



## Active surveillance for Aujeszky's disease and biosecurity aspects of swine farms in Espírito Santo State, Brazil

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**ABSTRACT:** Aujeszky's Disease (AD) is a highly infectious swine disease caused by Suid herpesvirus 1 (SuHV 1). The present study evaluated the biosafety of commercial swine farms and carried out the first serological survey for Aujeszky's Disease in Espírito Santo State, Brazil. A total of 55 farms in 27 municipalities of the state were selected, where 416 swine serum samples were collected to be submitted to serological diagnosis for AD. Finally, a survey was carried out using a questionnaire to obtain information on the biosecurity of the farms. The results by the ELISA technique indicated the absence of antibodies for AD. Regarding the biosecurity of the farms, 56.4% of the farms did not meet the requirement of internal isolation, 67.3% did not have changing rooms, 72.7% did not provide specific clothing for employees and visitors, 85.5% did not require visitors bathe to access the sheds and only 10.9% of the farms controlled the flow of visitors and vehicles accessing the property. Another noteworthy factor is that only 25.5% of the farms had an area to quarantine replacement animals and 69.1% had an access ramp located close to the animal pens. With the results, it was concluded that there is a need to improve biosecurity measures on commercial farms in the state of Espírito Santo and that there was no presence of Aujeszky's Disease in the evaluated animals.

**Key words:** Aujeszky, pseudorabies, swine, serology, biosecurity, surveillance.

## Vigilância ativa para Doença de Aujeszky e aspectos sobre a biosseguridade de granjas suínas no Estado do Espírito Santo, Brasil

**RESUMO:** A Doença de Aujeszky (DA) é uma enfermidade de suínos altamente infecciosa, causada pelo herpesvírus suíno 1 (SuHV 1). O presente estudo objetivou avaliar a biosseguridade das granjas comerciais capixabas de suínos e realizar o primeiro inquérito sorológico para Doença de Aujeszky no Estado do Espírito Santo, Brasil. Foram selecionadas 55 granjas em 27 municípios do Estado, onde se coletou 416 amostras de soro de suínos para serem submetidos ao diagnóstico sorológico para DA. Por fim, foi realizado inquérito, mediante questionário para obter informações sobre a biosseguridade das granjas. Os resultados pela técnica de ELISA indicaram ausência de anticorpos para DA. Em relação à biosseguridade das granjas, 56,4% das granjas não atenderam o requisito de cerca de isolamento interno, 67,3% não possuíam vestiários, 72,7% não disponibilizavam roupas específicas para funcionários e visitantes, 85,5% não exigiam a prática de banho dos visitantes para acesso aos galpões e somente 10,9% das granjas faziam o controle do fluxo de visitantes e veículos que acessam a propriedade. Outro fator destacado é que apenas 25,5% das granjas apresentavam área para realizar quarentena dos animais de reposição e 69,1% possuíam o embarcador localizado próximo às baias dos animais. Com os resultados, concluiu-se que há necessidade de melhoria das medidas de biosseguridade nas granjas comerciais no Estado do Espírito Santo e que não houve presença da Doença de Aujeszky nos animais avaliados.

**Palavras-chave:** Aujeszky, pseudorabia, suínos, sorologia, biosseguridade, vigilância.

## INTRODUCTION

Pig farming plays an important role in Brazil's economy. Currently, the country ranks fourth in the ranking of pork production and export. With approximately 4.701 million tons produced. In 2022, 1.137 million tons were exported, destined for 86 countries. In addition, 76% of pork production is destined for the domestic market, where per capita consumption

is 16.7 kg (ABPA, 2022). In Espírito Santo, 303,255 pigs were officially slaughtered in 2022, producing a total of 25,000 tons of products that were destined for the supply of the state itself (BRASIL, 2022).

Aujeszky's Disease (AD) is an infectious-contagious disease caused by Suid herpesvirus 1 (SuHV 1), or Aujeszky's disease virus (VDA), which mainly affects pigs, animals of the genus *Sus scrofa domesticus* and *Sus scrofa scrofa* (European wild

boar). The disease causes several economic losses due to high perinatal mortality, abortion, repeated estrus, infertility, temporary weight loss after weaning and mortality of piglets that can reach 66% within 72 hours after birth (KRZYANIAK et al., 2002).

The main characteristic of VDA is the ability to establish latency in ganglia of the peripheral nervous system of pigs (ROMERO et al., 2003). Due to the persistence of the virus it is necessary to adopt control measures on the farms, such as isolation of the sheds, quarantine period for the entry of new pigs and adoption of hygiene protocols for visitors (MORES et al., 2007). Such biosecurity measures aim to prevent the introduction and spread of infectious agents in herds, also contributing to the reduction of financial losses resulting from possible outbreaks (SILVA et al., 2019).

The objective of this study was to assess the biosecurity level of commercial pig farms installed in Espírito Santo State, identifying the critical points and their protection capacity. And also carry out the first serological survey for Aujeszky's Disease in the commercial pig herd of the State.

## MATERIALS AND METHODS

### *Sample selection*

An initial survey of the existing registry of commercial swine farms in the Agricultural Integration System of the Animal and Forestry Defense Institute of the State of Espírito Santo (IDAF) was conducted. After obtaining the owners' consent, 55 farms were selected (48 complete-cycle farms and 7 finishing farms) spread across 27 municipalities in the state of Espírito Santo, representing 72.4% of the total number of registered commercial farms.

To calculate the minimum representative sample size, the total estimated population of 241.550 pigs was used as a reference, according to data from IBGE (BRASIL, 2022). Using an estimated prevalence of 50%, a confidence interval of 95%, and a maximum error of 5%, statistical analysis using the Epi Info 7 program indicated that blood samples would be collected from 384 animals as the minimum representative sample size. Finally, a total of 416 swine serum samples were collected.

Due to differences in the herd size across farms, ranging from 17 to 2500 sows in the herds (with an average of 155 sows housed), proportional stratified sampling was performed, and samples were collected from 3–10 pigs per farm. The inclusion criteria for sample collection were cull sows, sows in the mid-gestation stage, and sows in the final stage of maternity. In the case of finishing farms, pigs ready for slaughter

were selected. Underweight animals and animals under any medication were excluded from the study.

### *Sample collection*

Field collections were conducted between April 2021 and March 2022. Each animal was restrained, and blood was collected by venipuncture from the right jugular fossa using a disposable needle. After clot formation, the tubes were centrifuged at 3,000 rpm (604 xg) for 10 min, and the serum was transferred to sterile 2.0-mL microfuge tubes, labeled, and stored in an ultra-low temperature freezer (-80°C) until further laboratory testing.

### *Laboratory tests*

Samples were processed at the Gerência de Diagnóstico Laboratorial of IDAF (GEDLAB) for serological diagnosis of Aujeszky's disease using a commercial blocking ELISA kit (IdexxSUHV1/ADV gl®, IDEXX Laboratories, Westbrook, ME, USA), which detects the presence of anti-gpl antibodies, indicating exposure to field strains or vaccines containing the gpl antigen. The analysis was performed according to the manufacturer's instructions. The ELISA test is one of the methods recommended by the Normative Instruction No.08 of 2007 for diagnosing Aujeszky's Disease in swine in Brazil (BRASIL, 2007).

### *Biosecurity data collection*

During farm visits, observations were made regarding the physical structure and sanitary management related to the implemented measures. For this survey, a pre-established questionnaire was used, based on the Normative Instructions 19/2002 and 44/2017 (BRASIL, 2002; BRASIL, 2017); and the questionnaire was designed by SILVA et al. (2019), covering issues such as presence of internal isolation fences, feed mill supply, precautions for potential visitors, and an all-in all-out (AIAO) management.

### *Farm risk classification*

A scoring system was established for each item in the questionnaire, following the same pattern as that of IN 19/2002 (BRASIL, 2002), but with addition of other variables on biosecurity, as mentioned in table 1. Finally, farms were classified as follows: farms with high vulnerability, farms with moderate vulnerability, farms with low vulnerability, and well-protected farms.

## RESULTS

Of the 416 animal samples subjected to serological diagnosis, antibodies for SuHV1

Table 1 - Frequency of biosecurity measures adopted in commercial pig farms in Espírito Santo (n = 55).

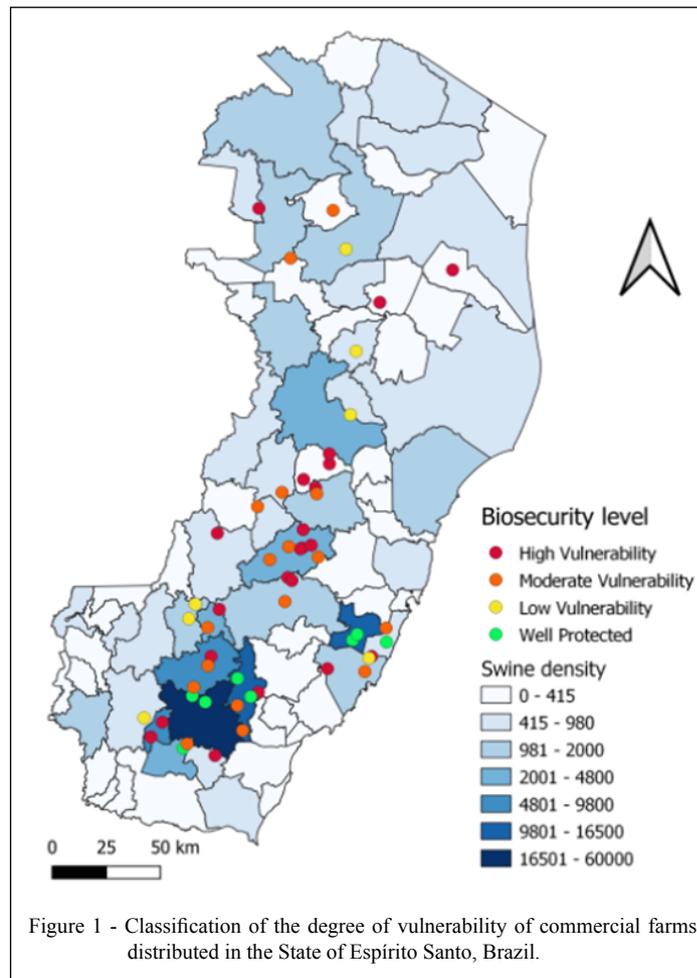
Biosecurity measures	-----N-----	-----(% )-----	-----IC 95%-----
Segregation		44.7	
Presence of internal isolation fence	24	43.6	(30.5 – 56.7)
External supply of feed mill	32	58.2	(45.1 – 71.2)
Boarding dock located more than 5 m from the animal stalls	17	30.9	(18.7 – 43.1)
Has its own vehicle to transport the pigs	36	65.5	(52.9 – 78.0)
Presence of Quarantine Station	14	25.5	(13.9 – 37.0)
Sanitization		36	
Performs sprinkling of vehicles upon arrival at the farm	8	14.5	(5.2 – 23.9)
Disinfection of materials arriving at the farm	7	12.7	(3.9 – 21.5)
Chlorine treatment of the farm water	21	38.2	(25.3 – 51.0)
Performs All in/All out between batches	44	80	(69.4 – 90.6)
Reuses perforating materials	19	34.5	(22.0 – 47.1)
Management		73.3	
Presence of other animals on the property (commercial farming)	49	89.1	(80.9 – 97.3)
Presence of other species with direct contact with pigs	41	74.5	(63.0 – 86.1)
Workers work exclusively with the pigs	45	81.8	(71.6 – 92.0)
Presence of traces of pests in the feed mill	34	61.8	(49.0 – 74.7)
Rodent control measures	50	90.9	(83.3 – 98.5)
Last reform aimed to improve biosecurity	23	41.8	(28.8 – 54.9)
Sanitation		53.6	
Has technical assistance	53	96.4	(91.4 - 100)
Reuses perforating materials	19	34.5	(22.0 – 47.1)
Records the zootechnical data of the batches	34	61.8	(49.0 – 74.7)
Presents respiratory problems	27	49.1	(35.9 – 62.3)
Presents reproductive problems	21	38.2	(25.3 – 51.0)
Has knowledge about notifiable diseases	23	41.8	(28.8 – 54.9)
Access to the shed/barn		29.1	
Records the flow of visitors and vehicles	6	10.9	(2.7 – 19.1)
Requires visitors to wash to access the shed	8	14.5	(5.2 – 23.9)
Requests quarantine of visitors	14	25.5	(13.9 – 37.0)
Has a dressing room	18	32.7	(20.3 – 45.1)
Provides visitors with boots and clothing	15	27.3	(15.5 – 39.0)
Does not perform any basic biosecurity measures	35	63.6	(50.6 – 76.3)

(Aujeszky's disease) were not detected in any sample. Regarding vulnerability of the 55 commercial farms evaluated (Figure 1), 21 were classified as farms with high vulnerability, 17 as farms with moderate vulnerability, 7 as farms with low vulnerability, and 10 as well-protected farms. Commercial farms with a relatively higher biosecurity risk were concentrated in the mountainous central microregion.

Regarding animal biosecurity, 56.4% of farms had no internal isolation fences, and 67.3% had no changing rooms (Table 1). Further, 72.7% of farms did not provide specific clothing for employees and visitors. Overall, 85.5% of farms did not practice bathing for visitors before accessing the barns. Notably, only 10.9% of farms controlled the flow of visitors and vehicles accessing the property.

In 34.5% of farms, vehicles used in the farms were shared with other properties. Regarding sanitation practices for vehicles with access to the establishment, 4.5% of farms sprayed the vehicles, and only 12.7% disinfected the materials introduced into the establishment. In addition, 65.5% of farms claimed to reuse perforating materials.

Another noteworthy finding was that only 25.5% of farms had a pre-designated area for quarantine of replacement animals. In addition, 69.1% of properties had loading docks located near animal stalls, allowing close contact between vehicles and housed animals. Regarding feed delivery, 58.2% of farms relied on external food supply, without the need to access the internal area of the farm. The use of chlorinated water was noted only in 38.2% of farms.



## DISCUSSION

The serological negativity in all samples tested in this study corroborates the absence of suspected cases of the disease in Espírito Santo. However, Aujeszky's disease has been reported in many other Brazilian states, such as Ceará, Mato Grosso do Sul, Santa Catarina, São Paulo, Rondônia, and, in 2018, in Paraná (BRASIL, 2021). In addition to cases in production pigs, several authors have reported Aujeszky's disease in wild pigs (CUNHA et al., 2006; PAES et al., 2013; CHIARI et al., 2015; KMETIUK et al., 2020).

PAES et al. (2013) reported a prevalence of 47.3% in free-living pigs in the Pantanal of Mato Grosso and identified viral latency and vertical transmission of the agent as important factors for epidemiology of the disease. The authors also warned about the possibility of transmission to other animal

species from asymptomatic carrier wild pigs. This characteristic reinforces the need for increased attention to biosecurity procedures in commercial farms, especially in terms of avoiding the possibility of direct contact between wild pigs and commercial breeding pig populations.

In the Cerrado region of the state of Goiás, a positive serological diagnosis was made in a free-living pig (1/36), suggesting the coexistence of SuHV1 circulation among the susceptible wild, production, and domestic animals (KMETIUK et al., 2020). The absence of cases in Espírito Santo excludes the need for vaccination of pigs against Aujeszky's disease, according to the Normative Instruction No. 8 (BRASIL, 2007), which allows the practice of vaccination only in properties with positive laboratory diagnosis under the control of the Official Veterinary Service. Thus, the possibility of false positives in screening serological analysis of the herd is minimized.

Other authors have reported contrasting results. In a serological study conducted on 829 animals in the city of Goiânia, SOUZA et al. (2002) detected the presence of antibodies in 2.17% of samples collected from animals under extensive production who were housed in unsatisfactory sanitary conditions. In Argentina, DIBARBORA et al. (2017) reported that 11.7% of the establishments had positive results for Aujeszky's disease, associating the serological findings to various biosecurity failures in farms. Similarly, MURCIA et al. (2022) diagnosed SuHV1 in 13.39% of swine serum samples, concluding that 29.59% of family farms evaluated in Argentina housed at least one animal with positive result.

The implementation of official strategies and programs for the control and eradication of Aujeszky's disease contributes to the prevention and early detection of the disease, avoiding extensive losses to pig farms (NONAKA, 2012). In addition, measures such as control of swine transit imposed by Normative Instruction No. 19 (BRASIL, 2002), which allows the commercialization of swine breeders only from Certified Pig Breeding Farms (CPBF) that are free of Aujeszky's disease, contributes to the maintenance of herds free of Aujeszky's disease. The occurrence of outbreaks in commercial farms represents a significant risk to national swine production due to the agent's ability to remain latent in pigs. Outbreaks in two CPBF were reported by MORES et al. (2007), in which seropositive animals contaminated 37 farms 45 days before the initial diagnosis.

Another strategy to reduce the emergence of diseases, such as Aujeszky's disease, is through strict adherence to biosecurity protocols. The presence of isolation fences, monitoring of factors related to visitor hygiene practices, and visitor and vehicle flow records are essential components for appropriate functioning of biosecurity programs. Such measures inhibit and restrict the free access of vehicles, people, and other animals that can be potential sources of pathogens (MASSOTTI et al., 2017).

Regarding the use of shared vehicles between establishments, MORES et al. (2007) highlight the use of shared vehicles between establishments as a possible means of transmission of Aujeszky's disease in CPBF. Thus, vehicles that are not exclusive to the farm should be washed and disinfected at the entrance gate, and vehicles transporting feed should be parked outside the production center, loading the supply from the outside (AGUILAR et al., 2015). This measure is one of key variables that can differentiate the biosecurity level of the farm, according to SILVA et al. (2019).

All materials introduced into the farm must be either fumigated or washed and then disinfected. In addition, when introducing new animals to the herd, they should be quarantined and clinically observed for an average period of 30 days before being introduced to the farms, thus avoiding possible asymptomatic carriers (BONATTI & MONTEIRO, 2008). Failure to implement basic sanitary measures, such as not quarantining replacement animals, exchanging animals between properties without sanitary control, and the possibility of contact with wild pigs, are risk factors pointed out by DIBARBORA et al. (2017). Another critical element influencing animal production is water quality, which must be of known source and chlorinated (DORNELAS, 2015).

The assessment of certain characteristics, such as location, production system, available budget, scale, and structural condition, is important for correcting and implementing biosecurity measures and disease control, thus facilitating the trade of animals and animal products (DORNELAS, 2015).

## CONCLUSION

The findings of this study indicate there are no problems related to Aujeszky's disease in the commercial farms of Espírito Santo State evaluated in this survey and that the owners comply with the regulations by not vaccinating their herds. Regarding biosecurity, there is a need for improvement in the minimum biosecurity measures in commercial farms in the state of Espírito Santo to reduce risk factors that contribute to the spread of infectious diseases.

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## DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

## AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception, design, and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

## BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

This research was approved by CEUA No. 9721120521 of the Universidade Federal Fluminense.

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