hydrated lime on feral swine carcasses delayed the onset of scavenging, a survival analysis was performed using R package “survival” (Therneau 2021). For each feral swine carcass, the number of days elapsed from the start of the trial to the first scavenging event was determined (Table 1). Five of the carcasses were not scavenged during the 10-d study period; therefore, these replicates were right censored. Given that the censored carcasses had the same survival prospects as noncensored carcasses (i.e., censoring was non-informative), carcasses had equal survival probabilities because they were not recruited early or late in the study, and the exact time and date of the first scavenging events were recorded via motion activated trail cameras, the Kaplan-Meier

method was used to estimate a survival curve for each treatment (Bland and Altman 1998; Goel et al. 2010). Median survival time for each treatment was calculated as the time point in which the survivor probability was ≤ 0.5 (Goel et al. 2010). A log-rank test, which has the same assumptions as the Kaplan-Meier method, was then conducted to evaluate whether there was a difference in the survival probabilities between the two treatment groups (Bland and Altman 2004). Due to the small sample size, data were not stratified based on study site. To assess scavenger species at each study site, raw counts of scavenging and non-scavenging events were recorded for each species.

RESULTS

Ten sets of paired carcasses, five per state, were placed on the landscape in Louisiana and Texas in April of 2020. Another five sets of paired carcasses were placed on the landscape in Missouri in April of 2021. Temperatures for the Louisiana study site were similar to those for the Texas study site; however, these sites differed greatly in terms of precipitation (Table 2). Temperatures were slightly cooler at the Missouri study site as compared to Louisiana and Texas, though the precipitation for Missouri resembled that of the Louisiana study site (Table 2).

One set of paired carcasses in Missouri was only left on the landscape for 9 d; however, these data were retained to avoid diminishing the sample size. In total, there were 508, 1,215, and 1,818 recorded events (i.e., photo bursts) for Louisiana, Missouri, and Texas, respectively. Note that these raw counts

TABLE 2. Climatologic data averaged across the study period for each study site (Louisiana, Missouri, and Texas, USA) in which feral swine (*Sus scrofa*) carcasses were placed on the landscape in the spring of 2020 and 2021 to monitor the behavior of scavenging animals in response to the treatment of carcasses with hydrated lime.

Study site	Study period	Temperature (°C)			Precipitation (mm)	
		Mean daily max ^a	Mean daily min	Daily mean	Daily mean	Total
Louisiana	13 April 2020–24 April 2020	22.17	10.89	16.56	12.70	154.94
Missouri	1 April 2021–7 May 2021	19.33	3.78	11.56	5.08	144.78
Texas	8 April 2020–17 April 2020	24.72	8.39	16.61	0.00	0.00

^a Climatologic data are reported from the National Oceanic and Atmospheric Administration weather station nearest to the study site (<https://www.weather.gov/wrh/climate>).

TABLE 3. One-tailed paired sample *t*-tests comparing the scavenging frequency of untreated feral swine (*Sus scrofa*) carcasses (control) to the scavenging frequency of hydrated lime-treated feral swine carcasses at three study sites (Louisiana, Missouri, and Texas, USA) during the spring of 2020 and 2021.

Study site	Estimate ^a	<i>t</i> -test		<i>P</i> -value	95% CI ^b
		Statistic	df		
Louisiana	11.8	1.49	4	0.105	−5.05 to ∞
Missouri	97.4	5.67	4	0.002	60.80 to ∞
Texas	124.8	2.49	4	0.034	17.94 to ∞

^a Statistical estimate; mean of the differences.

^b CI=confidence interval.

include events in which no animal was observed. The scavenging detection rate was ≥ 0.82 for all study sites; therefore, image count per event was not accounted for in subsequent analyses. All study sites passed the Shapiro-Wilk test of normality ($P > 0.05$); therefore, a one-tailed paired sample *t*-test was performed for each site. There was no difference in the frequency in which paired control and hydrated lime-treated carcasses were scavenged in Louisiana ($P > 0.05$); however, control carcasses were scavenged at a higher frequency than hydrated lime-treated carcasses in Missouri and Texas ($P < 0.05$; Table 3). There was no difference in survival probabilities between the two treatments (control and hydrated lime) using the log-rank test ($\chi^2 = 3.6$; $P = 0.06$); however, the median survival time for the control carcasses was 1 d compared to 6 d for the hydrated lime-treated carcasses (Fig. 1).

Turkey Vultures (*Cathartes aura*) were the most common scavenger across all three study sites and the first species detected scavenging on 10/30 carcasses (Table 4, Fig. 2). Corvids (*Corvidae*) were the second most-observed scavenger in Missouri and Texas and the first species detected scavenging on 8/30 carcasses. Mesocarnivores, including coyotes (*Canus latrans*), raccoons (*Procyon lotor*), foxes (*Vulpes* spp.), and opossums (*Didelphis marsupialis*), were detected across the three study sites. Feral swine were observed at all three study sites; however, no feral swine were

observed scavenging on their conspecifics (Fig. 3). Interestingly, cattle were found to be scavenging (contacting) carcasses in Texas (Fig. 3).

DISCUSSION

In this pilot project, we identified a statistically significant difference in the frequency of scavenging on hydrated lime-treated carcasses as compared to control carcasses in Missouri and Texas; however, this difference was not observed in Louisiana. These results suggest that applying hydrated lime to feral swine carcasses may reduce scavenging behavior; however, additional studies are needed to confirm the utility of hydrated lime in deterring scavenging. Interestingly, carcasses at the study sites in Missouri and Texas had 2–3 times the number of recorded events (i.e., photo bursts) as compared to the study site in Louisiana. A survey of the area surrounding the study site in Louisiana revealed a landfill in close proximity to the study site (approximately 25.4 kilometers from the study site centroid), and it is likely that this landfill represents a food source for a variety of species (Oro et al. 2013).

The survival analysis, in which the median time for first contact by scavengers (i.e., median survival time) among control carcasses was 1 d compared to 6 d for hydrated lime-treated carcasses, suggests that the application of hydrated lime delays the onset of scavenging; however, these differences only approached statistical significance ($P = 0.06$). Although the time to scavenging was consistent with expectations, the lack of statistical significance was probably attributable to our small sample size (Rich et al. 2010). Given preliminary indications that the addition of hydrated lime delayed scavenging, continued investigation is warranted; however, future studies would benefit from larger sample sizes.

Disease management in wildlife is a challenging endeavor; the potential for environmental contamination and pathogen vectoring

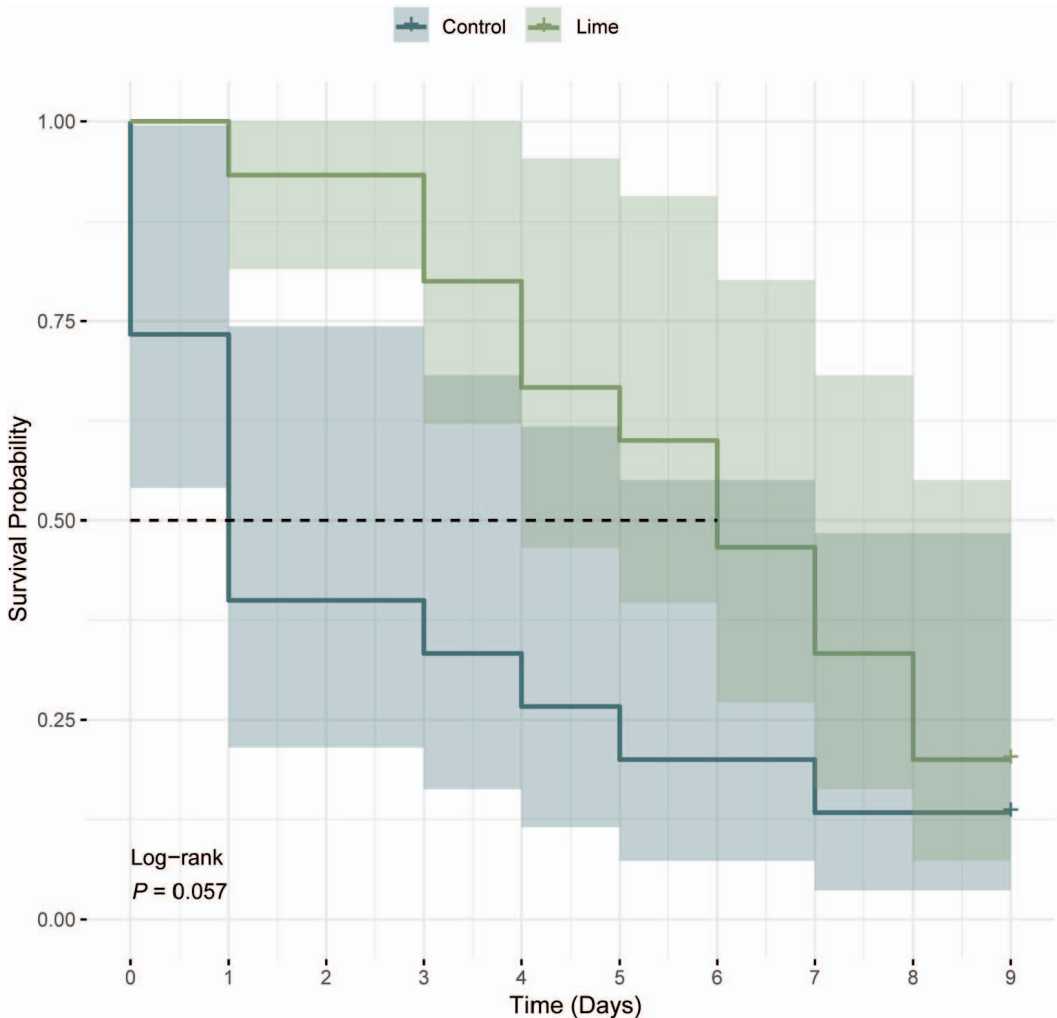


FIGURE 1. Kaplan Meier survival curves with 95% confidence intervals for feral swine (*Sus scrofa*) carcasses in two treatment groups (control and treated with hydrated lime) placed on the landscape during the spring of 2020 and 2021 to evaluate the behavior of scavenging animals relative to hydrated lime-treated carcasses. Median survival time is represented by the horizontal dashed line. Censored replicates are indicated by plus signs.

by scavengers further complicates disease management efforts. Previous studies have observed wild boar making direct contact with dead conspecifics and the soil surrounding their decomposing carcasses (Probst et al. 2017; Lim et al. 2022), which is problematic because contaminated carcasses on the landscape may serve as a reservoir of infection until decomposition is complete (Chenais et al. 2019). We did not observe feral swine scavenging or making direct contact with the carcasses; however, feral swine were observed

at all three study sites; this could result in the spread of ASFV via a contaminated environment if the disease were introduced into the US.

In our study, a plethora of species across a variety of taxonomic Classes scavenged on both control and hydrated lime-treated carcasses, including animals with large home ranges. In Missouri, a coyote was observed scavenging on a carcass and dragging the carcass outside of the camera's field of view (Fig. 3). During similar studies, raccoons,

TABLE 4. The number of times a species was detected scavenging on a feral swine (*Sus scrofa*) carcass during the spring of 2020 and 2021 using motion-activated trail cameras partitioned by study site (Louisiana, Missouri, and Texas, USA) and treatment (control and treated with hydrated lime).

Study site	Treatment	Species	Scientific name	Detections
Louisiana	Control	Coyote	<i>Canus latrans</i>	1
Louisiana	Control	Opossum	<i>Didelphis marsupialis</i>	10
Louisiana	Control	Turkey Vulture	<i>Cathartes aura</i>	62
Louisiana	Lime	Coyote	<i>Canus latrans</i>	2
Louisiana	Lime	Opossum	<i>Didelphis marsupialis</i>	1
Louisiana	Lime	Turkey Vulture	<i>Cathartes aura</i>	11
Missouri	Control	Black Vulture	<i>Coragyps atratus</i>	33
Missouri	Control	Corvid	Corvidae	61
Missouri	Control	Coyote	<i>Canus latrans</i>	11
Missouri	Control	Fox (General)	<i>Vulpes</i> spp.	1
Missouri	Control	Opossum	<i>Didelphis marsupialis</i>	3
Missouri	Control	Raccoon	<i>Procyon lotor</i>	52
Missouri	Control	Turkey Vulture	<i>Cathartes aura</i>	445
Missouri	Lime	Corvid	Corvidae	1
Missouri	Lime	Owl	Strigiformes	1
Missouri	Lime	Raccoon	<i>Procyon lotor</i>	2
Missouri	Lime	Turkey Vulture	<i>Cathartes aura</i>	84
Texas	Control	Birds (general)	Aves	4
Texas	Control	Black Vulture	<i>Coragyps atratus</i>	48
Texas	Control	Corvid	Corvidae	180
Texas	Control	Crested Caracara	<i>Caracara plancus</i>	104
Texas	Control	Turkey Vulture	<i>Cathartes aura</i>	553
Texas	Lime	Cattle	<i>Bos taurus domesticus</i>	3
Texas	Lime	Corvid	Corvidae	6
Texas	Lime	Crested Caracara	<i>Caracara plancus</i>	3
Texas	Lime	Opossum	<i>Didelphis marsupialis</i>	9
Texas	Lime	Turkey Vulture	<i>Cathartes aura</i>	27

raccoon dogs (*Nyctereutes procyonoides*), foxes, and Ravens (*Corvus corax*) have been observed dispersing elements of wild boar carcasses (Probst et al. 2019; Lim et al. 2022). Movement of contaminated carcass material via scavengers is concerning as it may lead to dispersal of ASFV (Probst et al. 2019; Pepin et al. 2020). While the role of mechanical vectors in the spread of ASFV remains largely unknown, they may be epidemiologically important for other pathogens (Siembieda et al. 2011; Vicente and VerCauteren 2019) and should be considered for further study.

It is important to note that scavenging may help to reduce the pathogen burden by

contributing to carcass breakdown and decomposition (Probst et al. 2019; O'Neill et al. 2020). For instance, scavenging of ASFV-contaminated wild boar carcasses by wolves reduces the environmental burden of the pathogen because the virus is degraded in the gastrointestinal tract (Szewczyk et al. 2021). The use of hydrated lime is intended to be in addition to carcass removal, not in lieu of it. If feral swine carcasses were to be left on the landscape indefinitely, the use of hydrated lime may negate the benefits of scavenging; however, hydrated lime may prevent the translocation of ASFV-contaminated carcass materials by scavengers in the interim between carcass detection and car-

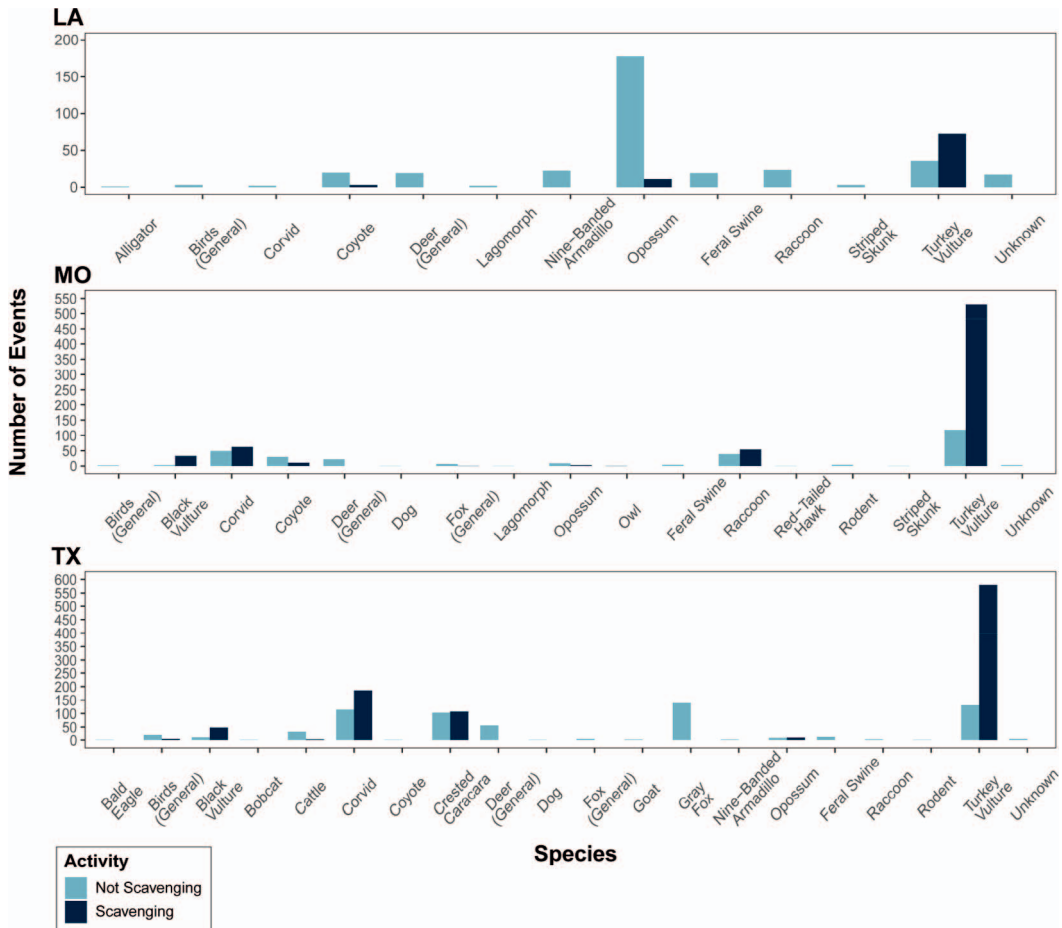


FIGURE 2. Bar chart depicting the number of times a species was detected via motion activated trail cameras scavenging or not scavenging on a feral swine (*Sus scrofa*) carcass at three study sites in Louisiana, Missouri, and Texas, USA during the spring of 2020 and 2021. Species observed included alligator (*Alligator mississippiensis*), Bald Eagle (*Haliaeetus leucocephalus*), birds (Aves), Black Vulture (*Coragyps atratus*), bobcat (*Lynx rufus*), cattle (*Bos taurus domesticus*), corvids (Corvidae), coyote (*Canus latrans*), Crested Caracara (*Caracara plancus*), deer (Cervidae), dog (*Canis lupus familiaris*), feral swine (*Sus scrofa*), fox (*Vulpes* spp.), goat (*Capra aegagrus hircus*), gray fox (*Urocyon cinereoargenteus*), lagomorphs (Lagomorpha), nine-banded armadillo (*Dasypus novemcinctus*), opossum (*Didelphis marsupialis*), owls (Strigiformes), raccoon (*Procyon lotor*), Red-tailed Hawk (*Buteo jamaicensis*), rodents (Rodentia), striped skunk (*Mephitis mephitis*), and Turkey Vulture (*Cathartes aura*).

cass removal. The epidemiology of carcass-based transmission is significant enough to warrant a stop-gap measure if the ASFV-contaminated carcass cannot be immediately removed from the landscape. Treatment of carcasses with hydrated lime provides an additional tool for wildlife professionals seeking to mitigate the spread of ASFV in feral swine populations.

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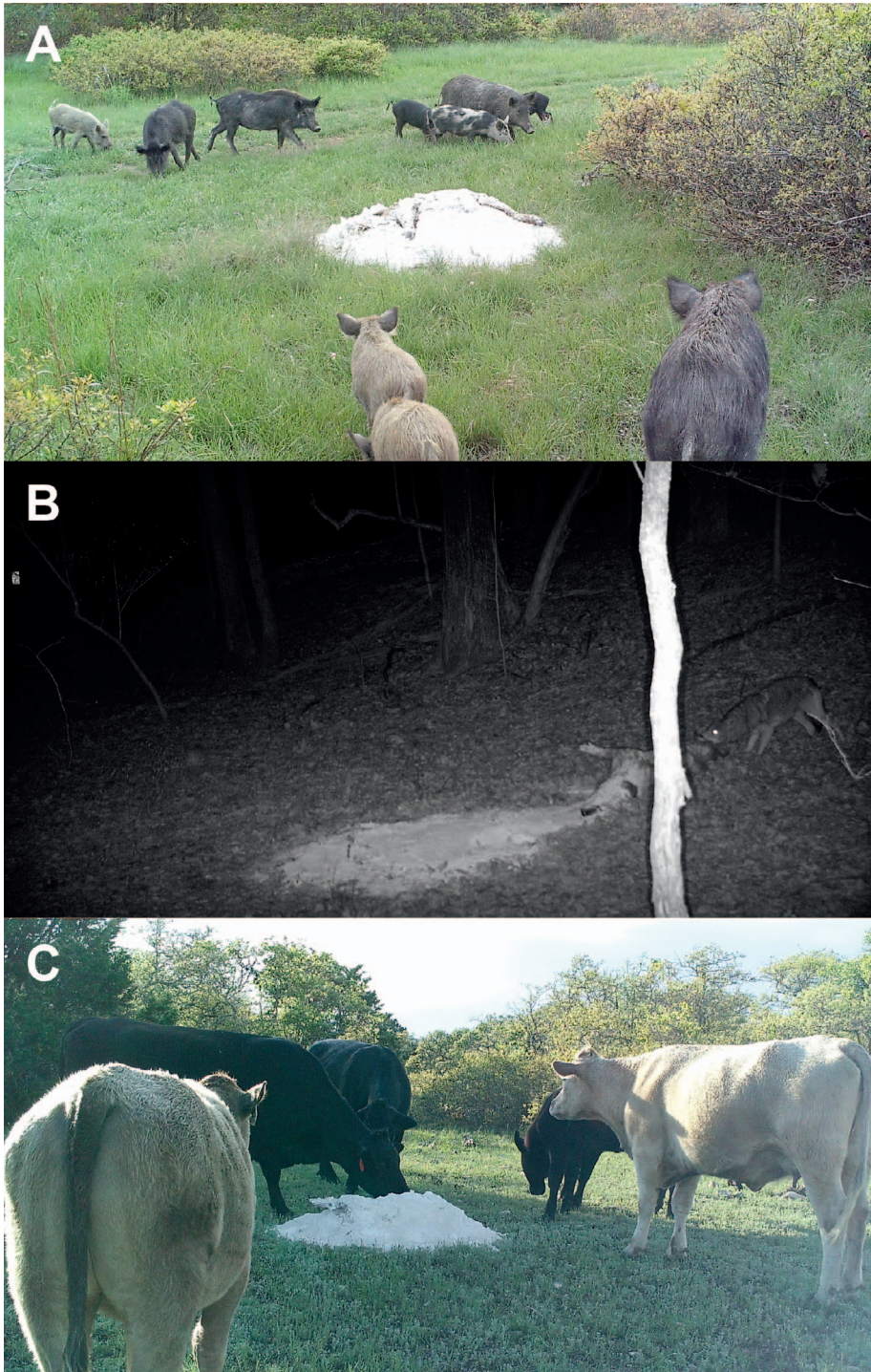


FIGURE 3. Photographs collected in the spring of 2020 and 2021 using motion-activated trail cameras during a pilot project in which the behavior of scavenging animals was evaluated in response to hydrated lime-treated feral swine (*Sus scrofa*) carcasses. A) Feral swine (*Sus scrofa*) observed near hydrated lime-treated feral swine carcass in Texas, US; B) coyote (*Canis latrans*) scavenging on hydrated-lime treated feral swine carcass in Missouri, US; C) cows (*Bos taurus domesticus*) making contact with hydrated lime-treated feral swine carcass in Texas, US.

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