

A retrospective investigation of risk factors associated with loads of pigs positive for Senecavirus A at a midwestern US packing plant during the summer of 2017

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Summary

This study describes a spatio-temporal cluster of Senecavirus A (SVA) outbreaks reported in a midwestern US slaughter plant during the summer of 2017. Data was collected on multiple site characteristics to conduct risk factor analysis. On June 8, 2017, 6 of 10 finishing pig lots delivered to the plant tested positive by reverse transcription-polymerase chain reaction for SVA RNA. Subsequently, 88 lots presented vesicular lesions at the plant, and 74 lots tested positive between June 8 and July 10, 2017, which was a significant temporal cluster.

Keywords: swine, Senecavirus A, vesicular disease, market pigs, packing plants.

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Resumen - Una investigación retrospectiva de los factores de riesgo asociados con embarques de cerdos positivos para el Senecavirus A en una planta empacadora del medio oeste de los EE UU durante el verano de 2017

Este estudio describe un grupo espacio-temporal de brotes del Senecavirus A (SVA) notificados en una planta de sacrificio del medio oeste de los EE UU durante el verano de 2017. Se recopilaban datos sobre las características de múltiples sitios para realizar un análisis de factores de riesgo. El 8 de junio de 2017, 6 de cada 10 lotes de cerdos de engorda entregados a la planta dieron positivo por reacción en cadena de transcripción reversa de la polimerasa para el ARN del SVA. Posteriormente, 88 lotes presentaron lesiones vesiculares en la planta, y 74 lotes dieron positivo entre el 8 de junio y el 10 de julio de 2017, siendo un grupo temporal significativo.

Résumé - Enquête rétrospective sur les facteurs de risque associés avec des chargements de porcs positifs pour le Senecavirus A dans un abattoir du midwest Américain durant l'été 2017

La présente étude décrit un regroupement spatio-temporel de poussées de cas de Senecavirus A (SVA) rapportées dans un abattoir du midwest Américain durant l'été 2017. Les données furent amassées sur les caractéristiques de sites multiples afin de mener une analyse des facteurs de risque. Le 8 juin 2017, 6 des 10 lots de porcs amenés à l'abattoir testèrent positifs par réaction d'amplification en chaîne par la polymérase avec la transcriptase reverse pour l'ARN de SVA. Subséquentement, 88 lots amenés à l'abattoir ont présenté des lésions vésiculaires, et 74 lots ont testé positif entre le 8 juin et le 10 juillet 2017, ce qui représente un regroupement temporel significatif.

Senecavirus A (SVA) is a virus of the genus *Senecavirus* of the family Picornaviridae. The virus causes vesicular lesions around the snout, mouth, and hooves of pigs,¹ and was first identified in North America in 2002 as a cell culture contaminant.^{2,3} In 2014 and 2015, SVA infection was also associated with outbreaks of neonatal pig mortality in Brazil and in the United States.^{4,7}

Clinical signs associated with SVA include erosions, ulcerations, and vesicular lesions of the snout, oral mucosa, and coronary band of distal limbs. Clinically, SVA may be indistinguishable from foot-and-mouth disease (FMD) and other swine vesicular diseases. Since FMD is designated as a foreign animal disease (FAD) by the US Department of Agriculture (USDA), every clinical case with lesions characteristic of SVA or FMD, including cases recognized at

packing plants, must be investigated to rule out the occurrence of an FAD. According to the USDA's Veterinary Services Guidance Document 7406.3, an FAD investigation must be conducted by state or federal animal health officials.⁸ These investigations take time and resources from state and federal animal health officials and market personnel because pigs and products cannot move until tests confirm the absence of an FAD.

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Currently, there is limited data on the transmission and spread of SVA in the swine industry. This report describes an assessment of spatio-temporal dynamics, as well as an investigation of risk factors associated with a spike in the number of lots of pigs testing positive for Senecavirus A at a midwestern US packing plant during the summer of 2017. The retrospective analysis conducted of all farms that provided animals to the packing plant during the investigation period (April 24, 2017 to August 8, 2017). The investigation, which was completed after the USDA ruled out FMD and confirmed SVA, aimed to describe epidemiological factors associated with the spike in the number of lots of pigs testing positive for Senecavirus A at a packing plant.

Case description

SVA investigation background

On June 8, 2017, 10 lots of finishing pigs were detected with vesicular lesions at a midwestern US packing plant. After the FAD Diagnostic Laboratory (FADDL) ruled out FMD, the lots were tested by reverse transcription-polymerase chain reaction (RT-PCR) for SVA RNA and 6 of the 10 lots were confirmed SVA positive. Between June 9, 2017 and July 10, 2017, 74 lots presenting vesicular lesions at the same plant were confirmed SVA positive by RT-PCR. All cases were negative for FMD based on diagnostic testing at an FADDL. Following the confirmation of SVA-positive cases, an investigation was conducted to describe the cluster of cases and to identify factors that may have contributed to the spread of the virus. The investigation was conducted using data provided by the packing plant. The suppliers of pigs to the plant were not contacted nor were any of the sites from which pigs originated visited.

Case definition

A lot of pigs was defined as all pigs from a single supplier in a truck load, consisting of up to approximately 170 market weight pigs. For the purpose of this study, the SVA status of each lot was used to classify the pig supplier (farm of origin). A case was defined as a pig supplier, which had a lot of pigs test positive for SVA RNA by RT-PCR after arriving at the packing plant. A single truck load with pigs from multiple suppliers would have multiple lots. During the investigation period, all lots that had clinical

signs suggestive of vesicular disease were tested for SVA RNA by RT-PCR unless a previous test on pigs from the same supplier had already tested positive.

Data

During the investigation period (April 24 to August 8, 2017) retrospective information on all suppliers that delivered pigs to the study packing plant were obtained from the plant records. The investigation period was broken into 3 periods based on the data obtained by the packing plant, the pre-outbreak period from April 24 to June 7 (45 days), the outbreak period when SVA-positive cases were reported from June 8 to July 10 (32 days), and the post-outbreak period after the last positive case was reported from July 11 to August 8 (35 days). The supplier code, supplier address, and harvest date were provided for 237 suppliers that delivered lots of pigs to the packing plant during the investigation period. For each lot, the packing plant identified if the pigs were delivered to the packing plant through a buying station and whether the pigs for a single supplier originated from multiple sites. For suppliers with multiple sites, the exact number and address of all the sites was unknown. Therefore, the single address provided for the supplier by the packing plant was used to represent the multiple sites within the same geographic area. The harvest dates of the lots were collected to evaluate spatial-temporal clusters of SVA-associated swine vesicular disease cases during the outbreak. The address of each pig supplier was provided by the packing plant and subjectively assessed using Google Earth maps to verify that it was a swine site and to assess if pigs were raised outdoors (absence of confinement buildings, presence of fences and walls forming outdoor pens, or presence of hoop structures) or indoors (presence of a confinement barn). This assessment was subjectively based on the type of animal housing facilities present in the satellite image.

To describe weather conditions during the investigation period, mean daily measurements were compared against the mean from 30-year historical data on a weekly basis and described as a percentage of the mean historical value. The data for temperature, relative humidity, and rainfall precipitation were collected from a single weather station 25 kilometers north of the packing plant using Iowa Environmental Mesonet.⁹ The mean historical data for

temperature and rainfall at the same weather station was extracted from Weather Underground¹⁰ and for humidity from Current Results.¹¹

Data analysis

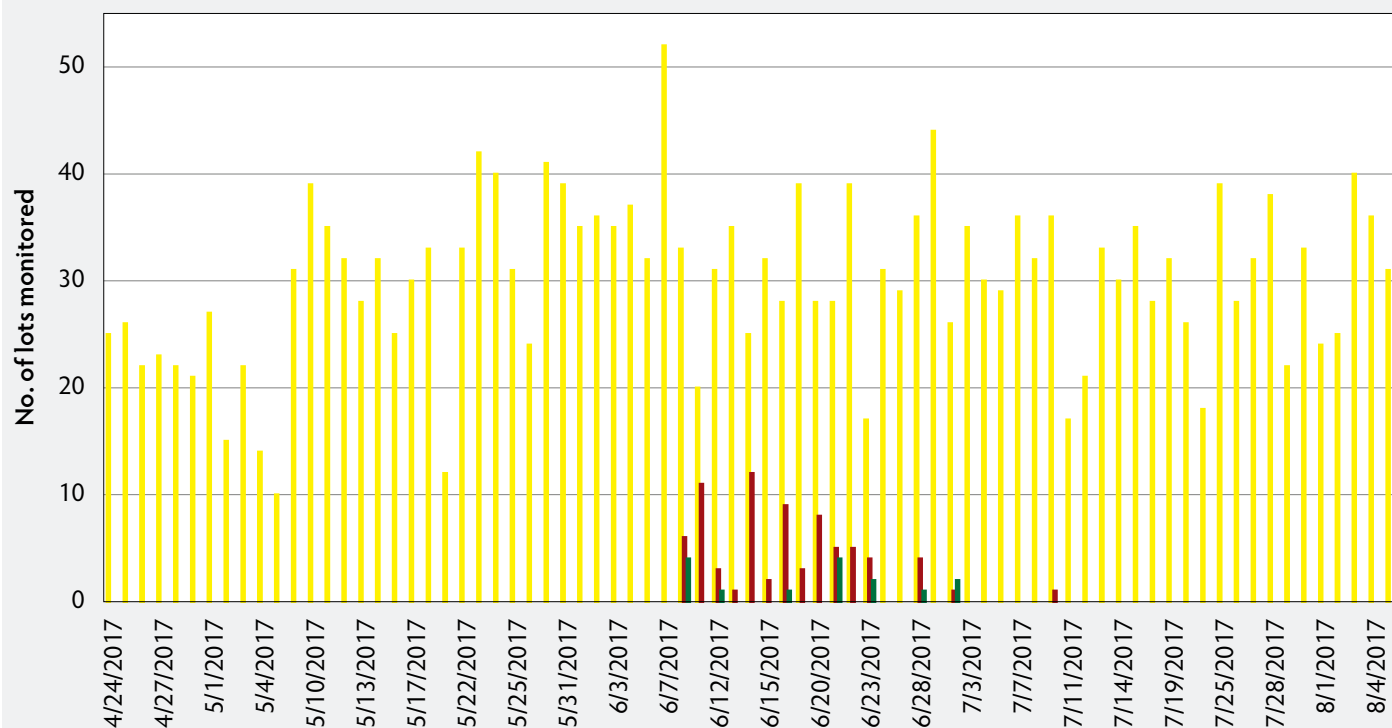
Descriptive analysis was performed on all data collected and odds ratios from univariate logistic regressions were computed to assess risk factors ($P < .05$). The univariate analyses were done using the R software (version 3.3.3; R Core Team) and the R Stats Package. The risk factors were: if the supplier raised pigs outdoors (Yes or No), if the pigs from the supplier were delivered to the packing plant through a buying station (Yes or No), and the supplier location type (multiple or single). Pigs from suppliers with a single location type all originated from a single site. Pigs from suppliers with a multiple location type originated from multiple sites within the same 1-mile geographic area.

Retrospective local space-time clustering was evaluated using the Bernoulli model of the space-time scan statistic, which compares the number of observed cases occurring within all possible cylindrical windows with the expected number of cases falling in that window under the null hypothesis of random distribution of cases. The scan analysis was run assuming a maximum window size set for up to 50% of the population at risk and with a temporal window of 1 day because the outbreak investigation period was short. The spatial-temporal analyses were performed using SaTScan software (Kulldorf and Information Management Services) and the spatialization of the sites were performed in QGIS software (QGIS Development Team). Eligibility criteria to include suppliers in the analyses were: 1) the address listed for the supplier could be located in Google Earth, 2) swine facilities were present at the location, and 3) the supplier had delivered one or more lots of pigs to the study packing plant during the investigation period.

Results and Discussion

Data was obtained from the packing plant for 237 suppliers who sent pigs to the plant during the investigation period (Figure 1). Data on lots of pigs from 44 of the suppliers were excluded from the analysis because the address listed for the supplier could not be located using Google Earth or because the location at the address appeared to lack

Figure 1: Lots of finishing pigs monitored for SVA at a midwestern US packing plant from April 24 to August 8, 2017. Lots tested positive for SVA by RT-PCR (n = 74; red bars), tested negative for SVA by RT-PCR (n = 14; green bars), or not tested (n = 2290; yellow bars) throughout the investigation period. SVA = Senecavirus A; RT-PCR = reverse transcription-polymerase chain reaction.



swine facilities. The remaining 193 suppliers sent 2378 lots of pigs to the packing plant during the investigation period. Of the 193 suppliers, 66 had at least 1 lot of pigs that was tested for SVA by RT-PCR because vesicular lesions were observed at the packing plant, and 61 had lots that were confirmed SVA positive by RT-PCR between June 8, 2017 and July 10, 2017. The onset of the outbreak and all the lots monitored during the investigation period are described in Figure 1. The timing of SVA cases was consistent with a seasonal peak in cases during the summer months.¹²

Of the 193 suppliers, 38 raised pigs outdoors, 22 sent pigs through a buying station, and 60 sent pigs from multiple sites (Table 1). One of the risk factors evaluated was the frequency of SVA-positive pigs from lots going through buying stations compared to those coming directly to the plant from the site where the pigs were raised. However, there was no difference in the odds of testing positive for SVA by RT-PCR between lots of pigs delivered through a buying station and those directly shipped from the site where they were raised. The odds of a supplier that raised pigs outdoors having a lot that

tested positive for SVA was 0.34 (95% CI, 0.12-0.81, $P = .01$), or 66% less compared to suppliers that raised pigs indoors. The odds of suppliers with single type sites having a lot that tested SVA positive was 0.58 (95% CI, 0.34-1.1, $P = .09$), or 42% less compared to suppliers with multiple type sites.

Combined, those 2 'protective' risk factors (outdoor pigs and originating from single sites) may be explained by these suppliers likely being smaller, not part of a larger production system, and having less contact with other sites (eg, shared equipment or trucks). Fewer connections may serve as a protective factor since the frequency of events in a swine farm (eg, frequency of feed delivery and rendering dead pigs) has been shown to be a significant risk factor for disease transmission and spread.^{13,14}

The relative humidity in weeks 1 to 6 was above the mean historical values. In weeks 2, 3, and 6, daily high temperatures were above historical mean values and greater than mean historical rainfall events occurred in weeks 1 and 4 (Table 2). While it is unclear why cases of SVA tend to increase in summer months, one possible hypothesis is that SVA

is transmitted from one herd to another by flying insects. However, vectors are of negligible importance in the epidemiology of the disease.

Joshi et al¹⁵ conducted a diagnostic investigation in 2 SVA-affected herds and detected SVA in environmental samples, mice, and houseflies. The results of this investigation do not challenge that hypothesis since the warm and humid weather conditions before and during the cluster of SVA cases at the packing plant were favorable for flying insects to live and reproduce.¹⁶ Humidity and temperatures remained at or above the 30-year mean during the outbreak and another rainfall event led to above normal rainfall in week 8. Although descriptive, our findings support that weather conditions were favorable for the reproduction of flying insects, which may have contributed to the spread of SVA between sites. However, the finding that pigs raised outdoors was a protective risk factor may contradict that hypothesis since pigs raised outdoors are generally more accessible to flying insects.

Table 1: Risk factors associated with lots testing positive for SVA by RT-PCR at a midwestern US pork plant between April 24 to August 8, 2017

	Lots tested for SVA by RT-PCR				OR (95% CI) [†]	P [‡]
	Negative or not tested, No.*	Negative or not tested, %	Positive, No.	Positive, %		
Pigs raised outdoors						
Yes (n = 38)	32	84.2	6	15.8	0.34 (0.12-0.81)	.01
No (n = 155)	100	64.5	55	35.5		
Buying station						
Yes (n = 22)	17	77.3	5	22.7	0.60 (0.19-1.61)	.34
No (n = 171)	115	67.3	56	32.7		
Supplier location type						
Single site (n = 133)	96	72.2	37	27.8	0.58 (0.34-1.10)	.09
Multiple sites (n = 60)	36	60.0	24	40.0		

* Only lots of pigs showing clinical signs of vesicular disease were tested.

[†] Odds ratio of a lot testing positive for SVA by RT-PCR if the supplier had (yes) the factor evaluated.

[‡] Presence of lots testing positive (yes or no) were compared using logistic regression with a model that included the risk factor (pigs raised outdoors, buying station and supplier location type) as the main effect.

SVA = Senecavirus A; RT-PCR = reverse transcription-polymerase chain reaction; OR = odds ratio.

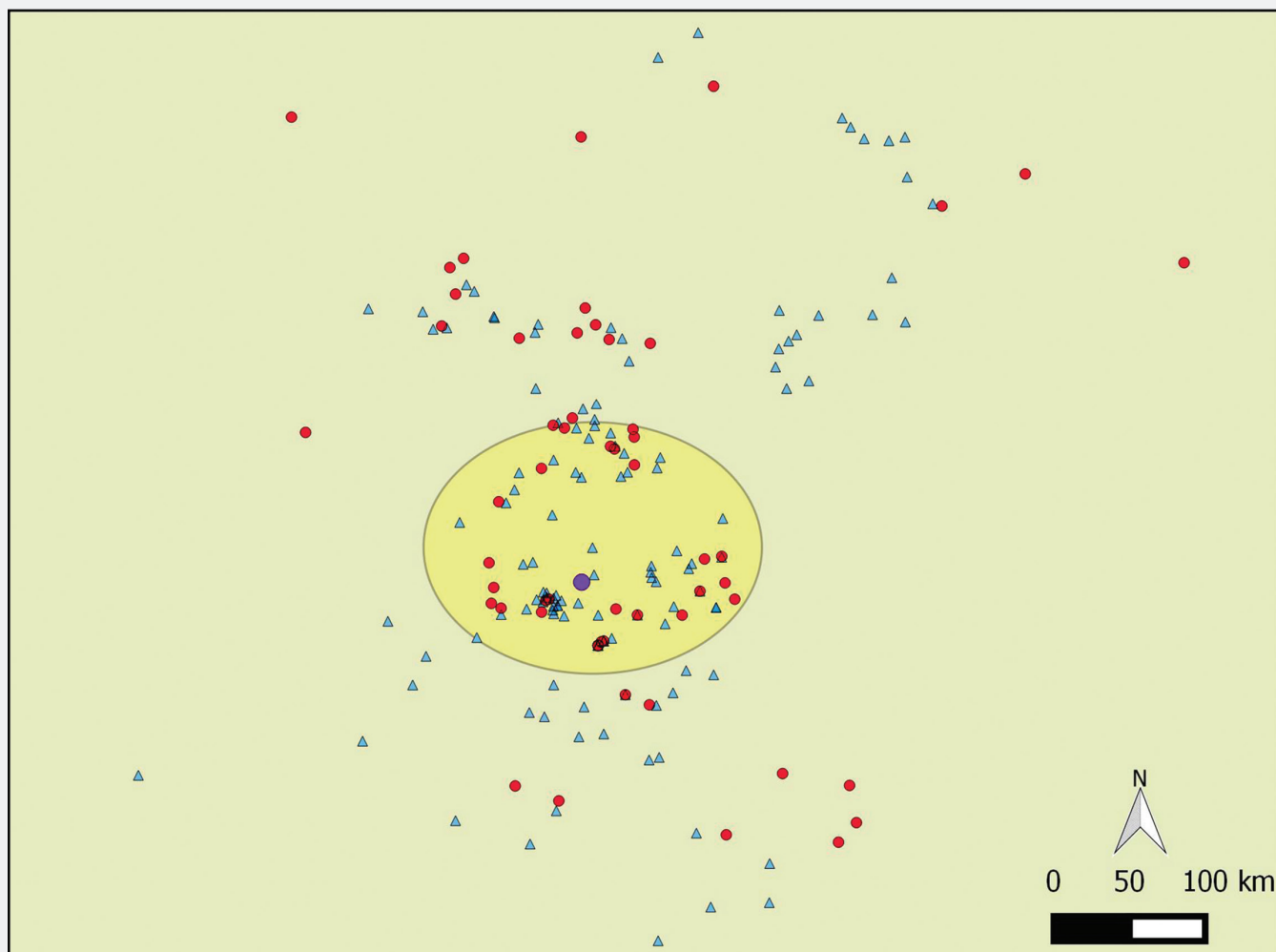
Table 2: Weekly weather data compared to the 30-year mean over the investigation period for humidity, temperature, and rainfall precipitation.

Week	Investigation period*	No. of cases	Humidity high, %		Temperature high, °C		Rainfall precipitation, mm	
			2017	% of historical mean [†]	2017	% of historical mean [†]	2017	% of historical mean [†]
1	4/24-4/30	0	86.6	111	10.0	76	8.3	327
2	5/1-5/7	0	84.4	106	21.7	103	1.2	42
3	5/8-5/14	0	82.6	104	25.0	109	2.1	69
4	5/15-5/21	0	93.0	117	17.9	88	12.3	367
5	5/22-5/28	0	86.0	108	21.7	95	0.1	3
6	5/29-6/4	0	88.7	110	27.9	106	0.0	0
7	6/5-6/11	17	75.3	93	31.5	111	0.0	0
8	6/12-6/18	27	88.3	109	30.3	106	5.7	161
9	6/19-6/25	25	79.4	98	28.3	99	0.0	0
10	6/26-7/2	5	87.9	108	28.5	97	3.3	99
11	7/3-7/9	0	86.9	105	32.1	104	0.0	0
12	7/10-7/16	1	91.6	111	31.9	103	3.1	113
13	7/17-7/23	0	90.1	109	33.5	106	0.1	3
14	7/24-7/30	0	91.6	111	29.8	100	4.5	171
15	7/31-8/6	0	92.6	109	25.1	90	3.3	121
16	8/7-8/9	0	98.5	116	25.8	92	0.0	0

* The investigation period occurred from April 24 to August 8, 2017 and consisted of 3 periods: pre-outbreak (week 1-6); outbreak when Senecavirus A-positive cases were reported (week 7-12); and post outbreak after the last positive case was reported (week 13-16).

[†] Values in bold are above the 30-year mean for that week.

Figure 2: Cluster map showing the location of suppliers during an SVA outbreak between June 8 and June 28, 2017. Red dots represent supplier addresses that had at least one lot of pigs that tested positive for SVA by RT-PCR at the packing plant, blue triangles represent supplier addresses with lots of pigs that tested negative for SVA by RT-PCR at the plant, and the purple dot is the packing plant. The yellow circle is the geographic cluster containing 81 supplier locations, 32 of which had at least one SVA-positive lot of pigs delivered to the packing plant. SVA = Senecavirus A; RT-PCR = reverse transcription-polymerase chain reaction.



One significant cluster in time and space ($P < .001$; Figure 2) was detected with the spatial-temporal analyses. The time frame of the cluster was from June 8 to 28, which was nearly the entire outbreak period, and the cluster covered a region with a radius of 83 km. The packing plant was located about 23 km south of the cluster center (Figure 2). Thirty-two of the 81 sites (39.5%) in this cluster had at least one lot of pigs that tested positive for SVA by RT-PCR. The cluster results confirmed that the number of observed cases in this cluster were over 3 times higher than the number of expected cases, suggesting that the proximity to the packing plant may be associated with a higher than expected incidence of lots

testing positive for SVA. Thus, the presence of the packing plant inside the cluster highlight that it may serve as an indirect contact between the sites since packing plants can act as a potential reservoir of bacterial, viral, prion, and parasitic pathogens capable of infecting animals and fomites.^{17,18}

However, a comprehensive investigation of all possible routes of transmission of the virus was not conducted. Therefore, the role the packing plant played in the spread of the virus can only be speculated. There were other limitations in this outbreak investigation as well. Due to the large number of suppliers involved ($n = 193$),

suppliers were not contacted and site visits were not carried out. Only information provided by the packing plant was used. To validate the geographic location of suppliers provided by the plant, Google Earth images were used to verify the supplier address and subjectively assess the presence and type (indoor or outdoor) of swine facilities. Although the most recent images were used, the possibility of outdated images or errors in characterizing the facilities may have led to some classification bias.

Implications

Under the conditions of this study:

- A cluster of SVA cases occurred at a plant between June 8 and June 28, 2017.
- Pigs with SVA were less likely from single site suppliers or kept outdoors.
- Weather conditions pre-outbreak may have favored insect multiplication.

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Conflict of interest

None reported.

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