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TWO-YEAR EPIDEMIOLOGICAL SURVEILLANCE OF EQUINE INFECTIOUS ANEMIA IN AN INSTITUTIONAL HORSE STABLES IN CHIAPAS, MEXICO

Oscar León-Velasco

Benemérita Universidad Autónoma de Chiapas, Escuela de Estudios Agropecuarios Mezcalapa, Campus XI. Copainalá, Chis. ORCID 0009-0000-4060-2784.

Humberto León-Velasco*

Benemérita Universidad Autónoma de Chiapas, Facultad de Ciencias Agronómicas, Campus V. Villaflores, Chis. ORCID 0009-0000-4202-3081.

*Corresponding Author.

Carlos Alberto Castellanos-Hernández

Benemérita Universidad Autónoma de Chiapas, Escuela de Estudios Agropecuarios Mezcalapa, Campus XI. Copainalá, Chis.

Horacio León-Velasco

Benemérita Universidad Autónoma de Chiapas, Facultad de Medicina Veterinaria y Zootecnia, Campus II. Terán, Chis. ORCID 0009-0001-1759-3856.

Esaú de Jesús Pérez-Luna

Benemérita Universidad Autónoma de Chiapas, Facultad de Ciencias Agronómicas, Campus V. Villaflores, Chis. ORCID 0000-0002-8721-8621.



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Abstract. The horse (*Equus caballus* L.) is of particular importance due to its economic, social, and cultural value to institutions, associations, and agricultural lands in Mexico. Over a period of two years, an observational study was conducted at the Llano San Juan de Ocozocoautla de Espinosa Cavalry in Chiapas, with the aim of identifying the incidence of Equine Infectious Anemia Virus (EIAV) through serological laboratory tests. Initially, 90 horses owned by the state government and used in public safety activities were evaluated. Two laboratory diagnostic tests were used: one ELISA and one agar gel immunodiffusion. In the first year, three seropositive animals were identified, and in the second year, two more; in both cases, they were removed in accordance with the established health protocol. Based on the results, the cumulative incidence during the period was 5.56%, reflecting a downward trend in the presentation of new cases. Specifically, in the first year, a cumulative incidence of 3.3% was recorded, and in the second year, 2.3%, showing a downward epidemiological trend. These findings suggest that the control and biosecurity measures applied in the unit have been effective in limiting the spread of the virus and maintaining a stable health status. It is concluded that epidemiological surveillance, timely diagnosis, and the elimination of positive animals are essential tools for the prevention and control of the disease in institutionally managed equine populations, highlighting that these actions are in line with national technical recommendations aimed at consolidating the downward trend of the disease in the central region of the state of Chiapas, Mexico.

Keywords: Equine Infectious Anemia, cumulative incidence, epidemiological surveillance, horses.

INTRODUCTION

Horses (*Equus caballus* L.) are highly im-

portant in the country's production systems, as they are used for work, transport, and support in agricultural activities, as well as occupying a prominent place in Mexico's social and cultural life. Horses are a symbol of national identity and tradition; the so-called cuacos are prized for their strength, docility, and endurance.

Their economic value is high, as they represent a significant investment for producers and breeders. Therefore, preserving their health and preventing diseases such as equine infectious anemia is essential to maintaining their productivity and the role they play in the rural economy and national culture. In short, comprehensive management focused on animal welfare is essential, regardless of the zootechnical purpose for which these highly prized animals are intended.

Equine Infectious Anemia (EIA) is a viral disease that affects horses worldwide. This infection, initially known as swamp fever, is caused by a lentivirus belonging to the Retroviridae family, Orthoretrovirinae subfamily. The host's immune response manifests itself with the production of precipitating antibodies against the Equine Infectious Anemia Virus (EIAV), which can be detected by serological tests such as ELISA and Agar Gel Immunodiffusion (AGID) (WOAH/OIE, 2019).

In Mexico, the disease was first diagnosed in 1999 in the state of Baja California. Subsequently, in 2003, an outbreak was reported in the same state, with an estimated economic loss of one million dollars. Some reports indicate that the prevalence of EIA in the country has been estimated at 1.45% in Federal Inspection Type (FIT) slaughterhouses, while in the North, Northwest, Gulf Coast, and Isthmus of Tehuantepec regions, the figures have varied between 0.3 and 13.8% (AMMVEE, 2024).

Meanwhile, in a study conducted on working equines (horses and donkeys), Sán-

chez-Contreras *et al.* (2018) reported a prevalence of 37.7% in horses in the municipality of Veracruz, Veracruz, also using serological tests for the detection of EIAV. These findings coincide with those of Ibarra *et al.* (2025), who showed a seroprevalence of 38.4% in Sonora, Mexico, as well as the records of Villamancera *et al.* (2024), who published a seroprevalence of 15.25% in Puebla and 20.49% in Veracruz.

Taken together, the results of different studies show an upward trend in the detection of seropositive cases, suggesting the persistence and possible spread of the virus in different regions of the country. In this context, the objective of this study was to evaluate the incidence and epidemiological indicators of equine infectious anemia in the Llano San Juan de Ocozocoautla de Espinosa cavalry in Chiapas, in order to contribute to the knowledge of its behavior and the implementation of more effective health control strategies.

MATERIALS AND METHODS

STUDY AREA

The municipality of Ocozocoautla de Espinosa, Chiapas, is located between the geographical coordinates 16° 25' - 17° 10' north latitude and 93° 11' - 93° 52' west longitude. According to the 2020 Population Census conducted by INEGI, its population is 97,397 inhabitants, its altitude varies between 100 and 1800 m, the average temperature between 20 and 28°C, with annual precipitation ranging from 900 to 3000 mm. The prevailing climate is warm and subhumid, with rainfall in summer and dry conditions in winter (INEGI, 2021).

MICRO-LOCATION

The Llano San Juan Cavalry, belonging to

the Chiapas State Police, is located on the Tuxtla-Ocozocoautla Highway at kilometer 1.5, at the former Llano San Juan Airport.

TARGET POPULATION

The study population was addressed through a census, i.e., all animals in the sampled unit were included, in accordance with the methodological definition proposed by Hernández-Sampieri *et al.* (2014).

In this study, 90 clinically healthy male and female horses were considered and were included in a 2-year observational, longitudinal, and prospective study, in accordance with the methodological criteria described by Bonita *et al.* (2006), Hernández-Sampieri *et al.* (2014), Thrusfield and Christley (2018), and Friis and Sellers (2021) to identify new cases and estimate epidemiological indicators of equine infectious anemia in horses at the Llano San Juan de Ocozocoautla de Espinosa equestrian unit in Chiapas.

BLOOD SAMPLING

According to the Mexican Association of Veterinary Specialists in Equines (AMMVEE, 2024), to obtain blood samples, the animals were securely restrained using a halter and, if necessary, a rope or halter was used to facilitate handling. The puncture site was located in the neck region, at the level of the jugular vein, which was carefully disinfected with a swab impregnated with 70% alcohol.

The jugular vein was then located by manual compression, and the puncture was performed with a Vacutainer® needle placed with the bevel facing upwards, inserting it gently and continuously to minimize bleeding and stress to the animal. Approximately 10 mL of blood was drawn, sufficient for serological testing, using tubes without anticoagulant. After collection, the needle was removed and the puncture site was gently massaged to prevent bruising. The samples were left in an inclined

position to achieve efficient h y separation of the serum, taking care to avoid contamination or hemolysis.

During the first year, 90 serum samples were collected, and 87 during the second year. Each sample was kept refrigerated at 4°C until it was sent to the laboratory authorized for the diagnosis of Equine Infectious Anemia Virus (EIAV), where serological tests were performed to detect specific antibodies.

SHIPPING OF SERUM SAMPLES

For shipping, the serum samples were placed in a properly identified and protected cooler. Cooling material (natural ice and cooling Gel) was added to maintain the appropriate temperature during transport. The samples were packed using a double box system, where the inner container was made of Styrofoam for its insulating capacity and was hermetically sealed with adhesive tape. The outer box was then closed, securing all joints to reinforce the structure and maintain thermal insulation. Finally, a label with the sender and recipient details was placed on the top of the package (AMMVEE, 2024).

DIAGNOSTIC TESTS

Two internationally recognized tests were used for the serological diagnosis of Equine Infectious Anemia (EIA). Initially, the samples were analyzed using the ELISA test, which was used as a screening method due to its high sensitivity for detecting antibodies against the virus. Positive or doubtful results were confirmed by laboratory analysis using Agar Gel Immunodiffusion (AGID), considered the gold standard for the official diagnosis of the disease. Both tests are recommended by the World Organisation for Animal Health for the confirmation of EIA (WOAH/OIE, 2019).

CALCULATION OF EIA INDICATORS

To determine the epidemiological behavior

of the disease during the study, the Cumulative Incidence (CI) was estimated, considering only animals at risk at the beginning of each year and new cases detected in the analyzed interval. This indicator was obtained by dividing the number of horses that tested positive for the first time among the initial population examined, expressing the result as a percentage. The methodology used for this calculation follows the epidemiological principles described by Thrusfield and Christley (2018), who point out that CI is the proportion of initially healthy individuals who develop the disease during a defined follow-up period. They emphasize that this indicator requires a group of animals free of the disease at the beginning and continuous monitoring to record the appearance of new cases. It also states that CI is a measure of risk, as it expresses the probability that a susceptible individual will become ill during that time interval.

Formula according to Martin *et al.* (1997) and Thrusfield and Christley (2018).

$$CI = \frac{C}{N} \times 100$$

Where:

- CI = Cumulative Incidence (in %)
- C = Number of new cases during the observation period
- N = Number of animals at risk at the start of the study

Incidence Density (ID) was used to estimate the rate at which new cases appeared during the observation period, based on the explanation provided by Thrusfield and Christley (2018) that not all animals are exposed during the same period, so a measure that incorporates animal-time is required, and is calculated using the following formula.

Formula according to Martin *et al.* (1997), Dohoo *et al.* (2003), and Thrusfield and Chris-

tley (2018).

$$ID = \frac{\text{Number of new cases}}{\text{Sum of time at risk for all individuals}}$$

From this epidemiological perspective, it is considered that each animal contributes different amounts of time, which is applicable given that during the observation period, three seropositive animals were eliminated in the first year and two EIAV-positive animals were eliminated in the second year of observation.

Therefore, Thursfield and Christley (2018) emphasize that this measure is not a proportion but a rate because it includes the behavior of the disease across populations or periods, even when the number of animals observed or their follow-up time is not homogeneous. There are similarities with Dohoo *et al.* (2003), who also mention that this rate is expressed in units such as “cases per animal-year” or “cases per animal-month,” depending on how the exposure time is measured. In the case of the present study, the rate of manifestation of EIA pathology is expressed in cases per animal-year.

It was necessary to identify the comparison of EIA risk between the two observation periods, for which the Relative Risk was estimated, following the arithmetic procedure described by Thursfield and Christley (2018). This indicator was obtained by dividing the incidence observed in each year by the incidence corresponding to the reference period, which made it possible to assess whether there was a change in the risk of new cases appearing between the two evaluation moments.

To estimate the strength of association between exposure and the occurrence of new cases, the Relative Risk (RR) was calculated following the epidemiological criteria described by Dohoo *et al.* (2003) and Thursfield and Christley (2018). The RR was obtained by comparing the risk of disease in exposed animals with the risk observed in unexposed animals, using the ratio between the two

cumulative incidences. This indicator made it possible to assess whether the condition evaluated increased, decreased, or did not change the probability of developing the infection during the follow-up period.

Formula according to Dohoo *et al.* (2003) and Thursfield and Christley (2018).

$$RR = \frac{R_1}{R_0}$$

Where:

- R_1 = Risk or CI in the exposed group.
- R_0 = Risk or CI in the unexposed group.

RESULTS

During the two-year epidemiological observation period for Equine Infectious Anemia (EIA), the horses located on the Llano San Juan property in Ocozocoautla de Espinosa, Chiapas, were evaluated. This site housed a total of 90 horses owned by the state government, which were used for public security operations and served as study subjects for laboratory diagnosis of the Virus that causes the disease known as Equine Infectious Anemia (EIAV).

Table 1 shows the results for the first year in a herd of 90 horses, where three animals were diagnosed as seropositive, i.e., with antibodies compatible with the virus through serological tests performed in the laboratory. The animals that tested seropositive for Equine Infectious Anemia Virus (EIAV) were removed from the at-risk population to prevent the spread of the disease.

In the second year of observation and diagnostic monitoring of EIAV, now in a herd of 87 horses, two animals were detected as seropositive for the disease under study, which were also removed from the stables as part of the epidemiological protocol.

Based on the results obtained during the two years of observation and diagnosis,

through serological tests, five positive results for Equine Infectious Anemia Virus (EIAV) were recorded. Consequently, the study period yielded a Cumulative Incidence (CI) of 5.56% for the Llano San Juan Cavalry in Ocozocoautla de Espinosa, Chiapas (Table 2); this value corresponds to five new pathologically confirmed cases: three in the first year, within a population of 90 animals, and two in the second year in a population of 87 horses. In both cases, the animals confirmed as positive were removed in accordance with the epidemiological protocol, with the aim of reducing the cumulative incidence in the stables.

Year	Horses observed	Positive cases	Removed
1	90	3	3
2	87	2	2
Total	177	5	5

Table 1. Annual distribution of seropositive horses removed by EIA.

Considering that the most accurate information is that provided by the incidence rate or Incidence Density (ID), an arithmetic calculation was performed, which was obtained by dividing the total number of new cases of Equine Infectious Anemia recorded during the study and epidemiological surveillance period by the sum of the time that the animals remained at risk of becoming ill.

Table 2 shows that, for this calculation, 90 horses observed during the first year and 87 during the second year were taken into account, which is equivalent to a total of 177 horse-years of follow-up and field observation and clinical diagnosis. Thus, at the end of the observation or research work, five new seropositive cases were diagnosed, detected during the period, in which the Incidence Density was 0.028 cases per horse-year, equivalent to 2.8 cases per 100 horses exposed during two years of epidemiological observation at the Llano San Juan de Ocozocoautla de Espinosa stable in Chiapas.

It is important to mention that the ID or incidence rate takes into account new cases,

as well as the exposure time of clinically healthy animals to the risk of acquiring the disease under study. Therefore, its denominator considers not only animals but also animal-time (horse-years). Thus, it cannot be estimated proportionally, as it reflects the rate at which new cases occur per unit of time, as shown in Table 2.

In other words, Table 2 also indicates the incidence rate (positive cases) of EIA during a period of risk of exposure to the disease in the cohort of 177 horse-years.

Indicator	Formula	Result	Interpretation
Cumulative Incidence	5/90	5.6	5.56% of horses became ill in 2 years
Incidence Density	5/177	0.028/ horse-year	Approximately 2.8 cases per 100 horses per year

Table 2. Epidemiological indicators of Equine Infectious Anemia

The epidemiological indicators shown in Table 3 are relevant for summarizing and presenting the results obtained from serological samples from 90 horses in the first year and 87 cattle in the second year of observation, in which three and two samples, respectively, were found to have antibodies compatible with the virus that causes EIA. These results were verified by the official certified animal pathology laboratory located in Tuxtla Gutiérrez, Chiapas, Mexico.

A total of 90 animals served as the baseline for this two-year observation study. Table 3 shows a downward trend from the first to the second year of observation of clinically ill animals, which is a good indicator of the animal health or epidemiological management of the herd studied.

During the two-year period of observation of animals susceptible to the virus that cau-

Year	Horses in Observation	Positive Positive	Incidence Cumulative (%)	Incidence density (cases/horse-year)	Relative relative	Interpretation
1	90	3	3.3	0.033	-	Base year for comparison
2	87	2	2.3	0.023	0.69	The risk of infection decreased by 31% compared to the first year.
Total (2 years)	90	5	5.6	0.028	-	Low overall rate of new cases (2.8 per 100 horses-year)

Table 3. Summary of epidemiological indicators recorded during two years of EIA observation

*The initial number of animals at risk (90) is considered.

ses equine infectious anemia at the Llano San Juan de Ocozocoautla de Espinosa stable in Chiapas, not only were slight differences in the occurrence of new cases of the disease calculated between the first year and the following year, as indicated in Table 3, where the presence of the disease shows a downward trend, but the relative risk was also observed, as this indicator is essential for comparing the probability of infection or contagion of the disease between two periods or groups of animals at risk. Thus, the relative risk is described as 0.69, representing a 31% decrease in the risk of infection during the second year compared to the first, i.e., the comparison of both horse-year groups. This proportion suggests that epidemiological strategies such as control and elimination of positive animals may have had a favorable influence on reducing the transmission of the infectious agent.

Overall, as shown in Table 3, the epidemiological indicators show downward trends in the incidence of equine infectious anemia within the observation period. Without detracting from the importance of animal health surveillance and reinforcing preventive measures, the presence of the virus as the causative agent of equine infectious anemia can continue to be controlled in the stable studied for two years.

DISCUSSION

Given that most of the research on equine infectious anemia conducted in Mexico ex-

presses its results in terms of prevalence, the incidence parameter calculated in this study was compared with those values.

The results of the present study show a low occurrence of positive cases of Equine Infectious Anemia (EIA) during the two years of observation, with three positive horses in the first year and two in the second. This reflects a downward trend and a low incidence in the population evaluated, equivalent to 5.6% during the analy y period.

These findings differ from those reported by Martínez (2010), who evaluated the blood serum of 300 horses belonging to the Mexican Presidential General Staff Sports Field and did not detect any animals positive for Equine Infectious Anemia (EIA) using the ELISA and Agar Gel Immunodiffusion (AGID) tests, considered by national regulations to be the official diagnostic method. From this epidemiological perspective, the results suggest that the stable studied remains free of viral circulation, thanks to the effective application of health, management, and biosecurity measures, as recommended by the (WOAH/OIE, 2019).

On the other hand, according to reports by SAGARPA (CONASA, 1995), which recorded a 2.11% positivity rate in samples from slaughterhouses in different states, this study shows a favorable scenario that reflects the positive impact of veterinary supervision and epidemiological prevention in institutional equine units.

In accordance with Murga (2008), the na-

tional incidence of Equine Infectious Anemia (EIA) in Mexico was estimated at 1.25%, a figure considered low when compared to outbreaks recorded since 2003, in which prevalence ranged from 7% to 20% in horses diagnosed by serological tests. This variability indicates that the disease maintains a localized behavior, with its presence limited to regions and populations with unfavorable sanitary conditions or poor epidemiological surveillance.

However, in a study conducted on working equines (horses and donkeys), Sánchez-Contreras *et al.* (2018) reported a prevalence of 37.7% in horses in the municipality of Veracruz, Veracruz, Mexico, also using serological tests for the detection of Equine Infectious Anemia Virus (EIAV).

Similarly, serological evaluations carried out in 2003 by Barajas (2006) indicate that, in the state of Chiapas, Mexico, a true prevalence of 13.88% was observed using the Agar Gel Immunodiffusion test, as well as an apparent prevalence of 21.11% using the ELISA test; the latter showed a sensitivity and specificity of 100%, as it detected animals in the subclinical phase or with mild infection by the Equine Infectious Anemia Virus (EIAV).

When comparing our findings with previous reports by SAGARPA (CONASA, 1995), Murga (2008), and Martínez (2010), a progressive decrease in the incidence and prevalence of EIAV in different regions of Mexico can be seen, which shows the positive impact of the surveillance and control programs implemented. However, the study by Sánchez-Contreras *et al.* (2018) reported a prevalence of 37.7% in working horses in the municipality of Veracruz, Veracruz, which is consistent with the findings of Ibarra *et al.* (2025), who report a 38.4% seroprevalence of EIAV in Sonora, Mexico. Similarly, Villa-Mancera *et al.* (2024) published a seroprevalence of EIA in horses in the states of Puebla and Veracruz of 15.25% and 20.49%, respectively. Bar-

jas (2006) observed an actual prevalence of 13.88% and an apparent prevalence of 21.11% with different tests, respectively, in Chiapas, Mexico. These figures demonstrate the persistence of the virus in equine populations with less sanitary control and greater exposure to vectors, which underscores the importance of maintaining preventive strategies and epidemiological monitoring.

These values are considerably higher than those observed in the present study, which can be explained by differences in management conditions, lack of health control, and exposure to vectors in working animals, in contrast to the horses of the Caballería Llano San Juan de Ocozocoautla de Espinosa, where stricter surveillance and biosecurity protocols are in place.

In this regard, the results of Sánchez-Contreras *et al.* (2018) and Ibarra *et al.* (2025) reinforce the importance of the epidemiological context and type of management when interpreting the occurrence of positive cases, as environmental conditions and animal mobility directly influence the persistence of the virus in certain regions of Mexico.

The marked difference between the values reported in the states of Veracruz and Sonora and those observed at Caballería Llano San Juan demonstrates the effectiveness of the preventive measures applied in our institutional livestock units, where vector control and the timely elimination of positive animals allow low levels of viral circulation to be maintained. Therefore, active surveillance and health management continue to be the most effective tools for maintaining a favorable health status with regard to EIAV (WOAH/OIE, 2019).

Likewise, serological evaluations carried out in 2003 by Barajas (2006) indicate that, in the state of Chiapas, Mexico, a true prevalence of 13.88% was observed with the Agar Gel Immunodiffusion test, as well as an apparent prevalence of 21.11% using the ELISA test; the latter showed a sensitivity of 100%, as it detected animals in the subclinical phase or with mild infection by the Equine Infectious Anemia Virus (EIAV).

The difference between the two prevalences can be attributed to the fact that the ELISA test, being more sensitive, can yield false positive results. However, its use is recommended as an initial diagnostic tool, as it allows the identification of recently infected animals.

Comparing the above results with those of the present study, it can be seen that there are no high prevalences, suggesting a significant decrease in EIAV circulation in the population evaluated. This decrease may be due to the implementation of health programs, the systematic elimination of reactor animals, and epidemiological surveillance, as recommended (De la Sota *et al.*, 2005).

According to Spickler *et al.* (2010), the transmission of the virus among equids does not occur primarily through direct contact, but rather through blood-feeding arthropods. Therefore, by eliminating seropositive animals, the risk of contagion among susceptible individuals is reduced, which explains the observed decrease in positive cases and demonstrates the effectiveness of the prevention strategies implemented in this study.

In accordance with Murga (2008), the national incidence of Equine Infectious Anemia (EIA) in Mexico was estimated at 1.25%, a figure considered low when compared to the outbreaks recorded since 2003, in which prevalence ranged from 7% to 20% in equines diagnosed by serological tests. This variability indicates that the disease maintains a localized behavior, with its presence limited

to regions and populations with unfavorable sanitary conditions or poor epidemiological surveillance.

The results of this study show a similar incidence to those published by Murga (2008) and Martínez (2010), reinforcing the idea that the control and early detection programs implemented by health authorities have contributed to reducing viral circulation. The elimination of positive animals and the use of official diagnostic tests such as ELISA and Agar Gel Immunodiffusion (AGID), standardized by SADER and SENASICA, are effective measures for maintaining low levels of infection in exposed livestock (WOAH/OIE, 2019).

Taken together, these observations support the importance of maintaining active surveillance and mandatory reporting of positive cases in order to prevent resurgence and consolidate the downward trend of the disease at the national level.

However, a critical animal health control point still remains: in Mexico, there are no animal inspection points on highways or at borders where EIAV-free certificates are required for the movement of horses between Mexican states. This situation poses a health risk, as is the case at the Horse Fair in Texcoco, where horses from different states converge without isolation zones or health certification requirements, as reported by SAGARPA (CONASA, 1995).

Even so, it is worth mentioning that the Manual of Procedures for the Epidemiological Management of Equine Infectious Anemia (SENASICA, 2017), published periodically, has contributed to the control and focal or regional eradication of the disease, as explained in this study.

Finally, Murga (2008) highlights that the epizootiology of Equine Infectious Anemia (EIA) remains an issue that has not been fully resolved by the authorities and associations linked to the equine sector, which results in

periodic outbreaks in different regions of the country. This underscores the need to continue strengthening surveillance and biosecurity programs to maintain control of this disease in Mexico.

CONCLUSIONS

The evaluation of equine infectious anemia carried out at the Llano San Juan de Ocozacoatlá de Espinosa Cavalry in Chiapas indicated a cumulative incidence of 3.3% in 2017 and 2.3% in 2018. In other words, in the two years of epidemiological observation of this pathology, the cumulative incidence was 5.6%.

The results obtained over two years of field and laboratory observation show a low frequency of positive cases and a downward epidemiological trend in Equine Infectious Anemia (EIA), suggesting that the disease is under control in the population evaluated. The timely elimination of reactive animals and the application of appropriate animal health measures contributed to preventing the spread of the virus within the herd.

In this regard, the difference in prevalence values highlights that management practices, biosecurity, and veterinary supervision are determining factors in the presence of the virus. Therefore, constant surveillance, periodic serological diagnosis, and control of horse movement are essential activities for maintaining a favorable animal health status and preventing the reemergence of outbreaks.

The results obtained through the epidemiological management of this study will strengthen prevention and control operations through training and dissemination among regional livestock associations and their affiliates in Chiapas, charro associations, horse riders, and farmers, as well as the College of Veterinary Zootechnicians. promoting greater animal health awareness and the active participation of owners and users in epidemiological surveillance.

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