Contribution to a study on the seroprevalence of Equine Infectious Anemia in Tiaret province northwestern Algeria

Contribuição para um estudo sobre a soroprevalência da Anemia Infecciosa dos Equídeos na província de Tiaret, no noroeste da Argélia

Contribución a un estudio sobre la seroprevalencia de la Anemia Infecciosa Equina en la provincia de Tiaret, en el noroeste de Argelia

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ABSTRACT
A serological survey was conducted in equines in the province of Tiaret, in order to estimate the prevalence level of infection of equine infectious anemia (EIA). This survey has allowed to study the rate of infection by *EIAV* equine, to clarify the current situation of the EIA in the province of Tiaret, constitute a first step towards a national approach to control the emergence of this disease. Serum samples were collected from 293 horses and donkeys 30 and tested by ELISA technique. All results obtained by the ELISA test proved totally negative for all the samples studied. The equine infectious anemia is a topical disease. It is not well known and rarely sought on the ground. Yet the consequences of an outbreak can be very heavy on the economy.

Keywords: Equine Infectious Anemia, *EIAV*, serological survey, ELISA, Algeria.

RESUMO
Uma pesquisa sorológica foi realizada em equinos na província de Tiaret, com o objetivo de estimar o nível de prevalência da infecção por anemia infecciosa equina (AIE). Este levantamento permitiu estudar a taxa de infecção pelo EIAV em equinos, esclarecer a situação atual da AIE na província de Tiaret e constituir um primeiro passo em direção a uma abordagem nacional para controlar a emergência dessa doença. Amostras de soro foram coletadas de 293 cavalos e jumentos e testadas pela técnica ELISA. Todos os resultados obtidos pelo teste ELISA foram totalmente negativos para todas as amostras analisadas. A anemia infecciosa equina é uma doença atual. Não é bem conhecida e raramente é buscada no campo. No entanto, as consequências de um surto podem ser muito pesadas para a economia.

Palavras-chave: Anemia Infecciosa Equina, EIAV, encuesta serológica, ELISA, Argelia.

RESUMEN
Se realizó una encuesta serológica en equinos en la provincia de Tiaret, con el objetivo de estimar el nivel de prevalencia de la infección por anemia infecciosa equina (AIE). Esta encuesta permitió estudiar la tasa de infección por EIAV en equinos, aclarar la situación actual de la AIE en la provincia de Tiaret y constituir un primer paso hacia un enfoque nacional para controlar la aparición de esta enfermedad. Se recogieron muestras de suero de 293 caballos y 30 burros, las cuales fueron analizadas mediante la técnica ELISA. Todos los resultados obtenidos en el test ELISA resultaron totalmente negativos para todas las muestras estudiadas. La anemia infecciosa equina es una enfermedad actual. No es bien conocida y rara vez se busca en el campo. Sin embargo, las consecuencias de un brote pueden ser muy graves para la economía.

Palabras clave: Anemia Infecciosa Equina, EIAV, encuesta serológica, ELISA, Argelia.

1 INTRODUCTION

Equine infectious anemia (EIA) is a viral, transmissible, and potentially fatal disease, also known as "swamp fever" or "marsh fever" (Ishii & Ruizo, 1975). It has been documented in various geographic regions and is now considered a cosmopolitan disease found worldwide.

EIA is caused by infection with a Lentivirus, EIAV (Equine Infectious Anemia Virus), which belongs to the Retroviridae family. Only equids are susceptible to infection by EIAV, including horses, mules, zebras, and donkeys (Sendel, 2010). However, donkeys do not appear to develop clinical disease, unlike horses (Hans et al., 2012). A unique characteristic of EIAV infection in

equids is that it can be controlled by the immune response after one or several febrile episodes, remaining completely asymptomatic for the rest of the animal's life (Cadoré et al., 2007).

Clinical signs associated with the infection appear after an incubation period ranging from 7 to 21 days, depending on the host's immune status and the viral load at the time of infection. These signs mainly include fever, anemia, edema, and various symptoms of lethargy.

Following infection, several clinical forms may occur successively: an acute form characterized by severe clinical signs that may lead to death, a chronic form marked by recurring clinical episodes (fever, anemia, lethargy, thrombocytopenia, etc.), and finally an asymptomatic form (Hammond et al., 2000; Leroux et al., 2001). After the initial infection, equids remain infected for life and will continue to be sources of contagion for other animals, even in the absence of clinical signs (Issel et al., 1982). Asymptomatic animals may also develop clinical signs under stress (Hans et al., 2012).

EIA is classified as a vector-borne disease, meaning that a vector is required to transmit the pathogen. In this case, the virus is spread by flies such as horseflies or stable flies, and possibly some mosquitoes. It can also be transmitted via blood from an infected animal to a susceptible animal through a syringe, needle, non-sterile medical equipment, or during mating via the semen of infected stallions (Hawkins et al., 1973; Foil et al., 1983).

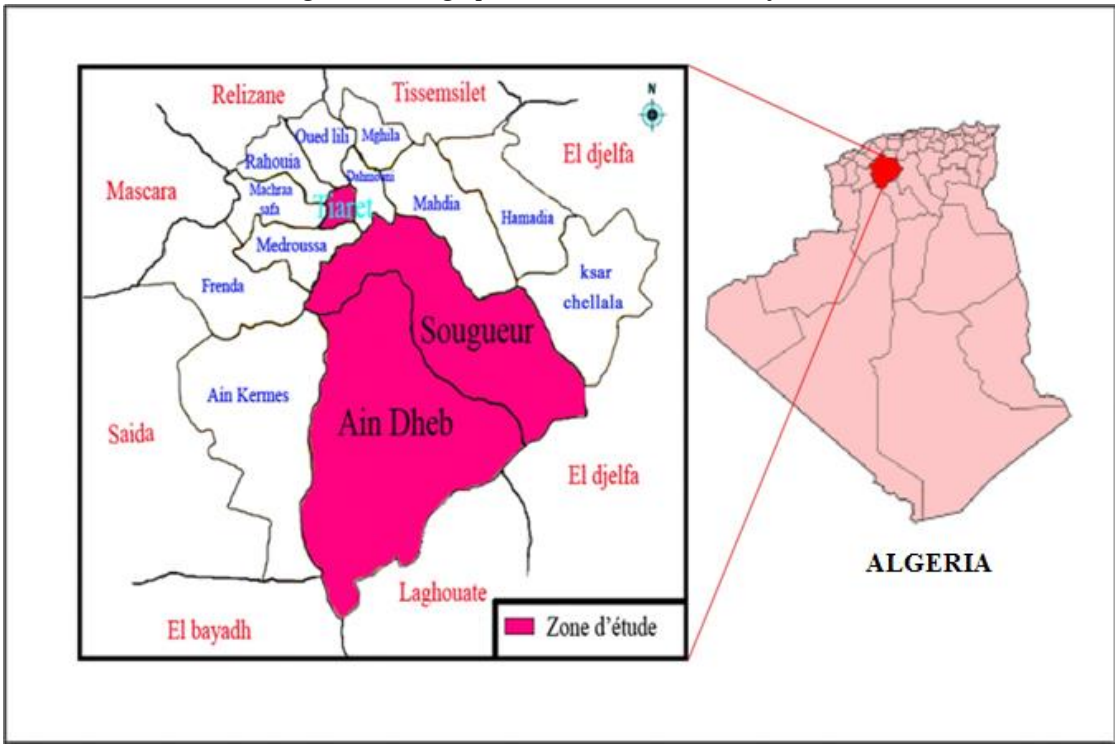
The officially recognized serological test for EIA worldwide is the agar gel immunodiffusion test (AGID; Coggins test) (Coggins et al., 1970). However, since the 1990s, the EIA-ELISA test has become the most widely used serological test in various countries. Its speed, specificity, and performance compared to the Coggins test have been key factors in validating this technique as the first-line method (Charles et al., 1993; Paré & Simard, 2004).

This study was undertaken with the aim of assessing the rate of EIAV infection in equids, clarifying the current status of EIA in the Tiaret region, and subsequently formulating recommendations to control the emergence or re-emergence of this disease.

2 MATERIAL AND METHODS

The study was conducted in the province of Tiaret (35° 22′ 30″ N, 1° 19′ 30″ E), situated in the northwest region of Algeria. Covering a total area of 200,505 km², Tiaret is located 300 km from the capital, Algiers. (Figure 01)

Figure 01: Geographical Situation of the Study Area



Source: Adapted from the geographical map of Algeria.

2.2 STUDIED EQUINE POPULATION

2.2.1 Calculation of the base sample size

For a survey model based on simple random sampling, the required sample size is calculated using the following formula (Falissard, 2005): $n=t^2 \times p(1-p)/m^2$

Three factors essentially determine the size of a sample:

- **p** = estimated prevalence of the variable studied
- **t** = targeted confidence level of 95% (typical value of 1.96)
- **m** = acceptable margin of error of 5% (typical value of 0.05).

The prevalence "p" considered in this study is represented by the highest prevalence found in the literature to date, which was 9.63% (p = 0.093) (Hans et al., 2012).

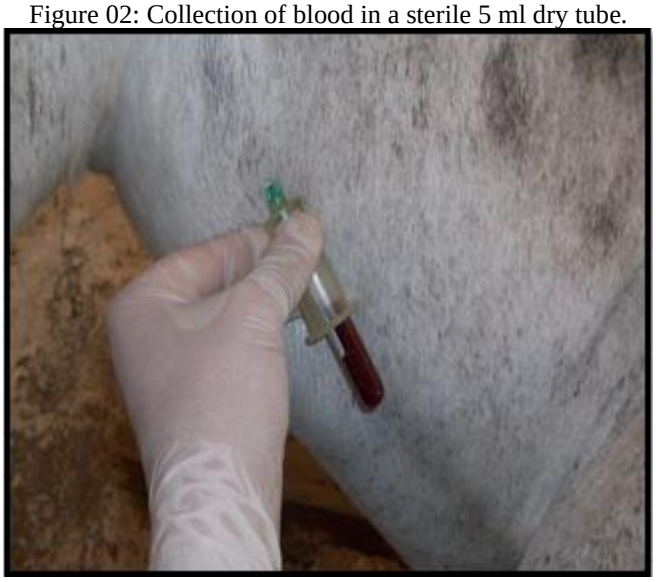
2.2.2 **Adjustment for difference and consideration of error risks**

The sample size was multiplied by two because oversampling is necessary in areas with high equine populations, and an additional 5% was added to the calculated sample size to account for registration error risks (Falissard, 2005). A total of 323 equids, including 293 horses and 30 donkeys, were sampled.

2.2.3 Collection of biological material

Each animal is held by its owner to undergo blood sampling. This is performed on a standing animal, at the level of the jugular vein with a stretched neck. Manual pressure is applied at the base of the neck with one hand while the other hand performs the venipuncture (Figure 02). The collected blood is placed in a sterile 5 ml dry tube and then centrifuged at 2500 RPM for 10 minutes.

Figure 02: Collection of blood in a sterile 5 ml dry tube.



Source: Prepared by the authors themselves.

The obtained serum is collected and stored in Eppendorf tubes, then labeled and frozen at -20°C until it is used for serological tests.

2.3 SEROLOGICAL TEST (ELISA)

The ELISA test was performed using a commercial kit ID Screen® (Equine Infectious Anemia Double Antigen/ID.VET innovative diagnostic, Montpellier, France). This kit detects anti-P26 antibodies in serum samples.

The reaction is quantified by reading at a spectrophotometer at 450 nm. Results are provided as optical density (OD) values. The positivity threshold is set at an index value ≥ 0.6 (60%). The interpretation of results provided by the manufacturer (ID.VET) based on the index value is as follows: ≤ 0.50: negative result; between 0.51 and 0.59: doubtful result; ≥ 0.60: positive result. Two horse sera were used as controls to validate the test: one positive control with an OD > 0.85 (85%) and one negative control < 0.40.

3 RESULTS AND DISCUSSIONS

This study represents the first investigation into the seroprevalence of EIA among the equine population in the Tiaret region of Algeria, where horse breeding is a strong tradition. Equids, particularly horses, play a major role in human life. Therefore, it is important to reduce the potential risk of economic losses caused by certain infectious diseases, including EIA.

The results obtained using the ELISA test on 323 serum samples showed that all tested equids were free of EIA. The overall seroprevalence of EIAV infection among equines was not significant, despite the conditions being conducive to the possible emergence of this disease. To better understand the nature of these results, several questions arose: Do the absence of positive results truly reflect the region's situation? Was the sample size of the equine population estimated with sufficient accuracy? Does this negative result represent a positive prospect for the future of the equine population? Can it be extrapolated to other regions in Algeria? Finally, are horses with non-significant results on the EIA-ELISA test truly free from infection?

This study has shown that, despite the significant equine population in Tiaret, the EIA virus is not widespread in this part of Algeria. The non-significant results do not necessarily indicate the absence of the disease in Algeria. These results suggest that: (i) EIA is not present in this part of the country, despite the abundance of hematophagous vectors, which are important risk factors; (ii) the sampled equids may not yet have developed an immune response against EIAV, as hosts develop an immune response during infection that keeps the viremia below the critical threshold for pathogenicity, allowing it to go unnoticed; and (iii) the method used (EIA-ELISA) can only detect infection 21 to 45 days after inoculation.

Compared to studies conducted in other Mediterranean countries, our results are similar to those reported by Albayrak and Ozon (2010) in Turkey, who collected 8,947 serum samples from equids, including 8,769 horses and 178 donkeys in northern Turkey. None of the sampled equids showed anti-EIAV antibodies. Similarly, no outbreaks have been officially declared in Albania or Spain to date (Yapkic et al., 2007; Kinmizigul et al., 2009; Albayrak & Ozan, 2010).

The lack of studies, publications, or information on the actual situation of EIA in Algeria makes it difficult to discuss the disease's emergence. Many infected equids are asymptomatic carriers and therefore represent a difficult-to-identify source of infection. However, this health threat appears relative, given that: (i) although the prevalence of EIA is unknown, it is likely very low, and (ii) observed outbreaks around the Mediterranean basin confirm the very low transmissibility of the infection by vectors, as long as infected equids do not show clinical symptoms (Issel & Foil, 1991).

Several studies, including those by Issel (2007), have shown that the probability of transmission by arthropods depends on several factors: (i) The viremia status of the donor horse is crucial. If the horse is in the acute phase with a febrile syndrome, experimental transmission is highly successful (one in seven horses is infected after a single bite). In contrast, in a latently infected horse without symptoms, the risk is much lower, or almost negligible. (ii) The vector species: the *Tabanus* genus is the most effective vector since it transmits a large volume of blood. (iii) Vector density: logically, the higher the number of vectors, the greater the risk of transmission. This density is higher in warm and humid regions and during the summer season. (iv) The population density of equids and the distance between individuals. An arthropod can only act as a vector if its blood meal on an infected horse is interrupted, and it continues feeding on a healthy horse, as the virus's infectivity in the arthropod does not seem to persist beyond four hours. (v) Favorable climatic conditions for the activity and survival of potential disease vectors, which likely create an ideal ecosystem for their reproduction. (vi) Hygiene and the environment in which the equid lives play a major role in the presence and intensity of the vector. Even though EIA was not detected in the study site, it remains an excellent breeding ground for larvae and provides the ideal biogeographical setting for the vector's life cycle. The site mainly consists of manure, contaminated by decomposing organic matter.

Nevertheless, even though the disease was not detected in the Tiaret region, and transmission of the infection appears to be a rare event, the importance of EIA should not be underestimated given the hidden transmission potential of the lentivirus and the associated consequences for equine professionals (euthanasia of infected equids and movement restrictions). Unlike in Algeria, where no data on the current status of the disease exists, EIA has been reported in almost all Mediterranean countries, with relatively low prevalences, ranging from 4.5% in Greece to 0.12% in Palestine (Dauphin et al., 2005; Sublime, 2006; Hans et al., 2012). The number of reported cases of EIAV-infected horses in Tunisia, Morocco, and Libya is relatively low, with prevalence rates of 0.22%, 0.6%, and 0.04% respectively in horses and slightly higher in donkeys and mules.

EIA is also present in the United States and Asia (Lew et al., 1993; MALLIGA et al., 2001). These differences in observed prevalence are primarily due to the investigation periods separating the various studies, with over 20 years between some studies (Pearson & Knowles, 1984; Camargo, 2012) and this one, and to the screening techniques used.

DETECTION OF INFECTION

During epidemiological investigations, detecting an infection, particularly a viral infection like EIA, the subject of this study, requires a method that can identify not only symptomatic cases

but also asymptomatic ones. Serological techniques, which highlight markers of infection, are well-suited for this type of investigation. Among the most commonly used techniques in serological diagnosis, agar gel immunodiffusion (AGID), or the Coggins test, is considered the reference method recommended by the World Organization for Animal Health.

Several factors have contributed to changes in screening methods. In recent years, the EIA-ELISA test has been the most widely used by various laboratories worldwide (Charles et al., 1993; Issel et al., 2013). The ELISA test targets the p26 antigen of the EIAV virus, which is found on the surface of the virus and is relatively specific to EIA (Alvarez et al., 2007b).

The speed of the ELISA test compared to the Coggins test (AGID) has been an important factor for the equine industry. Indeed, ELISA test results are available in a few hours, whereas the AGID requires 48 hours. Laboratories authorized to screen for EIA have also expressed a preference for ELISA: this efficient method allows for the accurate analysis of large numbers of serum samples in a relatively short time (Paré & Simard, 2004; Issel et al., 2013).

This test has the advantage of being less subjective than the AGID and could be fully objective if an ELISA reader is used. The antibody status of a horse can be determined in two hours by comparing the color produced by the test sample to that produced by a weakly positive sample (Matsushita et al., 1989). Indeed, the EIA-ELISA test primarily detects immunoglobulin M (and to a lesser extent IgG), which decrease over time after infection. It is therefore possible that the test does not detect all chronically infected animals, whose immunoglobulin M levels are very low (Soutullo et al., 2001).

According to the current protocol, when a laboratory authorized to screen for EIA reports a negative result on an EIA-ELISA test, the horse is considered negative, and no further analysis is required. However, when a laboratory obtains a positive or atypical result in an EIA-ELISA test, the laboratory declares the ELISA test inconclusive and indicates that the result must be confirmed by the AGID (OIE, 2013).

Both the AGID and ELISA are simple, accurate, and reliable methods for detecting infection by the EIA virus. With ELISA, the detection of antibodies is possible a little earlier and at lower titers than with the AGID, but ELISA can occasionally give false positive results. Therefore, the World Organisation for Animal Health (OIE) recommends that a positive ELISA result requires confirmation through an AGID test (OIE, 2013)

SHOULD WE FEAR THE EMERGENCE OF EIA?

The number of reported cases of horses infected with EIAV is relatively low. However, it is important to note that despite this infection being a notifiable contagious disease, few or no

screening tests are performed in relation to the number of equids recorded in the Tiaret region and the number of transactions carried out. While the economic impact seems to be minimal in Algeria, at least on the surface, the detection of an outbreak can, in some cases, take on unexpected proportions, given that infected equids must be euthanized. Moreover, in almost all outbreaks observed in recent years, mainly in Europe, it has never been possible to formally explain the origin of the episode despite numerous investigations carried out by various authors (Cadoré et al., 2007; Hans et al., 2012; Issel et al., 2013).

On one hand, the frequent and numerous movements of equids are a specific characteristic of this species compared to other livestock, making epidemiological investigations complex, broad, and non-specific. On the other hand, the observed outbreaks have highlighted management challenges, particularly due to the special status of horses, which range from companion animals to livestock, as well as sport or working animals. These various statuses (which can change over the course of an animal's life) determine multiple sectors that are highly interconnected but whose interests are not always aligned (Cadoré et al., 2007). These sectors show significant variability in structure and levels of professionalism, as well as in their awareness of certain regulatory requirements, such as the screening for specific infectious diseases.

An analysis of the results indicates that the EIA situation in Algeria, particularly in the Tiaret province, is difficult to improve. The equine industry is poorly organized, with limited state involvement. Even though the government is interested, it is not willing to invest significant funds. Many professionals are financially constrained and see no benefit in improving their health situation because they perceive no economic gain, except, of course, in the racing sector.

4 CONCLUSION

The current functioning of the equine sector and the state provisions do not currently allow for a regulatory evolution towards rigorous EIA screening. However, several measures could be implemented to increase epidemiological knowledge of EIA and raise awareness among industry stakeholders.

Thus, even though the incidence and prevalence of this infection are low in most countries, they remain significant from an epidemiological, medical, economic, and scientific standpoint. The various cases recently described in Europe should encourage vigilance regarding the circulation of this virus.

It is always difficult to fully understand the potential emergence of a new outbreak, as even though natural infection may remain asymptomatic after one or more febrile-anemic episodes, it can, in some cases, be very debilitating and lead to the death of the animal (Issel et al., 2013).When

an outbreak is identified, health control measures are strict, and any seropositive animal must be culled (Tuffert, 2011).

However, actions to be taken in the event of an emergence could include the following:

- Strengthening voluntary screenings through veterinarians. They should be encouraged to test horses more frequently in the case of a suggestive clinical picture, upon introduction into a herd, or during transactions. Focusing research on horses presenting clinical signs seems crucial since a horse with clinical signs presents the highest risk of transmitting the disease to other horses.
- Communicating with industry stakeholders about the potential existence or introduction of EIA from a neighboring infected country and the presence of cases in Europe in recent years, raising awareness about the implications of EIA, especially to encourage testing of horses when purchased.
- Improving knowledge of the actual epidemiological situation of the infection through screenings in equine populations considered at risk. If epidemiological investigations reveal outbreaks, they could be used to justify more regular testing

It is recommended to conduct additional epidemiological studies and expand the study area to improve the understanding of transmission and risk factors. This would allow for a better assessment of disease prevalence in various regions and help optimize control and prevention strategies.

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