Peer reviewed **Case Study**

Outbreak investigations of *Actinobacillus pleuropneumoniae* serotype 15 in central Iowa in the winter of 2021-2022

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Summary

From November 2021 to January 2022, 20 growing-pig sites in central Iowa reported severe clinical respiratory disease and weekly mortality of up to 50%. *Actinobacillus pleuropneumoniae* serotype 15 was identified as the causative agent of the clinical disease. Given that *A pleuropneumoniae* serotype 15 has been infrequently diagnosed in the United States, an outbreak investigation was conducted to explore biosecurity hazards and epidemiological aspects associated with the surge of cases. The investigations revealed that all farms had significant gaps in biosecurity and relied heavily on third-party service providers, which may have contributed to the pathogen spread.

Keywords: swine, *Actinobacillus pleuropneumoniae*, outbreak investigation, biosecurity hazard analysis

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Resumen - Investigaciones de brotes de *Actinobacillus pleuropneumoniae* serotipo 15 en el centro de Iowa en el invierno de 2021-2022

Desde noviembre de 2021 hasta enero de 2022, 20 sitios de engorda de cerdos en el centro de Iowa reportaron problemas respiratorios clínicos graves, y una mortalidad semanal de hasta el 50%. Se identificó al *Actinobacillus pleuropneumoniae* serotipo 15 como el agente causal de la enfermedad clínica. Dado que el *A pleuropneumoniae* serotipo 15 ha sido diagnosticado con poca frecuencia en los Estados Unidos, se llevó a cabo una investigación del brote para indagar los peligros de bioseguridad y los aspectos epidemiológicos asociados con el aumento de casos. Las investigaciones revelaron que todas las granjas tenían deficiencias importantes en la bioseguridad, y que dependían en gran medida de proveedores de servicios externos, lo que puedo haber contribuido a la propagación del patógeno.

Résumé - Enquêtes sur des poussées de cas causées par *Actinobacillus pleuropneumoniae* sérotype 15 en Iowa durant l'hiver 2021-2022

De novembre 2021 à janvier 2022, 20 sites de porcs en croissance du centre de l'Iowa ont rapporté des cas cliniques sévères de maladie respiratoire et de la mortalité hebdomadaire allant jusqu'à 50%. *Actinobacillus pleuropneumoniae* sérotype 15 a été identifié comme étant l'agent étiologique de ces cas. Étant donné qu'*A pleuropneumoniae* sérotype 15 n'a été diagnostiqué que peu fréquemment aux États-Unis, une enquête a été menée afin d'examiner les risques en biosécurité et les aspects épidémiologiques associés avec cette poussée de cas. Les enquêtes ont révélé que toutes les fermes présentaient des déficiences en lien avec la biosécurité et se fiaient beaucoup sur les services de tiers parties, ce qui pourrait avoir contribué à la dissémination de l'agent pathogène.

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PT: Iowa Select Farms, Iowa Falls, Iowa.

LJ: New Fashion Pork, Jackson, Minnesota.

LF: Protein Sources, Mapleton, Minnesota.

LG: TriOak Foods, Oakville, Iowa.

DB: Cactus Family Farms, Osceola, Iowa.

TB: The Maschhoffs, Carlyle, Illinois.

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Actinobacillus pleuropneumoniae is the etiologic agent of porcine pleuropneumonia, distributed worldwide in pig-producing countries, and causes economic losses and negative impacts on pig health due to exacerbated respiratory clinical disease and mortality.[1,2] This gram-negative bacterium is classified into biotypes I or II based on the nicotinamide adenine dinucleotide requirement for growth.[3] *Actinobacillus pleuropneumoniae* serotype 15, classified as biotype I, was described first in Australia, where it is the predominant serotype.[4] Although less frequent, *A pleuropneumoniae* serotype 15 has also been reported in North America, South America, and Japan.[5-7]

The introduction of *A pleuropneumoniae* into a swine herd typically occurs through direct transmission, mainly when introducing a carrier animal into the herd.[8] The carrier animal acts as a reservoir for the pathogen and can transmit it through direct nose-to-nose contact with susceptible animals. Indirect routes of *A pleuropneumoniae* transmission are uncommon.[9] Reports suggest that aerosol transmission over short distances (up to 2.5 m) can occur.[10-12] *Actinobacillus pleuropneumoniae* has a relatively short environmental survival time, particularly under dry and warm conditions. However, it can survive on frozen surfaces up to -20°C for over 17 weeks,[13] suggesting possible long-term survival in frozen pig carcasses and other frozen items, thus indicating the possibility of indirect transmission.

The incubation period for *A pleuropneumoniae* and the appearance of clinical signs can be extremely variable. In experimental studies with naive pigs, clinical signs, such as fever, dyspnea, and cough, were reported 1 to 10 days post *A pleuropneumoniae* inoculation.[14,15] Gross pathologic findings in the lungs, such as consolidation and hemorrhagic areas, can be found up to 24 hours after endotracheal or intranasal inoculation with *A pleuropneumoniae*.[16] Although antibiotic therapy has been used to treat porcine pleuropneumonia, animals that survive the acute phase often remain carriers for several months, mostly with the *A pleuropneumoniae* detected or isolated in the lungs and tonsils.[17-19]

Since 2010, *A pleuropneumoniae* serotype 15 has occasionally been diagnosed at the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL). From 2010 to 2020, *A pleuropneumoniae* serotype 15 was identified in affected lungs in 31 cases, and in 11 cases from January to October 2021. However, within a 60-day period from November 2021 to January 2022, an outbreak of 20 *A pleuropneumoniae* serotype 15 cases were diagnosed from grow-finish sites with weekly mortality of up to 50% of the pigs placed in the group. The cases from November 2021 to January 2022 were unusual from an epidemiological perspective since they originated from 9 unrelated production companies that submitted tissues for diagnostic workup from sites in a small geographic area (30-km radius) in central Iowa. Notably, no case reports outside of this area were observed in the ISU VDL during the same period. Therefore, an outbreak investigation was conducted to explore biosecurity hazards and epidemiological aspects associated with the surge of *A pleuropneumoniae* serotype 15 cases in central Iowa.

Animal care and use

An animal care protocol was not necessary as all samples used in this study were derived from routine diagnostic submissions to the ISU VDL. Animals involved in this case report were under the supervision of the herd veterinarian and were cared for in accordance with the Pork Quality Assurance Plus program.

Case description

Case definition

The case definition was applied to the site level. The start date of each case and age of affected pigs were determined by the first diagnostic submission to the ISU VDL or by the date on which clinical signs were first observed with subsequent diagnostic confirmation of *A pleuropneumoniae* serotype 15 as the etiology.

Thus, cases were eligible for this study if they met criteria based on clinical history, pathological findings (gross and microscopic evaluation), and diagnostic results. The clinical history provided by the herd veterinarians through the investigations included high acute mortality with or without the presence of respiratory clinical signs, such as dyspnea or respiratory distress. Macroscopic pathological findings were characterized by areas of firm to friable, dark red to black lung consolidation, and histologically by necrosis and hemorrhage rimmed by viable and degenerating neutrophils, often with streaming nuclei. Finally, diagnostic testing confirmed the growth of *A pleuropneumoniae* from affected lungs with subsequent gel-based serotyping polymerase chain reaction (PCR) confirming serotype 15.

Data collection

A total of 20 grow-finish sites from 9 production systems were classified as cases and included in the descriptive and spatial-temporal analyses. Epidemiological information for all 20 cases was collected from the herd veterinarian of each of the 9 companies. The information included the production stage, age of animals at the onset of the case, the number of pigs placed, mortality, diagnostic test results, the method used for carcass removal, and address for spatial analysis. Additionally, maps of the surrounding area of each case site were obtained using satellite images from Google Earth to evaluate the number of other swine sites within a 1.6 to 8 km radius and the number of public roads near each site. Moreover, the detection of *A pleuropneumoniae* serotype 15 by the ISU VDL in previous cases dating back to 2010 for the 9 companies was tracked as part of the investigation to assess any potential recurrences within the company.

Epidemiological investigation and biosecurity hazard analysis

Of the 20 cases that occurred in the 60-day period (November 2021 to January 2022), 7 cases, each from a different production system, agreed to share information through an intensive epidemiological investigation and biosecurity hazard analysis. Two companies declined to participate. In addition, information on clinical signs, mortality, treatment, unusual weather events and power outages, and characteristics of the herd, site, and area surrounding the site was collected. The investigation was conducted retrospectively as an epidemiological investigation and biosecurity hazard analysis of the production processes. The biosecurity hazard analysis consisted of a detailed assessment of the production processes and biosecurity control measures for each case. Information on circumstances and actions (or inactions) likely to result in failures that may have led to the introduction of *A pleuropneumoniae* serotype 15 into the herd was evaluated for each case.

An investigation form (available upon request from the corresponding author) was used to conduct the investigation interviews and report the results.[20] The

main objectives of the intensive investigations were to evaluate the biosecurity hazards and epidemiological aspects of the cases, explore potential routes of *A pleuropneumoniae* serotype 15 transmission, and comprehensively evaluate potential entry events, including swine movements, deliveries, people movements, and others that occurred during the investigation period. Briefly, the investigation form was organized by each type of epidemiological information and entry events. Entry events were defined by the entry of one or more pathogen-carrying agent(s) into the perimeter buffer area of the site. Pathogen-carrying agents were defined as any agent that 1) can be infected or contaminated with a pathogen and 2) carry the pathogen from one herd to another. Examples of pathogen-carrying agents include pigs, people, and livestock trailers. Based on *A pleuropneumoniae* transmission characteristics, incubation period, and severity of the disease, a 14-day investigation period was used for each investigation ending on the date clinical signs were first recognized or when a laboratory diagnostic result confirmed *A pleuropneumoniae* serotype 15. Only entry events that occurred during the investigation period were evaluated.

To identify the biosecurity hazards, the 3 failures concept was employed, which states that 3 failures must have occurred for *A pleuropneumoniae* serotype 15 to have been transmitted to a herd.[20] The 3 failures were 1) failure to prevent the infection or contamination of the pathogen-carrying agent with *A pleuropneumoniae* serotype 15, 2) failure to mitigate the contamination or infection of the pathogen-carrying agent, and 3) failure to prevent pigs in the herd from being infected with an infectious dose of the pathogen from the pathogen-carrying agent.

The intensive investigation interviews were conducted as an open-ended discussion of the production processes connected with each entry event as guided by the investigation form. The investigations were conducted from February to May 2022 by veterinarians from Iowa State University in collaboration with the herd veterinarians, site caretakers, production management, and members of the Swine Health Information Center's Rapid Response Team (RRT). The RRT consists of a nationwide network of veterinarians prepared and committed to moving within 24 hours notice to conduct epidemiological investigations.[21]

Data analysis

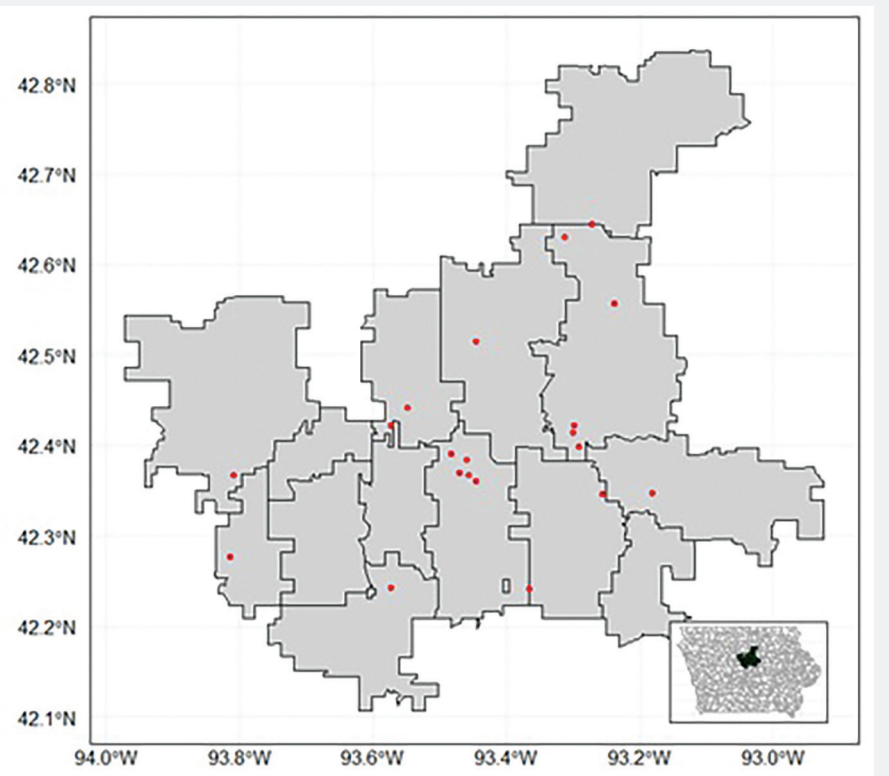
A descriptive analysis of the information collected during the investigation was done. Thereafter, a spatial-temporal statistical model was performed to evaluate the interaction between the distance and time of each *A pleuropneumoniae* serotype 15 case (n = 20). A space-time interaction K function (R package *splancs*) was used with geographic locations of sites (longitude and latitude, projection in datum NAD83) and time (case start and end dates).[22,23] For the case start dates, day 1 was defined by the herd veterinarian based on the observation of the first clinical signs or laboratory diagnostic tests. Although *A pleuropneumoniae* shedding is highly variable,[24] a recent study from our group (unpublished data) demonstrated that it was possible to detect *A pleuropneumoniae* by PCR from nasal swabs and tonsil scrapings up to 10 weeks after clinical signs were first observed. Thus, the lesser of 10 weeks or the time until the sites were emptied was used for the spatial-temporal statistical model. An area using Iowa postal zip codes that included at least one *A pleuropneumoniae* serotype 15 case was used to create a polygon delimiting the outbreak area (Figure 1). Analyses were performed in R program version 4.2.1 (R Core Team, 2022) with a $P \leq .05$ to establish statistical significance.

Discussion

The production phases of the 20 cases included wean-to-finish (n = 5) and finishing only (n = 15) sites where pigs were being raised for meat production. None of the cases occurred on gilt development, acclimation, or isolation sites. Pig ages ranged from 8 to 30 weeks of age at the onset of clinical signs with most cases occurring between 22 to 25 weeks of age (n = 7).

Typically, *A pleuropneumoniae* is directly transmitted by introducing a carrier animal into the herd.[8] Previous detections of *A pleuropneumoniae* serotype 15 by the ISU VDL were assessed to determine potential recurrences within a company. Five of the 9 companies involved had no previous detections of *A pleuropneumoniae* serotype 15. However, 4 companies did have previous detections: one company had 3 diagnoses in 2011, 2016, and 2020; another company had 2 diagnoses in 2021 through June; one company had a single diagnosis in 2017; and one company had 5 diagnoses in 2021 through September. Notably, 3 of the 4 companies that experienced recurrences had multiple cases in 2021-2022, while companies with no previous detections experienced a single case.

Figure 1: Polygon delimiting the outbreak area in the state of Iowa.



The magnitude of death loss varied among the 20 cases with a mean cumulative mortality of 25% (range, 2%-56%) and a mean single-week mortality peak of 17% (range, 1%-50%). Eleven of the 20 cases also had a porcine reproductive and respiratory syndrome virus (PRRSV) RNA-positive PCR result, one had an influenza A virus RNA-positive PCR result, and one had a *Mycoplasma hyopneumoniae* DNA-positive PCR result. The diagnosticians reported evidence of pneumonia consistent with PRRSV associated with *A pleuropneumoniae* serotype 15 lesions in 3 cases. Clinical signs of *A pleuropneumoniae* can be worsened when associated with other respiratory agents, such as *M hyopneumoniae*.[25]

Of the 20 growing-pig sites with cases of *A pleuropneumoniae* serotype 15 over the 60-day outbreak, 18 used the same third-party rendering service for carcass removal. Carcass removal from the sites followed specific routes within a rendering collection area (Figure 2). Rendering trucks and drivers were, with rare exceptions, dedicated to the rendering collection area. The carcasses were collected by trucks within the rendering collection area and were taken to a single collection point. After unloading the carcasses at the collection point, they were transferred to larger trailers for transport to the rendering plant located outside of the rendering collection area. All 20 cases were within the rendering collection area marked as a red square in Figure 2. The routes followed by the rendering trucks within the rendering collection area were unknown to the herd veterinarians, and the rendering company declined to provide more information on the timing of pickups and routes followed. In most cases, the carcass storage and rendering pickup locations were at the site's entrance. For all the cases, if the area around the carcass storage was contaminated with *A pleuropneumoniae* serotype 15 during pickup, the caretaker biosecurity procedures were insufficient to prevent the bacteria from being transmitted from the carcass storage area to the pigs in the barns.

The spatial-temporal analysis revealed a significant interaction between space and time of *A pleuropneumoniae* serotype 15 cases ($P < .01$). For the spatial-temporal analysis, the index case (a finishing site undergoing a case on November 25, 2021) was located at 0 km and 0 day in Figure 3. It was estimated that cases progressively occurred at all distances after 10 days of the first case (blue shade in Figure 3). The highest intensity of spatial-temporal interaction (red shade in Figure 3) occurred between 20 and 40 days after the index case (December 15, 2021 to January 4, 2022) and between 20 km and 40 km from the index case.

The descriptive results of the epidemiological investigations and biosecurity hazard analysis completed for 7 cases are shown in Table 1. The mean number of animals in the facilities was 4056 (range, 2445-5691 pigs). The total number of sources of pigs placed were either 1 (n = 4) or 2 (n = 3). Four cases were finishing only and 3 were wean-to-finish sites. The number of weeks after the pigs were placed on feed at the onset of each case ranged from 5 to 27 weeks. The mean death loss between these 7 cases was 18% (range, 2.2%-38.9%). The most common clinical signs reported were sudden death and high mortality, dyspnea, lethargy, anorexia, cough, and cutaneous hyperemia. Except for cases where pigs were actively being marketed (n = 3, range 3-8 loads prior to the case start date), the pigs were medicated with injectable antimicrobials (ceftiofur, oxytetracycline, and enrofloxacin) or orally administered antimicrobials (tiamulin, amoxicillin, and tilmicosin) after the onset of clinical signs.

In 2 of the 7 cases, not all barns on the site were affected. Clinical signs were observed in 1 of the 4 barns on the site in one case and 2 of the 3 barns on the site in the second case. In those 2 cases, the pigs remained on the site for 3 to 7 weeks after clinical signs were first observed, and it was reported that caretakers

Figure 2: The blue pin indicates the rendering collection point. The red square indicates the approximate rendering collection area: 30 km to the north, 130 km to the west, 70 km to the south, and 95 km to the east of the rendering collection point. The red pins indicate the locations of the *Actinobacillus pleuropneumoniae* serotype 15 outbreak cases.

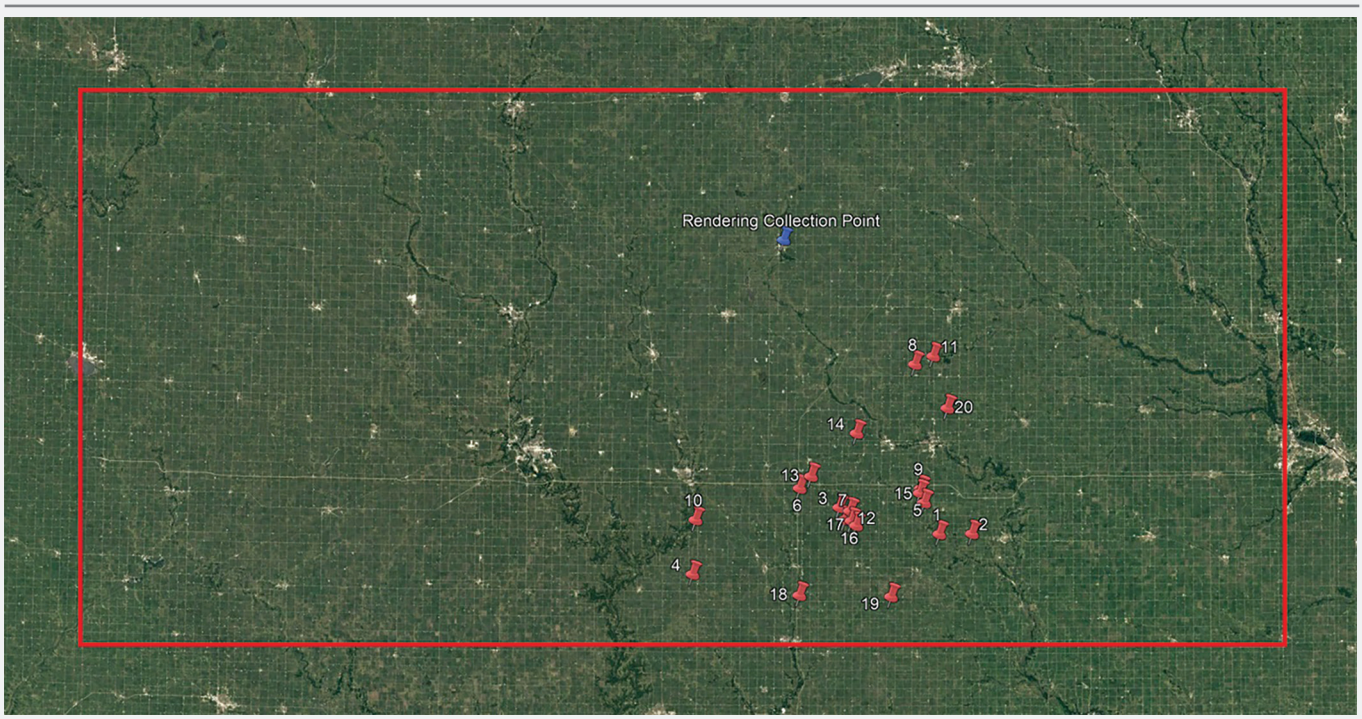


Figure 3: Contour plot showing the spatial-temporal clustering of *Actinobacillus pleuropneumoniae* serotype 15 cases in the outbreak. The cluster intensity from lowest (light blue) to highest (dark red) represents the correlation coefficient between the cases in terms of space and time ($P < .01$). The white area in the top right corner represents distance and dates with no significant correlation between cases ($P > .05$).

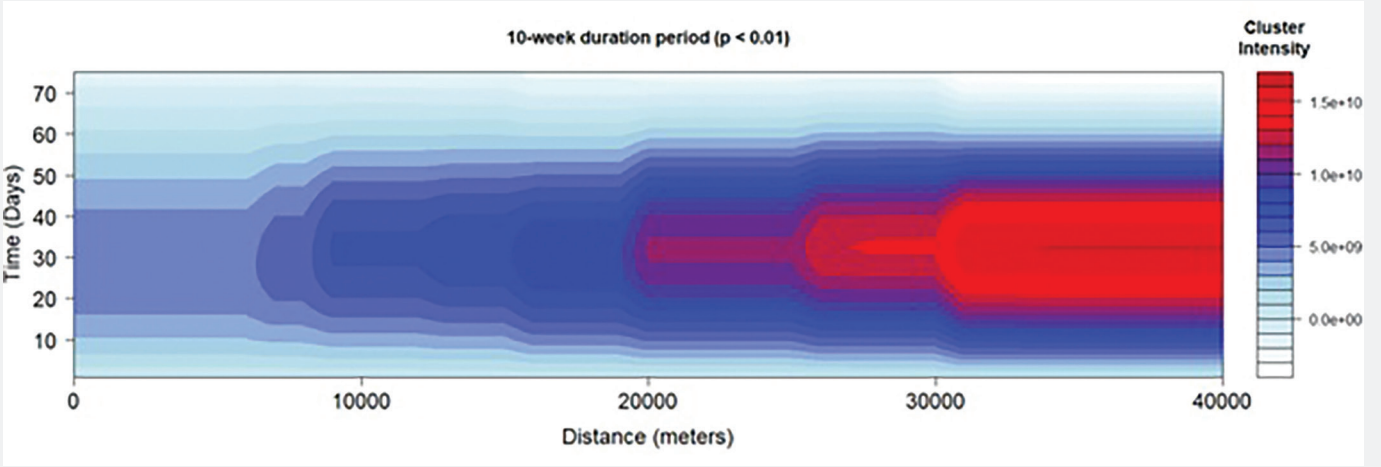


Table 1: *Actinobacillus pleuropneumoniae* serotype 15 outbreak information on 7 sites from 7 companies

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Outbreak start date	12/12/2021	12/16/2021	12/24/2021	12/30/2021	1/8/2022	1/9/2022	1/14/2022
No. animals placed	4800	3950	5691	4000	5057	2445	2451
No. animal sources	2	1	1	2	2	1	1
Production phase	Finisher	Finisher	Finisher	W-F	W-F	W-F	Finisher
Time of outbreak, wk post placement	8	17	12	24	5	27	10
Outbreak mortality, No. (%)	105 (2.2)	700 (18.3)	46 (2.8)	781 (19.8)	704 (14.3)	175 (30.0)	873 (38.9)
No. barns on site	4	4	2	3	2	2	2
No. affected barns	1	4	2	2	2	2	2
Stress events*	Yes	Yes	Yes	No	No	No	Yes
Market time†	No	No	Yes	Yes	No	Yes	No
History of *A pleuropneumoniae* serotype 15‡	Yes	No	No	No	Yes	No	Yes
Carcass removal method	Rendering	Compost	Rendering	Rendering	Rendering	Rendering	Compost

* Stress events included power outages, curtains failing to drop, water outages, and movement of pigs between barns.

† Market time means one or more loads of pigs had been marketed on or before the case start date.

‡ Diagnostic history from 2020 to 2021 was reviewed to identify previous detections of *A pleuropneumoniae* at the site.

W-F = wean-to-finish.

changed boots between barns and wore disposable gloves. No pigs were moved between the barns. This finding suggests that *A pleuropneumoniae* serotype 15 might not easily be indirectly transmitted on fomites and that biosecurity practices, such as changing boots between barns, may reduce the likelihood of transmitting *A pleuropneumoniae* to animals from different barns.[11,13]

Stress events are a potential cofactor in the pathogenesis of infectious diseases.[26,27] Stress events including power outages, curtains failing to drop, water outages, and movement of pigs between barns during the marketing phase were reported in 4 of the 7 cases up to 14 days before the case. Also, a notable weather event occurred on December 15, 2021. A derecho spawned tornadoes with wind speed up to 173 km/hr and moved quickly from the southwest to the northeast sections of Iowa according to the National Weather Service Forecast Office in Des Moines.[28] The herd veterinarian in one case reported that a power and water outage occurred 1 day after the derecho. These stress events could have exacerbated the magnitude of the outbreak.

The characteristics of the surrounding area were similar in the 7 cases. All cases occurred in pig-dense areas with an average of 6 known finisher sites within a 5-km radius and the nearest public road within 0.4 km from the case sites. Also, 6 of 7 cases were over 11 km from a collection point or a slaughter plant. All cases were in flat topography with none having full windbreaks. All used water from private wells with no water treatments.

Among the 7 cases, 3 were marketing pigs to multiple packing plants in Minnesota and in nearly every region of Iowa, all outside of the area where the outbreak occurred. The small geographic area where the outbreak occurred suggests that marketing events likely did not play a significant role in the transmission of *A pleuropneumoniae* serotype 15 from one herd to another. However, some very significant biosecurity hazards related to the transport of market pigs were discovered. Transport was conducted primarily by third-party contractors. The requirements for washing and decontaminating trailers between loads varied and were frequently absent. Oversight of the third-party contractors to verify that trailer washing and decontamination requirements were being met was absent. When washing and decontamination were required, downtime to allow the trailers to dry or thermal-assisted drying was absent.

Regarding movement of people, animal caretakers also cared for other finisher sites (up to 12 sites per person), with caretakers entering sites 1 to 3 times per day. The labor was contracted in 2 of the 7 sites. In one case, a caretaker also worked for a different swine production company, while the other 6 cases had caretakers visiting other growing-pig sites within the same company. In all cases, no other sites on their daily routes were known to be positive for *A pleuropneumoniae* serotype 15. However, from 2020 to 2021, there were reported cases of *A pleuropneumoniae* serotype 15 in 3 of the 7 companies. In 5 cases, off-farm employees, such as maintenance, visitors, and load crews, entered the barns during the investigation period. Six companies frequently relied on contracted third-party labor for loading pigs, maintenance, and vaccinations. Downtime for caretakers, off-farm employees, and other visitors before entering the sites was not required for any of the cases. Knowledge of how third-party contractors operated and their activities was lacking.

The investigation exposed other gaps in grow-finish biosecurity. Six cases had only one vehicle entrance, and none had a vehicle wash and disinfection area. Five cases received feed deliveries during the outbreak investigation period, all from different feed mills. One case had a propane delivery during the outbreak investigation period, 2 reported garbage collection, and 1 reported introducing tools and supplies from another pig site. The barn and office doors at the site of 4 cases were required to be closed and locked, the other 3 cases did not have this requirement. For 6 cases, personal items could enter the office and barns and required no decontamination procedures. Shower-in-shower-out of the site was optional in 5 cases, and a clear line of separation defining clean and dirty sides with a bench was in place in only 2 cases. If properly designed and used, a shower-in-shower-out structure with a bench system can significantly reduce the risk of introducing pathogens into the swine barn.[29]

After conducting intensive investigations, herd veterinarians from 6 of the participating production companies attended a meeting in April 2022 at Iowa State University. The primary objective of the meeting was to facilitate sharing of experiences and discuss pertinent information discovered during the investigations. It became evident during this meeting that companies shared connections among sites from different systems (labor) and poor biosecurity procedures were related to the lack of biosecurity auditing. Participants reported implementing various biosecurity control measures that may have mitigated further spread of *A pleuropneumoniae* serotype 15 among sites and companies. This meeting highlighted the indispensable role of effective and active communication among veterinarians and companies, which likely was essential to effectively respond to this emerging health challenge.

Cases of *A pleuropneumoniae* serotype 15 were clustered in time and space in a swine-dense area of Iowa and within a common rendering route. The outbreak investigations revealed a heavy reliance on contracted services, such as rendering and load-out crews, that created a potential for operational connections between production systems and exposing the facilities to biosecurity risks. However, one limitation of this study was that a case-control design was not employed. This limited our ability to gather information on risk factors and identify gaps in biosecurity among farms without *A pleuropneumoniae* serotype 15 cases in the same region during the same period. Consequently, this study provides valuable insights and speculative analysis regarding potential causes or risk factors associated with the observed cases. Moreover, the findings revealed a general need for more knowledge and compliance monitoring of biosecurity control measures on growing-pig sites. These gaps in grow-finish biosecurity can expose US swine production to emerging, re-emerging, and foreign animal diseases, such as the African swine fever virus.

Implications

The findings of this study suggest:

- Focus on carcass removal biosecurity must improve to prevent further outbreaks.
- Lack of biosecurity audits led to gaps that may facilitate pathogen introduction.
- The outbreak investigation facilitated a rapid response by veterinarians.

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Conflict of interest

None reported.

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