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THE OCCURRENCE OF VACCINIA VIRUS INFECTION IN CATTLE RAISED IN THE CAÉM-BAHIA REGION: A One Health Issue

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ABSTRACT

This study describes the occurrence of Vaccinia virus infection in cattle raised in the rural area of Caém, Bahia, highlighting the potential transmission to workers exposed to infected animals. The research was conducted from February to November 2008 on 14 farms located in the municipalities of Caém, Jacobina, Miguel Calmon, and Várzea do Poço. This observational descriptive study utilized geoprocessing mapping to identify affected areas and clinical evaluation of the animals. Samples collected included scabs, vesicles, and blood serum, which were sent for laboratory analysis focusing on differential diagnoses, such as foot-and-mouth disease and vesicular stomatitis. Results confirmed the presence of Vaccinia virus as the etiological agent. Approximately 14% of the cattle were affected, mainly dairy cows and calves, while adult male cattle showed no symptoms. The study emphasizes the economic and public health impacts of outbreaks in subsistence farming areas, highlighting the need for control and prevention strategies to protect human and animal health.

Keywords: Vaccinia virus, dairy cattle, human health, geoprocessing

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INTRODUCTION

Smallpox is a widely recognized term used to define an infectious disease characterized by vesiculopustular manifestations of varying severity, caused by a group of viruses capable of infecting numerous animal species. These viruses, also known as poxviruses, belong to the family Poxviridae, a complex and ancient group of DNA viruses that replicate in both vertebrate and invertebrate hosts. Currently, four of the eight known genera within this family are capable of infecting humans: Orthopoxvirus, Parapoxvirus, Yatapoxvirus, and Molluscipoxvirus (Jones et al., 2000; Schatzmayr et al., 2001; Regnery, 2007).

The species within the Orthopoxvirus genus are considered among the primary microorganisms responsible for diseases of medical and veterinary importance, including the human smallpox virus (Variola virus), which was declared eradicated worldwide by the World Health Organization (WHO) in 1980 (WHO, 2001). Another notable member is the Vaccinia virus, a pathogen capable of infecting humans (Roop et al., 1999) and, more commonly, dairy cattle (Schatzmayr et al., 2005).

The study of the Vaccinia virus holds significant importance for both human and bovine health, as it was historically used as a vaccine that led to the global eradication of smallpox, while also conferring immunity to other Orthopoxvirus species (Regnery, 2007; Roop et al., 1999; Stoot, 2003). In recent years, there have been extensive reports of cases in cattle populations and instances of zoonotic transmission to humans, making it an emerging occupational zoonosis (Lobato et al., 2005; Fonseca et al., 1998; Fonseca et al., 2002; Diniz et al., 2001; Damaso et al., 2000; Trindade et al., 2003; Nagasse-Sugahara et al., 2004; Leite et al., 2003).

In cattle, the characteristic lesions occur in dairy cows, affecting the udder and teats, while in nursing calves, lesions are found on the nostrils, mouth, and tongue. Although these lesions are generally localized, they can serve as sites for secondary infections. In humans, the disease primarily affects milkers with a history of direct contact with lesions on infected animals, resulting in lesions distributed on the hands, arms, and even the face (Lobato et al., 2005; Mapa, 2009; Schatzmayr et al., 2005; Simonetti et al., 2007).

In Brazil, outbreaks of Vaccinia virus infection have been reported since the 1960s across various states, consistently leading to significant economic losses for both producers and the government. These losses arise not only from decreased milk production and impaired animal development, which increase veterinary costs, but also from additional expenses incurred by the state due to human cases. These cases often lead to interruptions in labor activities and the need for medical assistance (Batista et al., 2009; Damaso et al., 2000; lewis-jones, 2004; Shugara et al., 2004; Trindade et al., 2003).

In 2008, rural producers and veterinary supply store owners reported clinical cases of vesicular lesions in cattle and milkers in the regions of Jacobina, Miguel Calmon, Caém, and Várzea do Poço in the state of Bahia. Following these reports, an investigation into the disease was initiated, especially due to the initial suspicion of an infectious disease, which necessitates the implementation of a series of control and eradication plans. Thus, it is crucial to characterize this disease from clinical and epidemiological perspectives and to identify the etiological agent involved in the outbreaks. This investigation is of utmost importance for both human and veterinary health.

The objective of this study was to report the occurrence of the Vaccinia virus in dairy cattle raised in the rural area of Caém, Bahia, and to explore its impact on One Health.

MATERIALS AND METHODS

This is a descriptive observational case report study on outbreaks of the Vaccinia virus in cattle and humans on farms in the surrounding regions of Caém, Jacobina, Miguel Calmon, and Várzea do Poço in 2008.

The study was conducted from February 2008 to November 2008 on 14 dairy farms located in the municipalities of Caém, Jacobina, Miguel Calmon, and Várzea do Poço, in the northwest region of the state of Bahia. The study areas are detailed in Map 01 below.

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Map 01.

The regions where the disease outbreaks were identified are characterized by a semi-arid climate with low rainfall, as reported by the EBDA for all neighboring areas. In 2008, the municipality of Jacobina recorded a rainfall index of 757.5 mm, while Miguel Calmon registered 291.9 mm. The other neighboring regions showed similar values, as there were no significant variations (EBDA, 2008).

The study focused on clinical and laboratory analyses in cattle; therefore, other animal species present on the farms were not described and are not included in this research.

The confidentiality of farm owners and individuals affected by the infection was maintained. Considering the probable nature of a vesicular disease, the guidelines from the Ministry of Agriculture (MAPA, 2009) for vesicular diseases were followed. An official notice was sent to the State and Municipal Health Departments, informing them about the sick milkers, the location of the affected farms, and a brief description of the disease. The affected farms were quarantined, with restrictions on animal movement and trade.

In cases involving suspected vesicular disease, considered a veterinary emergency, it is essential to alert various authorities, such as the Ministry of Agriculture, Livestock, and Food Supply, the Municipal Health Department, and the Regional Health Directorate - 16th DIRES, among others.

The 16th DIRES informed local hospital units and clinics about the occurrence of bovine vaccinia and later communicated the laboratory results confirming the clinical diagnosis.

The Ministry of Agriculture was immediately notified, and samples collected from the affected farms were sent to the Secretariat of Agricultural Defense under the General Coordination of Laboratory Support, Virology Unit, registered as 0108/08, as well as to the Secretariat of Agriculture and Supply of the São Paulo Agency for Agribusiness Technology – Biological Institute, documented under FORM IN 2905-104 0001/2008 - Of.DDSA. 003/08.

To gather information, the FORM-IN (Initial Disease Investigation Form) and FORM-COM (Complementary Disease Investigation Form) were used (ANNEX I). These forms are mandatory for completion by the official veterinary service, even in cases where suspicions are dismissed during the initial visit (Mapa, 2009).

The forms must be completed to include descriptions of each property with the following identification details: area/region; purpose of farming; type of operation; and population data for the affected species, such as age range, animal movement, focus chronology, vaccination records, and sample collection data (species type, number of samples). Clinical signs in each animal on the property should also be recorded.

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The samples for this study were distributed across 14 dairy cattle properties located in the municipalities of Caém, Jacobina, Miguel Calmon, and Várzea do Poço in the State of Bahia.

To better characterize and maintain the confidentiality of the owners, the properties were identified by letters (A, B, C, D, E, F, G, H, I, J, L, M, N, and O) and subdivided into three groups (1, 2, and 3) based on temporal and spatial criteria. Additionally, the use of shared labor in cattle management was noted. All these details are part of the epidemiological survey and were incorporated into the geoprocessing analysis. Thus, Groups 1, 2, and 3 were organized as follows:

Tabela 1. Distribuição de surtos de infecção pelo vírus Vaccinia em propriedades de bovinos, por grupo e município

GROUP	PROPERTY	MUNICIPALITY	COORDENATE	DESCRIPTION
1	A	Caém	Lat. 11º10’47.0″ S, Long. 40º21’39.3″ O, Alt. 494 m	1130 bovines. Large for the region, with dual-purpose farming (meat and milk). Meat-producing animals are kept in a separate pen
	B	Caem	Lat. 11º11’04.7″ S, Long. 40º22’30.0″ O, Alt. 445,2 m	193 bovines, dedicated to dairy farming
	C	Jacobina	Lat. 11º12’53.1″ S, Long. 40º17’15.8″ O, Alt. 411,2 m	50 bovines
	D	Jacobina	Lat. 11º13’51.2″ S, Long. 40º17’16.3″ O, Alt. 434,0 m	63 bovines
	E	Jacobina	Lat. 11º13’44.6″ S, Long. 40º17’02.2″ O, Alt. 428 m	40 bovines. Subsistence farming
	F	Jacobina	Lat. 11º12’41.3″ S, Long. 40º17’56.5″ O, Alt. 408 m	78 bovines
	G	Jacobina	Lat. 11º14’00.2″ S, Long. 40º16’45.2″ O, Alt. 426,4 m	24 bovines
	H	Jacobina	Lat. 11º15’07.3″ S, Long. 40º17’00.1″ O, Alt. 440,8 m	10 bovines.
	I	Jacobina	Lat. 11º07’05.5″ S, Long. 40º31’59.0″ O, Alt. 490,8 m	97 bovines. Property with dual-purpose production (milk and meat). Twenty days before the outbreak, five cows were transferred from Farm J to Farm I.
	J	Miguel Calmon	Lat. 11º27’38.7″ S, Long. 40º24’00.6″ O, Alt. 553,0 m	93 bovines
2	L	Várzea do Poço	Lat. 11º28’59.4″ S, Long. 40º21’56.2″ O, Alt. 538,8 m	153 bovines. Located near Farm J, occasionally shares labor and has family ties among the owners.
	M	Jacobina	Lat. 11º09’28.9″ S, Long. 40º32’41.7″ O, Alt. 496,6 m	71 bovines. Pen structure used by neighbors for weighing, vaccination, and overnight stay.
	N	Jacobina	Lat. 11º09’05.1″ S, Long. 40º31’17.1″ O, Alt. 511 m	79 bovines
3	O	Jacobina	Lat. 11º14’06″ S, Long. 40º24’06.2″ O, Alt. 442,2 m	107 bovines, dedicated to dairy farming

Note: The properties were organized based on temporal and spatial criteria. Labor sharing practices among properties for cattle management were also taken into account.

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Sample Collection and Storage:

Samples were collected by an official veterinarian from the Bahia State Agricultural Defense Agency (ADAB), following the safety recommendations of the Ministry of Agriculture (Mapa, 2009). The following materials were obtained from bovines showing clinical signs:

Types of Samples:

- Crusts: Fragments of lesions from the mouth and udders.
- Vesicles: Vesicular epithelium from the edges of the lesions.
- Blood Serum: Blood samples collected from the jugular vein of both adult and young bovines.
- Collection Sites: Lesions on the snouts of young animals and udders of adult cows. No samples were taken from the hooves, as they showed no abnormalities.

Sample Storage

The storage process complied with MAPA (2009) regulations, using refrigerated thermal boxes. The specifications for each sample type were as follows:

1. **Oral Crusts**
   - Origin: Lesions from the mouths of 10 young bovines (50%).
   - Storage: Individual glass vials, preserved on ice.
2. **Udder Crusts**
   - Origin: Lesions from the udders of 10 cows.
   - Storage: Glass jars with lids, kept on ice.
3. **Samples for Clinical Testing**
   - Requested Tests: Foot-and-mouth disease, vesicular stomatitis, and bovine vaccinia.
   - Preservation Method: Crusts submerged in a hyper-saturated sugar solution; others kept in dry containers within an insulated box with ice.
4. **Blood Serum**
   - Collection: Blood samples obtained from the jugular vein of four cows and four young animals.
   - Storage: Glass tubes with vacutainers, kept on ice.

Destination of Samples and Procedures

The samples were sent for analysis to the National Agricultural Laboratory (LANAGRO) in Belém (PA) and the Biological Institute in São Paulo for PCR testing, utilizing the polymerase chain reaction (PCR) technique.

The collection and shipment processes followed the epidemiological surveillance guidelines for vesicular diseases established by MAPA (2007) and the PAHO Manual (2007), adhering to the updates in the Veterinary Sample Collection and Shipment Manual (Paho, 2010).

RESULTS

The first outbreak was recorded on February 19, 2008, at Farm A (Lat. 11º10’47.0″ S; Long. 40º21’39.3″ W; Alt. 494 m), located in the municipality of Caém, which borders Jacobina. Among the infected animals, only lactating females and some of their calves exhibited clinical symptoms. Beef cattle showed no signs of the disease.

As shown in Table 2, 44% of the calves younger than four months old (n=16) on the farm were diagnosed with bovine smallpox caused by the Vaccinia virus. Additionally, the disease was confirmed in 50 females (10.7%) older than 36 months.

Table 2. Distribution of Infected Animals in the First Outbreak (February 19, 2008), Farm A – Caém/BA.

Age Group	Total Animals		Affected Animals	
	Male	Female	Male	Female
< 4 monts	20	16	10	06
4 – 12 monts	150	60	—	—
12 – 24 monts	11	306	—	—
24 – 36 monts	09	90	—	—
> 36 monts	—	468	—	50
Total	**190**		**940**	

Eight blood serum samples were collected from four calves and four adult cows, all presenting characteristic lesions of the disease. The material was properly stored in an insulated container and sent for laboratory analysis.

Farm B, adjacent to Farm A, was the second property where bovine vaccinia was detected, as identified through epidemiological investigation. A total of 193 animals were examined, comprising 25 males and 168 females. Among the analyzed animals, 25 females (21%) over 36 months of age and in lactation exhibited clinical signs of the disease. No clinical signs were observed in the remaining cattle (Table 3).

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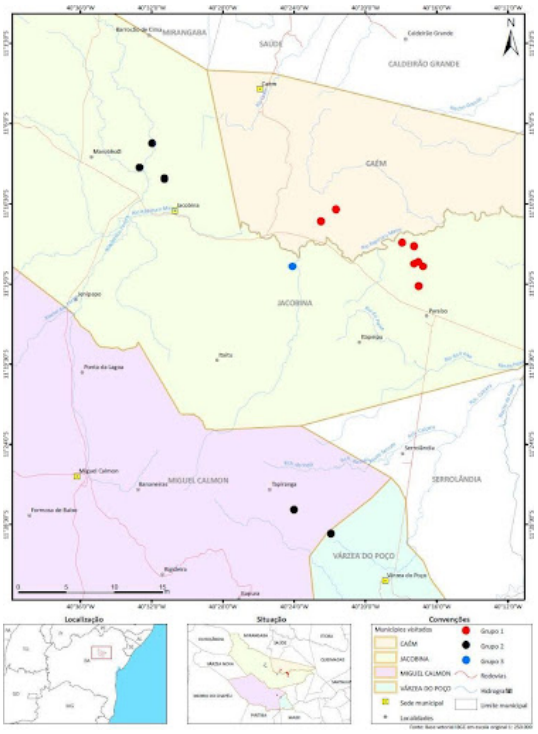

Map 02. Geoprocessing of Regions with Disease Occurrence.

Farm B, adjacent to Farm A, was the second property where bovine vaccinia was detected, as identified through epidemiological investigation. A total of 193 animals were examined, comprising 25 males and 168 females. Among the analyzed animals, 25 females (21%) over 36 months of age and in lactation exhibited clinical signs of the disease. No clinical signs were observed in the remaining cattle (Table 3).

Table 3. Distribution of Infected Animals in the Second Outbreak (02/26/2008), Farm B – Caém/BA

Age Group	Total Animals		Affected Animals	
	Male	Female	Male	Female
< 4 monts	—	—	—	—
4 – 12 monts	23	30	—	—
12 – 24 monts	—	—	—	—
24 – 36 monts	02	20	—	—
> 36 monts	—	118	—	25
Total	**25**	**168**	—	**25**

Farm C was the third identified outbreak site. A total of 50 animals were evaluated, of which 14 females over 36 months of age (73.7% of animals in this age group) exhibited clinical signs of the disease. No animals under 4 months of age were found for analysis (Table 4).

Table 4. Distribution of Infected Animals in the Third Outbreak (Farm C – Jacobina/BA)

Age Group	Total Animals		Affected Animals	
	Male	Female	Male	Female
< 4 monts	—	—	—	—
4 – 12 monts	08	60	—	—
12 – 24 monts	03	306	—	—
24 – 36 monts	01	90	—	—
> 36 monts	01	468	—	14
Total	**13**	**37**	—	**14**

Farm D, reported on 04/29/2008, is a small property in Jacobina and represented the fourth outbreak of bovine vaccinia. Of the 63 animals assessed, 8 females over 36 months old exhibited clinical signs of the disease. Conversely, at Farm E, corresponding to the fifth outbreak of the disease, 100% of the females over 36 months old showed clinical signs of bovine vaccinia (Table 5).

Table 5. Distribution of Infected Animals in the Fourth Outbreak (April 29, 2008) – Farm D – Jacobina, BA

Age Group	Total Animals		Affected Animals	
	Male	Female	Male	Female
< 4 monts	—	—	—	—
4 – 12 monts	02	03	—	—
12 – 24 monts	08	10	—	—
24 – 36 monts	10	02	—	—
> 36 monts	11	17	—	08
Total	**31**	**32**	—	

Conversely, at Farm E, corresponding to the fifth outbreak of the disease, 100% of the females over 36 months old showed clinical signs of bovine vaccinia (Table 6).

Table 6. Distribution of Infected Animals in the Fourth Outbreak (April 29, 2008) – Farm D – Jacobina, BA

Age Group	Total Animals		Affected Animals	
	Male	Female	Male	Female
< 4 monts	20	16	10	—
4 – 12 monts	03	02	—	—
12 – 24 monts	07	06	—	—
24 – 36 monts	08	90	—	—
> 36 monts	01	13	—	13
Total	**19**	**21**	—	**13**

Farm F represents the sixth outbreak of the disease, with a total of 78 animals (30 males and 48 females). Among the females over 36 months old, 18 (75%) showed clinical signs of the disease.

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Table 7. Distribution of Infected Animals in the Sixth Outbreak (04/29/2008) – Farm F – Jacobina/BA

Age Group	Total Animals		Affected Animals	
	Male	Female	Male	Female
< 4 monts	—	—	—	—
4 – 12 monts	—	—	—	—
12 – 24 monts	29	19	—	—
24 – 36 monts	—	05	—	—
> 36 monts	01	24	—	18
Total	**30**	**48**	—	**18**

Farm G, notification dated 04/29/2008, was identified as the seventh outbreak of the disease. Among the evaluated animals, 8 females over 36 months old showed clinical signs of bovine smallpox, as shown in Table 8.

Table 8. Distribution of Infected Animals in the Seventh Outbreak (04/29/2008) – Farm G – Jacobina/BA

Age Group	Total Animals		Affected Animals	
	Male	Female	Male	Female
< 4 monts	—	—	—	—
4 – 12 monts	03	04	—	—
12 – 24 monts	01	01	—	—
24 – 36 monts	—	06	—	—
> 36 monts	01	08	—	08
Total	**05**	**19**	—	**08**

The eighth outbreak was recorded at Farm H, where 10 animals (4 males and 6 females) were evaluated. Among them, 2 females aged between 24 and 36 months exhibited clinical signs of the disease.

Farm I has a total of 97 animals, including 71 males and 26 females. Among the females, 17 over 36 months old were diagnosed with bovine smallpox. None of the males showed clinical symptoms.

Table 9. Distribution of Infected Animals at Farm I – Jacobina/BA

Age Group	Total Animals		Affected Animals	
	Male	Female	Male	Female
< 4 monts	—	—	—	—
4 – 12 monts	08	09	—	—
12 – 24 monts	23	—	—	—
24 – 36 monts	40	—	—	—
> 36 monts	—	17	—	17
Total	71	26	—	**17**

At Farm J, which has 93 animals, 25 females over 36 months old were diagnosed with clinical signs of bovine vaccinia. No clinical symptoms were observed in other animals.

Table 10 Distribution of Infected Animals in the Ninth Outbreak (04/07/2008) – Farm J – Miguel Calmon/BA

Age Group	Total Animals		Affected Animals	
	Male	Female	Male	Female
< 4 monts	—	—	—	—
4 – 12 monts	16	—	—	—
12 – 24 monts	—	—	—	—
24 – 36 monts	01	—	—	—
> 36 monts	—	76	—	25
Total	**17**	**76**	—	**25**

Farm L, neighboring Farm J, has a total of 153 animals. Twenty-five females over 36 months old showed clinical signs consistent with bovine vaccinia, Table 11

Table 11. Distribution of Infected Animals at Farm L – Várzea do Poço/BA

Age Group	Total Animals		Affected Animals	
	Male	Female	Male	Female
< 4 monts	20	12	—	—
4 – 12 monts	04	03	—	—
12 – 24 monts	10	13	—	—
24 – 36 monts	—	—	—	—
> 36 monts	01	90	—	25
Total	35	118	—	25

At Farm M, which has a total of 71 animals, 100% of females aged between 24 and 36 months (n=30) were diagnosed with bovine vaccinia, as well as one female aged between 4 and 12 months, Table 10.

Table 12. Distribution of Infected Animals at Farm M – Jacobina/BA.

Age Group	Total Animals		Affected Animals	
	Male	Female	Male	Female
< 4 monts	10	12	—	—
4 – 12 monts	03	14	—	01
12 – 24 monts	02	01	—	—
24 – 36 monts	01	30	—	30
> 36 monts	—	—	—	—
Total	16	55	—	31

At Farm N, a total of 79 animals were recorded (22 males and 57 females). All females (n=22) over 36 months of age exhibited clinical signs of bovine vaccinia. The owner had arranged temporary housing for the cattle at Farm M approximately 30

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days before the disease was reported and had purchased two cows clandestinely from a livestock trader, as shown in Table 13.

Table 13. Distribution of Infected Animals at Farm N – Jacobina/BA

Age Group	Total Animals		Affected Animals	
	Male	Female	Male	Female
< 4 monts	—	—	—	—
4 – 12 monts	08	17	—	—
12 – 24 monts	12	07	—	—
24 – 36 monts	—	11	—	—
> 36 monts	02	22	—	22
Total	22	**57**	—	**22**

Farm O, Notification: 11/20/2008, was the third outbreak identified in the municipality of Jacobina. Among the evaluated animals, one aged 4 to 12 months and 24 females over 36 months were diagnosed with bovine vaccinia. No animal movement was reported, and only the milk produced was sold.

Table 14. Distribution of Infected Animals at Farm O – Jacobina/BA

Age Group	Total Animals		Affected Animals	
	Male	Female	Male	Female
< 4 monts	—	—	—	—
4 – 12 monts	23	29	—	01
12 – 24 monts	—	—	—	—
24 – 36 monts	—	—	—	—
> 36 monts	03	52	—	24
Total	26	**81**	—	**25**

Figure 1. Occurrence of Bovine Vaccinia in 14 Farms in the Jacobina/BA Region. Prevalence of Infected Bovines

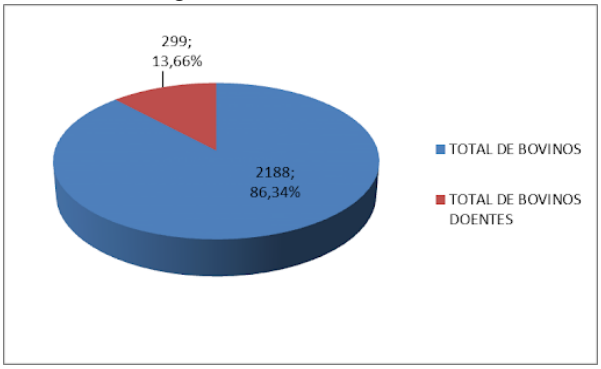
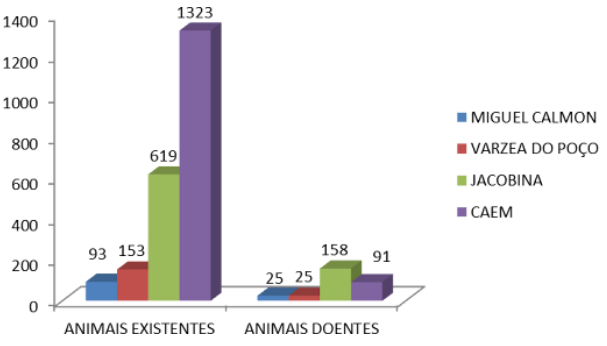


Figure 2: Infected Animals by Municipality in Farms Affected by the Vaccinia Virus Outbreak



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Clinical Presentation of Poxvirus Infection in Dairy Cattle

In the farms affected by the bovine pox outbreak, the animals exhibited typical lesions characterized by erythematous patches, vesicles, pustules, and crusts on the teats and udders. The lesions progressed to form hypochromic scars, with a duration of approximately 15 to 20 days from the appearance of the patches to the completion of the scarring process.

Photo 1: Crusts on Teats. 1st OUTBREAK - FARM A, CAÉM

Photo 2: Coalesced Pustules on Calf's Snout. 1st OUTBREAK - FARM A, CAÉM

Photo 3: Crust on Dairy Cow. 2nd OUTBREAK - FARM B, CAÉM

Photo 4: Crusts and Scars on Dairy Cow. 7th OUTBREAK - FARM G, JACOBINA

Individuals who had contact with cattle infected by bovine vaccinia presented clinical signs consistent with poxvirus infection. All milkers from the affected properties exhibited symptoms such as erythematous patches (reddened and inflamed skin lesions), vesicles (small superficial blisters causing pain and discomfort), and later crust formation at the affected sites as the lesions progressed.

During visits to Farms D, E, F, G, and H, it was observed that milkers often assisted each other with milking tasks, including on neighboring properties. This sharing of labor resulted in direct contact with the herds of multiple farms, increasing the likelihood of poxvirus transmission among workers and between herds across different properties.

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Photo 5: Vesicles on the hand of a milker. 1st OUTBREAK – FARM A – CAÉM

Photo 6: Vesicles on the hand of a milker working on Farms D, E, F, G, and H – JACOBINA

Photo 7: Vesicles on the anterior surface of a milker's hand at Farm G – 7th OUTBREAK – JACOBINA.

Photo 8: Milking stool – FARM O.

Laboratory Results

On February 23, 2008, the Ministry of Agriculture released the results of tests conducted at the National Agricultural Laboratory (LANAGRO) in Pará to investigate foot-and-mouth disease virus and vesicular stomatitis virus. Using the ELISA technique on epithelial samples, the results were negative for both viruses.

Regarding the diagnosis of Orthopoxvirus (Pox), on April 11, 2008, the Research and Development Center for Animal Health of the Bovine Virus Laboratory at the Biological Institute (São Paulo) provided the results of a virus-neutralization test performed on serum samples for Orthopoxvirus, Vaccinia Serotype, all of which were non-reactive. Subsequently, on April 16, 2008, the same center issued the results of a PCR test performed on epithelial samples (scabs) for the detection of Orthopoxvirus using DNA polymerase chain reaction (PCR), which yielded a positive result for the Vaccinia virus.

This series of tests confirmed the absence of foot-and-mouth disease and vesicular stomatitis but demonstrated the presence of Orthopoxvirus (Vaccinia virus) in the analyzed samples.

DISCUSSION

In 2008, during an inspection of agro-product resale shops in the region, an increase in the stock of iodophor-based products was observed. These products were reportedly used by a small group of dairy farmers for milking hygiene ("pre- and post-dipping") and for treating lesions caused by foot-and-mouth disease (Sharbauh, 1998; Amorim et al., 2006). Upon inquiry about the reason for this increase, store owners indicated that some local farmers were using the medication to treat wounds and warts on

cow udders. Based on this information, efforts were made to identify the owners and quickly locate properties that could serve as potential foci of vesicular disease (Table 1/Flowchart of Assistance).

On February 19, the author visited Farm A (Table 2), located in Caém. During the inspection, it was noted that only cattle exhibited inflammatory signs characterized by vesicles, pustules, and crusts (Photos 1, 2, 3).
Among these animals, only lactating cows (Photo 1), representing 10.68% of the total (n = 50) females in the same age group, showed lesions on the udder and teats. Simultaneously, some calves (n = 16) also displayed similar lesions on the gums, mouth, and muzzle. These calves were offspring of the affected cows and were still in the lactation phase. Additionally, some workers involved in milking these cows also developed vesicles and ulcers on their hands and forearms, suggesting a possible zoonotic transmission.

Upon investigating the clinical history of the disease in cattle, it was observed that although the animals displayed vesicles on the udder and teats, there were no signs or history of vesicles on their hooves, nor reports of lameness, diarrhea, or hyperkeratosis. The owners denied any associated deaths, and the animals were up to date with foot-and-mouth disease vaccination. Other animals on the property, such as horses, pigs, dogs, and cats, showed no symptoms.

Initially, the owners denied any connection between the lesions observed on the milkers and their handling of the animals, likely due to fears of work restrictions, labor complaints, or interruptions in milk production. However, the characteristics of the vesicles, pustules, and crusts, as well as the synchronicity of the lesions in both workers and animals, were strikingly similar. The milkers and their families, in a state of panic, eventually admitted to direct contact with lesions on the infected animals. Poxvirus-induced lesions are extensively documented in the literature (Reis et al., 1970; Mazur et al., 2000; Moss, 2001; Lewis; Jones, 2004). Based on the signs, symptoms (Breman; Henderson, 2002), and collected epidemiological data, bovine vaccinia was identified as the primary suspicion. Differential diagnoses of vesicular diseases, including foot-and-mouth disease and vesicular stomatitis, were necessary to rule out these conditions in the region (Schatzmayr et al., 2001). Consequently, all neighboring properties were investigated.

The investigation followed the Ministry of Agriculture's protocol for vesicular diseases (MAPA, 2007). This protocol, established by the Ministry of Agriculture, Livestock, and Supply, outlines procedures for handling suspected vesicular disease notifications. Accordingly, all appropriate measures were implemented, including notifying relevant animal and human health authorities, providing guidance to owners and milkers, collecting samples for laboratory testing, and issuing notifications and quarantines for the affected properties, with restrictions on animal movement and trade.

On February 23, results were received from the National Agricultural Laboratory in Pará (LANAGRO) regarding tests for foot-and-mouth disease and vesicular stomatitis viruses. The results were negative for the submitted samples, effectively ruling out these diseases and reinforcing the primary suspicion of bovine vaccinia.

On February 26, an inspection was conducted at Farm B, located in the municipality of Caém and adjacent to Farm A (MAP 02). Vesicular disease was observed exclusively in cattle (n = 193). Once again, only lactating cows (n = 25) exhibited signs of illness (Photo 4). The clinical manifestations were similar to those observed at Farm A, characterized by vesicles, pustules, and crusts on the teats and udders, alongside a temporal similarity in lesion progression among milkers (Schatzmayr et al., 2000; Damaso et al., 2000; Nagassane; Shugahara et al., 2004; Lewis; Jones, 2004; Leite et al., 2005; Lobato et al., 2005).

The results of the viral neutralization test were received on April 11, indicating a non-reactive outcome for Orthopoxvirus (POX) Serotype – Vaccinia in all samples. However, negative results should be interpreted cautiously, as animals may be in the incubation phase, during which neutralizing antibodies cannot be detected (Okuda, 2009). Therefore, confirmation of the disease awaited polymerase chain reaction (PCR) analysis.

On April 16, the Biological Institute at the Research and Development Center for Animal Health in the Laboratory of Bovidae Virology provided the PCR results, definitively confirming the presence of the Vaccinia virus as the causative agent.

Other six properties (Farms C, D, E, F, G, and H), located in the municipality of Jacobina but adjacent to Farms A and B (Map 2), were also inspected, and new outbreaks of bovine smallpox were identified, exclusively in lactating cows. On April 25, Farm C was inspected, and the cattle displayed smallpox lesions with the same characteristics as those found on Farms A and B. Of the fifty cattle evaluated, fourteen cows showed clinical signs of poxvirus infection. The other farms were also inspected, starting with Farm D, where eight cows with clinical signs of bovine smallpox were identified. At Farm E, corresponding to the fifth disease outbreak, thirteen cows displayed clinical signs of the

illness. At Farm F (Table 7), 72% of the lactating females were affected by smallpox. At Farm G, eight cows were diagnosed with clinical signs of bovine smallpox. Finally, for Farm H shows two lactating cows presenting clinical symptoms of bovine smallpox.

On the properties in Group 1, it was also observed that milkers worked on more than one farm, including Farms A and B, and all showed signs of bovine smallpox, indicating a possible transmission route. According to the literature, reports show that lesions caused by poxvirus present as erythema, skin patches evolving into vesicles and scabs on the teats of infected cows (Reis et al., 1970; Breman and Henderson, 2002). The condition can last between 15 and 20 days (Simonetti, 2007), and transmission to humans may occur (Okuda, 2009; Lewis and Jones, 2004), as well as transmission from humans to cattle, for instance, during milking (Schatzmayr et al., 2000 and 2009; Batista et al., 2009). In humans, the infection is characterized by an inflammatory process that starts with edema and local pain, followed by pustules with umbilicated lesions and ulcers on the hands, which may also extend to the forearm and face (Schatzmayr et al., 2000 and 2009; Damaso et al., 2000; Nagassane; Shugahara et al., 2004; Lewis and Jones, 2004; Leite et al., 2005; Lobato et al., 2005).

In relation to Farms A and B, the animals had contact not only due to the proximity between the farms but also through shared milkers, who showed vesicles and ulcers on their hands and forearms. It is known that, since the 1990s, some rural properties in Brazilian states have been affected by epizootic outbreaks affecting animals and humans, caused by the vaccinia virus. In these outbreaks, it was found that transmission among animals occurred mainly through the milkers' hands or mechanical milking equipment (Damaso, 2000; Lewis and Jones, 2004; Trindade G. et al., 2003; Schatzmayr et al., 2000).

Studies indicate that worker contamination occurs through direct contact with animal lesions, especially when protective equipment is not used, and there are micro-abrasions on the skin (Fagliari et al., 1999; Lewis and Jones, 2004). Additionally, it is known that the milkers' hands and milking equipment are the main transmission routes of poxvirus among cattle, allowing the virus to penetrate through skin abrasions and mucosal continuity, particularly in the teat and udder regions of animals (Donatele et al., 2007), which may have occurred at Farms A and B due to the inadequate use of protective materials and the milkers' precarious working conditions.

Another group, referred to as Group 2, was created following the identification of bovine smallpox outbreaks in the herds of five other properties. The infected animals were first identified on Farm I, located in Jacobina, after a report that the milker had hand injuries and the cattle displayed signs of bovine smallpox. On this farm, all lactating cows (n=17) were affected.

Upon investigating the possible entry route of the disease on Farm I, two relevant pieces of information were obtained. The first, found in the property owner's records, indicated that five cows had been transported from Farm J, which belongs to the same owner as Farm I, ten days before the inspection. Farm J is located in Miguel Calmon, and among its cattle, all lactating cows (n=25) exhibited clinical signs of bovine smallpox.

The second relevant piece of information is that Farm J is adjacent to Farm L. This Farm L had been inspected previously, and an outbreak of bovine smallpox was identified during the inspection of Group 1 in February 2008. However, it should be noted that the author chose not to include Farm L in Group 1 because, following its notification on February 19, 2008, another veterinarian conducted an inspection on February 28, 2008, confirming the presence of bovine smallpox at the time of the outbreak. During a subsequent inspection carried out by the author on April 14, 2008, it was found that twenty-five lactating cows continued to display clinical signs of bovine smallpox.

The probable cause of transmission on Farm L during the first outbreak was not precisely identified, as the owner did not provide detailed information for an accurate analysis. This owner is involved in cattle transportation in the region and has a community milk collection tank on the property.

In Group 2, two additional farms were inspected due to the clinical signs of the disease and their proximity to Farm I. On Farm M, 31 affected animals were identified, including 30 lactating cows and 1 sick calf. On Farm N, all cows (n=22) were diagnosed with bovine smallpox.

Finally, the group referred to as Group 3 included Farm O, where 24 dairy cows and 1 calf exhibited clinical signs of bovine smallpox. The owners are elderly, and the responsibility for animal care lies with the farmhands. It was not possible to identify any clandestine movement of animals, only the sale of raw milk for bulk processing.

For all properties affected by the outbreak, neighboring properties were investigated, analyzing the flow of animal entry and exit, transport vehicles, milk trade, and even manure sales or pasture rentals. It was found that, in general, the involved properties practiced manual milking and did not implement pre-dipping or post-dipping practices. During milking, it was

common to observe blood between the milker's fingers and in the milk bucket, highlighting poor hygiene conditions. In most cases, there was no water available at the facilities, nor an adequate structure for the activity.

During the epidemiological investigation, it was reported that Property A had clandestinely purchased ten cows from a cattle trader in the state of Minas Gerais. However, no concrete data was obtained to establish a connection with the other properties, nor was it possible to gather more information about the incident.

As this is a zoonotic disease (Mapa, 2009), an official notice was issued to the State and Municipal Health Departments, informing them about the presence of sick milkers, the location of the affected properties, and a brief description of the disease. The regional health authority formally notified hospitals and clinics in the area about the occurrence of bovine smallpox, and the laboratory results later confirmed the clinical diagnosis. The affected properties were placed under quarantine, and the sale of milk was suspended.

To raise awareness among workers, educational talks were conducted on protection and hygiene methods required for handling dairy cattle. Milkers with lesions were referred to health centers. A team from the regional health authority visited the properties to provide guidance to the milkers and their families.

The owners were informed about the severity of the disease, the potential economic repercussions, and their responsibility to comply with all established control and prevention regulations (Mapa, 2009). Additionally, talks with local doctors, meetings with farmers, and public announcements in the press were conducted.

CONCLUSION

Bovine smallpox has been consistently described in recent years by various authors across multiple states in the country. It is caused by the Vaccinia virus, establishing itself as an emerging disease. In the studied region, no public reports of the disease caused by the Vaccinia virus had been identified until now.

Despite unfavorable working conditions and widespread poverty observed in the state, which hinder a comprehensive epidemiological investigation, it was possible to delineate the affected areas during the outbreak using geoprocessing techniques. It was found that most of the cattle affected by the disease were located in the Jacobina region, where ten properties reported outbreaks.

Among the animals involved in the outbreak, approximately 14% of the cattle herd was infected, including 281 dairy cows, ten male calves, and eight female calves. No cases of disease were identified among adult male cattle. Laboratory tests confirmed Vaccinia virus as the etiological agent responsible for the infection in the animals and the affected rural workers.

The measures recommended by the Ministry of Agriculture were followed, and preventive actions were implemented as soon as vesicular disease with zoonotic potential was suspected. However, due to the challenges encountered, it was not possible to determine the origin of the outbreak or identify the initial source of the infection caused by the Vaccinia virus.

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