

Published: January 31, 2023

**Citation:** Dionísio L, Medeiros F, et al., 2023. Vaccination and Laboratory Diagnosis of Equine Influenza in Portugal in Comparison with Other European Countries, Medical Research Archives, [online] 11(1).

<https://doi.org/10.18103/mra.v11i1.3360>

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DOI

<https://doi.org/10.18103/mra.v11i1.3360>

ISSN: 2375-1924

## RESEARCH ARTICLE

### Vaccination and Laboratory Diagnosis of Equine Influenza in Portugal in Comparison with Other European Countries

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#### ABSTRACT

Equine Influenza (EI) is a disease caused by the genus A influenza virus, with a global distribution and under constant review. Equine Influenza is highly contagious and affects the respiratory tract. Vaccination in horses is an adequate prevention method, however some strains have the capacity to infect immunized horses, due to the mutagenic changes that the virus undergoes. This work aimed to study the Veterinary Medicine performance regarding Equine Influenza in Portugal in the years 2018 and 2019, and to compare the clinical practice in Portugal with that of other European countries. The study was based on the responses to a questionnaire consisting of nine questions on: 1) Geographical area of clinical practice; 2) Activity of equines assessed; 3) Number of suspected cases of Equine Influenza in the last two years; 4) Number of cases of Equine Influenza diagnosed with the support of laboratory tests in the last two years; 5) Number of vaccinations against Equine Influenza in the year 2018; 6) Number of vaccinations against Equine Influenza in the year 2019; 7) Whether the vaccination protocol used follows FEI/FEP standards; 8) Whether the vaccination protocol is annual or biannual; and 9) Use of laboratory tests for Equine Influenza in the last two years. The surveys were circulated online in Portugal and in Germany, France, Ireland, Italy, the Netherlands, the United Kingdom and Sweden for anonymous reply. In Portugal, 50 responses to the survey were obtained. Veterinarians reported suspected cases. Only four cases were confirmed using laboratory tests. 22 professionals applied more than 100 vaccines against Equine Influenza in the year 2018, and a decrease was observed in the year 2019. The Veterinarians did not reveal a preference for biannual or annual vaccination. Most professionals (94%) did not use laboratory tests to confirm the suspected infection. In Portugal it was possible to identify an opportunity to improve the clinical practice of Veterinarians in the use of laboratory tests for disease diagnosis and vaccination. The decree law that establishes the compulsorily notifiable diseases does not match the list of the World Organisation for Animal Health (OIE) and therefore the General Directorate of Food and Veterinary (DGAV) does not have the data needed to properly notify the disease. Equine Influenza needs greater attention in Portugal and there are a number of measures that can be adopted to improve disease management in the country.

**Keywords:** Diagnosis, Equine, Influenza, Vaccination

## Introduction

Influenza, also called the flu, is an extremely contagious respiratory disease caused by the influenza virus (IV). The virus predominantly affects the upper respiratory tract (nose and throat), and in some cases may affect the lungs.<sup>1</sup>

IVs capable of infecting humans and causing epidemics and pandemics are viruses of the genera *Influenzavirus* A, B and C, of the *Orthomyxoviridae* family. In addition to these, *Influenzavirus* D was identified in 2011. IVs infect different species: Type A infects humans and animals in general, such as horses, birds, pigs, and dogs; Type B infects humans, seals, and ferrets; Type C infects humans, dogs, and pigs, and Type D was identified in goats, sheep, pigs and cattle. However, antibodies for Type D have already been identified in horses and humans.<sup>2,3,4,5,6,7,8</sup> Although the pathogenesis of the latter virus has not been fully studied, some authors argue that humans, like pigs, can be infected with all IVs.<sup>9,10</sup> Pigs and chickens are considered two key reservoirs for IVs.<sup>11</sup> However, the classification of natural reservoirs belongs mainly to water birds and wild sea birds.<sup>12</sup>

The clinical diagnosis of this disease is based on the clinical signs presented by the infected animals, which can be confirmed by supplementary diagnostic tests.<sup>13</sup> Diagnosis using supplementary diagnostic imaging methods, such as ultrasound, is still not used frequently.<sup>14</sup> Among the most common clinical signs are mucopurulent nasal discharge, dry cough, fever, depression, and lack of appetite. The presence of these signs and their intensity may vary according to the age of the animal and the individual's susceptibility to the disease.<sup>13,14,15,16,17</sup>

Treatment of a horse with equine influenza (EI) is based on management of the disease and rest for the animal. Although Amantadine has been tested for the treatment of EI, the existence of a specific antiviral available on the market to treat this disease has not yet been described. The use of neuraminidase inhibitors is indicated at an early stage, as it reduces the spread of the virus and limits its transmission to other animals.<sup>18,19</sup> Although its efficacy is not fully known, animals with EI can be given mucolytics. The administration of corticosteroids and the use of antitussives is contraindicated due to their side effects and the possibility of masking complications, while the use of non-steroidal anti-inflammatory drugs should be considered.<sup>20</sup> The use of antipyretics with non-steroidal anti-inflammatory action is not contraindicated, and the following drugs can be used: phenylbutazone, flunixin meglumine or dipyrrone.<sup>21</sup>

Vaccine surveillance and updating programs remain the best way to prevent and

control EI.<sup>22</sup> In the event of outbreaks, a strategic vaccination programme can be carried out in order to control the spread of the disease.<sup>23</sup>

Given the health and economic relevance of EI and its possible prevention through the implementation of vaccination protocols, it was considered appropriate to study the medical-veterinary practice in Portugal regarding vaccination against EI and its diagnosis using laboratory tests, in the years 2018 and 2019. In order to allow a comparative analysis of Portugal in the European context to be made, the study also included data from some other European countries.

## Materials and Methods

The study was based on a prepared survey consisting of nine questions on:

- 1) Geographical area of clinical practice
- 2) Activity of the equines assessed (sport, leisure, or breeding)
- 3) Number of suspected cases of EI in the last two years (0-5; 6-10; 11-20; 21-50; >50)
- 4) Number of cases of EI diagnosed with the support of laboratory tests in the last two years
- 5) Number of vaccinations against EI in the year 2018 (0-20; 21-50; 51-100; >100)
- 6) Number of vaccinations against EI in the year 2019 (0-20; 21-50; 51-100; >100)
- 7) Whether the vaccination protocol used follows FEI/FEP standards
- 8) Whether the vaccination protocol is annual or biannual
- 9) Use of laboratory tests for EI in the last two years

The surveys were disseminated online for anonymous response by Veterinarians engaged in Equine clinical practice, in order to protect the identity of the professionals and their patients. In order to conduct a more comprehensive study and compare the clinical practice of Portugal with other European countries, the survey was disseminated in the following countries: Germany, France, Ireland, Italy, the Netherlands, Portugal, the United Kingdom and Sweden. These European countries were selected based on the occurrence of EI outbreaks in the years 2018 and/or 2019 reported by the OIE.

The study was carried out between 1 September 2019 and 16 March 2020, first in Portugal and then extended to the selected Member States. The survey was made available in Portuguese in Portugal and in English in the remaining countries. In Portugal, the survey was disseminated by the Portuguese Association of Equine Veterinarians and shared on a Facebook

group of Equine Veterinarians. The survey was disseminated by the British Equine Veterinary Association in the United Kingdom. In Ireland it was disseminated by the Equine Interest Group of Veterinary Ireland and by the publication of an article in the Veterinary Ireland Journal. An electronic message (Vetalert) was also sent to Equine Veterinarians as an incentive to participate in the study. The Deutschen Reiterlichen Vereinigung of Germany, the Koninklijke Nederlandse Hippische Sportfederatie of the Netherlands, the Association Vétérinaire Equine Française of France, the Società Italiana Veterinari per Equini of Italy, and the Sveriges Veterinarförbund of Sweden were also contacted.

### Statistical Analysis

In this study only the data collected through the model survey are referred to and analysed. The descriptive statistical analysis of the data was performed using Microsoft Excel (Microsoft, USA).

## Results

### Inclusion of European Countries in the Survey

In addition to Portugal, several Member States were invited to disseminate the survey online: Germany, France, Ireland, Italy, the Netherlands, the United Kingdom and Sweden. However, not all accepted the invitation to participate in the study, and no response was obtained to the contact requests sent by e-mail or to the survey itself from the *Deutschen Reiterlichen Vereinigung* (Germany), the *Società Italiana Veterinari per Equini* (Italy) and the *Koninklijke Nederlandse Hippische Sportfederatie* (the Netherlands).

The *Association Vétérinaire Equine Française* (France) did not respond to the e-mail invitation, but two survey responses were obtained. The *Sveriges Veterinarförbund* (Sweden) responded to the e-mail invitation, declining to disseminate the survey, but two survey responses were obtained.

The countries that actively participated in the study and disseminated the survey were Portugal, Ireland, and the United Kingdom (Figure 1).



**Figure 1.** Participation of Member States in the Study. The countries that agreed to participate in the study and in the dissemination of the survey are shown in green. Countries that declined to disseminate the survey, but from which survey responses were obtained, are shown in orange. Countries from which no response to the electronic contact or no response to the survey was obtained are shown in red.

### Responses to the Survey in the Various Member States

#### Question 1 - Geographical area of clinical practice

There were 87 responses to the survey from different sources. Most of them were from Veterinarians practicing in Portugal (57.5%), while the rest were from professionals practicing in the

United Kingdom (19.5%), Ireland (18.4%), Sweden (2.3%) and France (2.3%) (Figure 2A).

#### Question 2 – Activity of equines assessed

The clinical practice of Portuguese Veterinarians participating in the study is mainly with sport horses (24/50) (Figure 2B).

The veterinary practice of the professionals from other countries who replied to the survey focuses mainly on sport (14/37) and leisure equines (17/37) (Figure 5). Only 12 of the professionals replying to the survey have practices focusing on racing (4/37) and breeding (2/37) animals (Figure 2C).

### **Question 3 - Number of suspected cases of EI in the last two years**

Veterinarians active in Portugal reported the existence of suspected cases of EI in 2018 and 2019. Among these, 12 professionals reported 6 to 20 suspected cases, and only one practitioner reported a high number of suspected cases (>50 cases) (Figure 2D).

Of the 37 practitioners active in other European countries, 13 reported suspected cases of EI in 2018 and 2019. Of these, four professionals had a high number of suspected cases of the disease (>20 cases). The remaining 24 participants could not confirm any suspect cases (Figure 2E).

### **Question 4 - Number of cases of EI diagnosed with the support of laboratory tests in the last two years**

Of the 50 suspected cases of EI in Portugal, the diagnosis was confirmed by laboratory tests in only four cases (Figure 2F).

In the other countries where the study took place, 60 cases of EI were confirmed among the suspected cases reported by 37 professionals (Figure 2G).

### **Question 5 - Number of vaccinations against EI in the year 2018**

The Veterinarians who participated in the study in Portugal reported that they vaccinated equines against EI in the year 2018, with 22 of the professionals administering more than 100 vaccines (Figure 2H).

The replies from the other countries showed that 21 of the professionals applied more than 100 vaccines and only six professionals administered less than 51 vaccines in 2018 (Figure 2I).

### **Question 6 - Number of vaccinations against EI in the year 2019**

The data regarding vaccination against EI in Portugal in the year 2019 were similar to those observed in the year 2018. However, a decrease

was observed in the number of professionals who administered more than 100 vaccines and an increase in the number of professionals who administered 0-20 vaccines or 51-100 vaccines (Figure 2J).

As to the data of other professionals regarding vaccination against EI in the year 2019, the values are very similar to those observed in the year 2018, as are the data for Portugal. An increase can be observed in the number of professionals who administered more than 100 vaccines and a decrease in those who administered 0-20 vaccines (Figure 2K).

### **Question 7 - Whether the vaccination protocol used follows the FEI/FEP standards**

The majority of the Veterinarians in Portugal (90%) follow the vaccination protocol recommended by FEI. None of the professionals followed the recommendations of the Federação Equestre Portuguesa (FEP) (Figure 2L).

Most professionals from other countries (73%) follow the vaccination protocol recommended by FEI, while some professionals (24%) follow the protocols recommended by their country's federations or regulatory bodies. Only one Veterinarian stated that they did not to follow any recommendation (Figure 2M).

### **Question 8 - Whether the vaccination protocol is annual or biannual**

According to FEI, the Veterinarian can adopt two types of vaccination protocol: biannual or annual, on the basis of the activity or mobility of the horse. The professionals in Portugal did not reveal a preference for biannual or annual vaccination (Figure 2N).

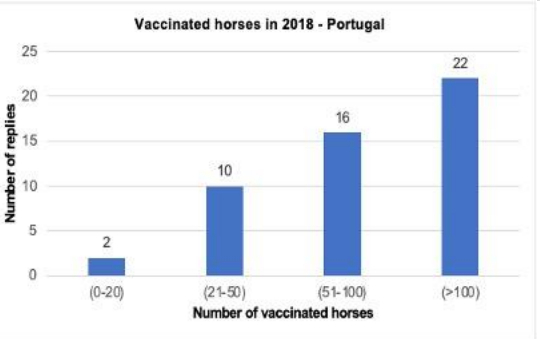
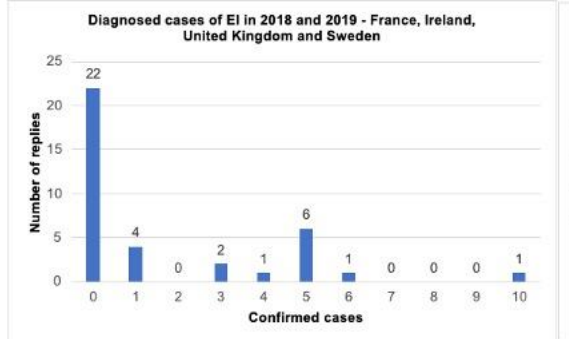
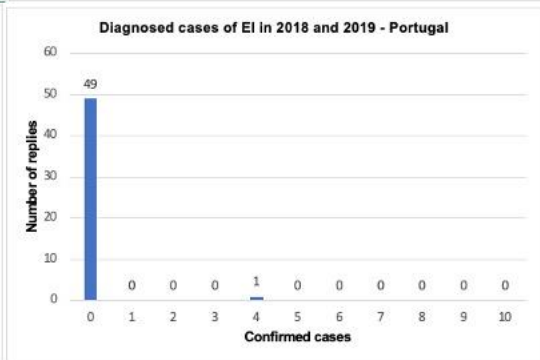
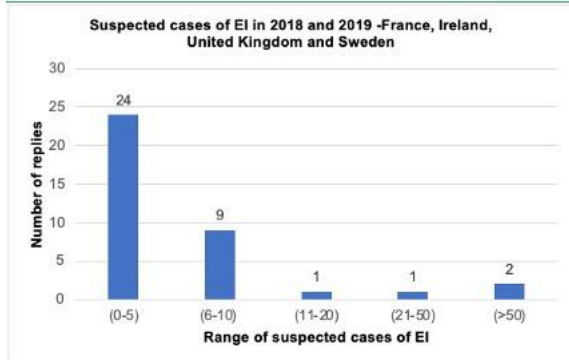
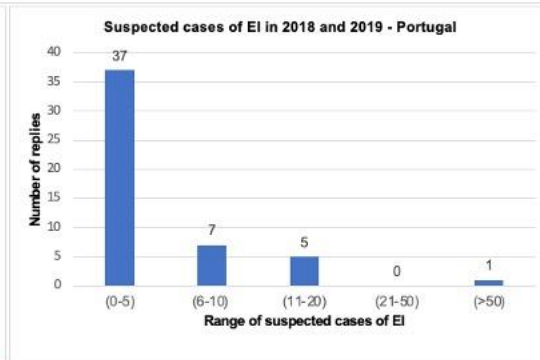
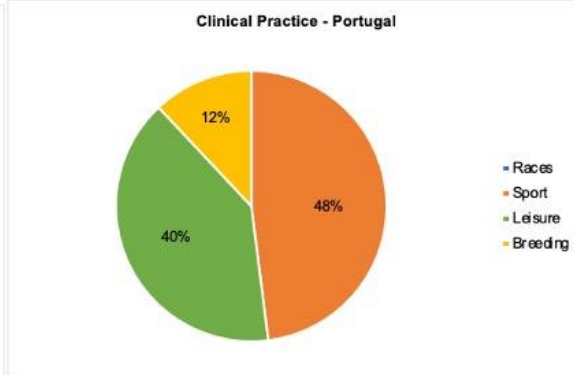
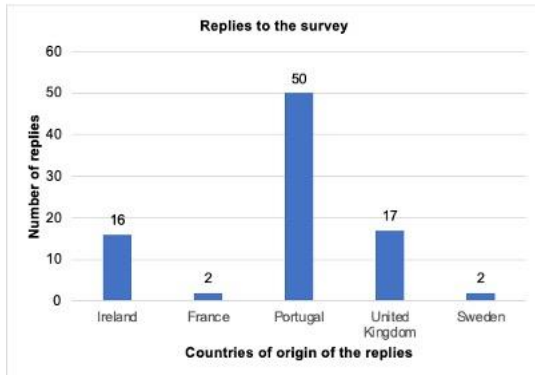
Professionals from other European countries (73%) showed a preference for the annual vaccination protocols (Figure 2O).

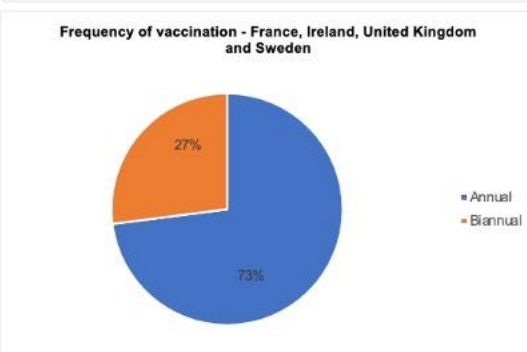
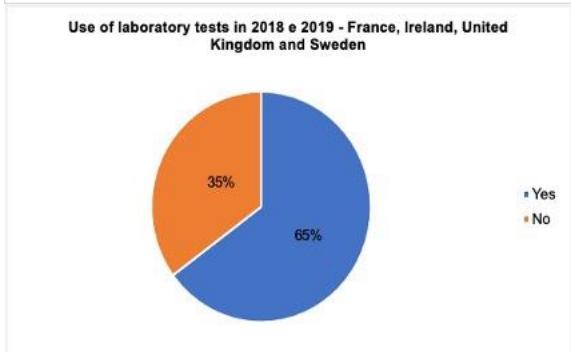
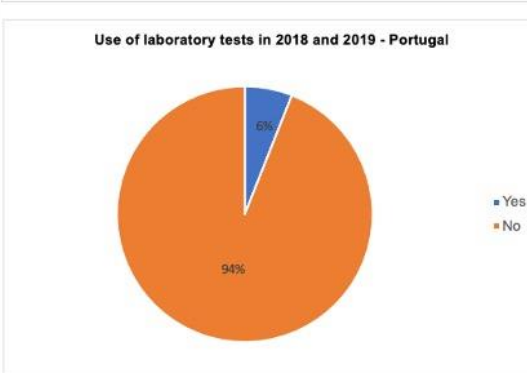
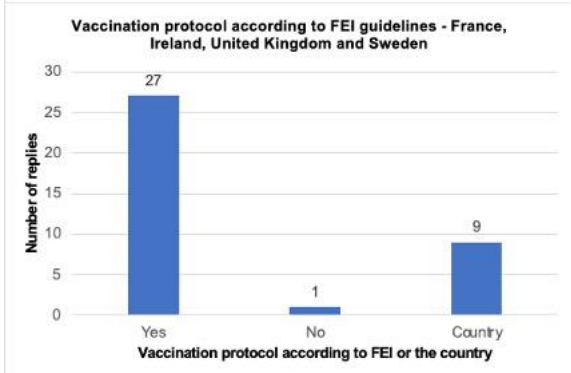
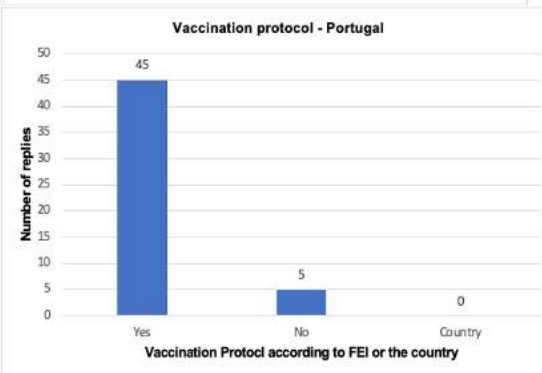
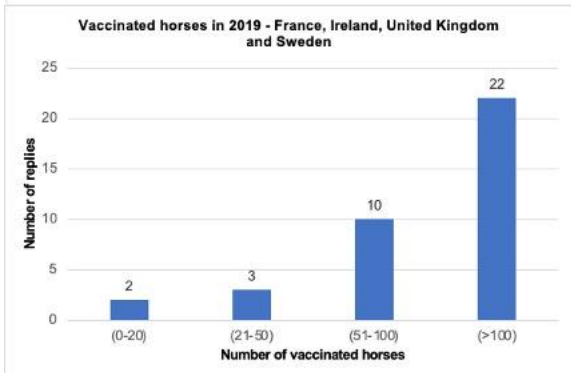
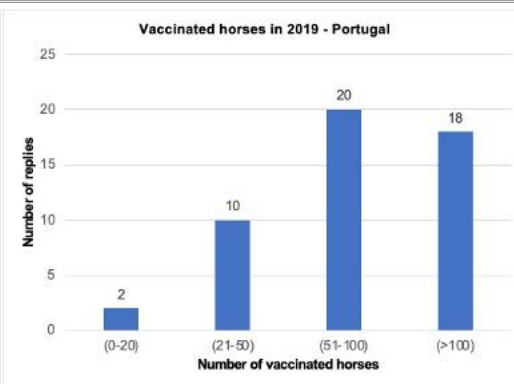
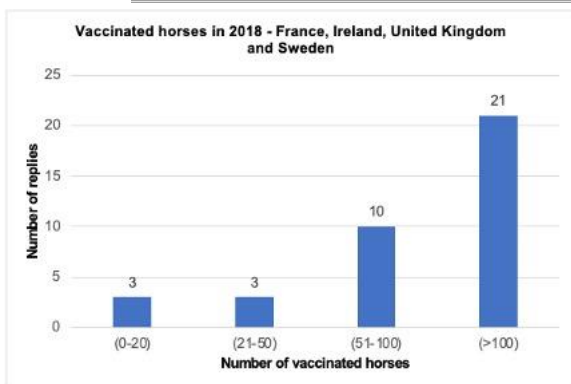
### **Question 9 - Use of laboratory tests for EI in the last two years**

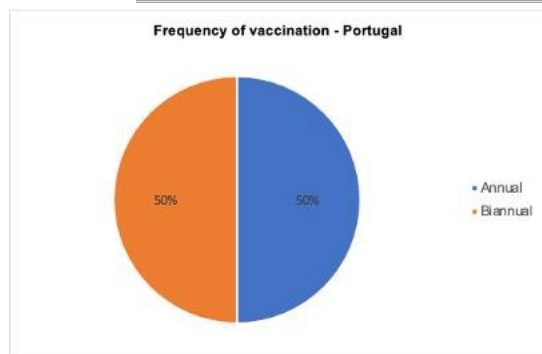
The majority of Veterinarians in Portugal (47/50) stated that they did not use laboratory tests to screen or confirm their suspected EI cases in the years 2018 and 2019 (Figure 2P).

Regarding professionals from other countries, the majority (24/37) stated that they used laboratory tests to screen or confirm their suspected EI cases in 2018 and 2019 (Figure 2Q).

Original results (replies to the questions)







**Figure 2.** Responses to the various survey questions. **A.** Countries where the participating veterinarians carry out their clinical activity; **B.** Activity of the equines treated by responding Veterinarians to the survey in Portugal. **C.** Activity of equines treated by responding Veterinarians in France, Ireland, United Kingdom and Sweden. **D.** Suspected cases of Equine Influenza (EI) in 2018 and 2019 in Portugal. **E.** Suspected cases of Equine Influenza (EI) in 2018 and 2019 in France, Ireland, the United Kingdom and Sweden. **F.** Cases of EI diagnosed in Portugal using laboratory tests. **G.** Cases of EI diagnosed in France, Ireland, the United Kingdom and Sweden using laboratory tests. **H.** Vaccination against Equine Influenza (EI) in 2018 in Portugal. **I.** Vaccination against Equine Influenza (EI) in 2018 in France, Ireland, the United Kingdom and Sweden. **J.** Vaccination against Equine Influenza (EI) in 2019 in Portugal. **K.** Vaccination against Equine Influenza (EI) in 2019 in France, Ireland, the United Kingdom and Sweden. **L.** Equine Influenza (EI) vaccination protocol adopted by Veterinarians in Portugal. **M.** Equine Influenza (EI) vaccination protocol adopted by Veterinarians in France, Ireland, United Kingdom and Sweden. **N.** Frequency of vaccination against Equine Influenza (EI) in Portugal. **O.** Frequency of vaccination against Equine Influenza (EI) in France, Ireland, the United Kingdom and Sweden. **P.** Use of laboratory tests for the diagnosis of Equine Influenza (EI) in Portugal in 2018 and 2019. **Q.** Use of laboratory tests for the diagnosis of Equine Influenza (EI) in France, Ireland, the United Kingdom and Sweden in 2018 and 2019.

## Discussion

Equine influenza (EI) is a highly infectious disease that affects the respiratory system of horses, with a high economic impact.<sup>24,25</sup> The constant contact of humans with animals, and between animals, is a predisposing factor for the spread of the disease, being considered an important public health problem. This study sought to provide information on the practices of Veterinarians in Portugal regarding EI and compare them with those of other Member States, providing alternatives that may be of value in the future.

The study was well accepted by Veterinarians and Veterinary Associations of equines in Portugal, the UK and Ireland. In Ireland, the *Equine Interest Group of Veterinary Ireland* showed a particular interest in the study and supported and promoted the dissemination of the survey through *VetAlert* and the *Veterinary Ireland Journal*. This interest was further expressed through a request for a specific individual report with the results of the survey for Ireland. The entities contacted in Germany, France, Italy, and the Netherlands did not reply to the invitation to participate in the study, while *Sveriges Veterinärforbund* (Sweden) declined to disseminate the surveys. The data provided by this institution did not match the questions asked, which prevented their data from being included in the comparative analysis. Despite the lack of response to the

invitation, responses to the questionnaire were obtained from France and Sweden.

Veterinarians answering the survey in Portugal mainly treat horses for sport, while professionals from other countries mainly treat horses for leisure. In Portugal and the other Member States included in the study, a lower percentage of Veterinarians were more directed to breeding equines. However, when looking at the figures for racing horses, no Veterinarian in Portugal indicated that their clinical practice focused on horses involved in this activity while, on the other hand, 11% of the professionals in the other Member States indicated that their professional activity involved these animals. This situation was foreseeable, as horse racing in other countries is more visible, and there are associations and entities which regulate this activity of high economic importance.

Evaluating the suspected cases, we can state that the Veterinarians in Portugal had fewer suspected cases in comparison to the professionals from the other European countries included in the study. We observed that only one Portuguese Veterinarian had more than 50 suspected cases in the last two years, while two professionals from the other countries had more than 50 suspected cases and one professional had between 21 and 50 suspected cases.

In Portugal, only one Veterinarian identified four cases of EI using laboratory tests in

the last two years. Regarding the other countries, where the declaration of the disease is compulsory, 60 cases of EI were identified using laboratory tests

Data collected in Portugal indicated that Veterinarians administered fewer vaccines in 2019 when compared to 2018.

The use of annual or biannual protocols does not imply a greater risk and they should be applied according to the equines' activities and their movements. In general, vaccination every six months is recommended so that the antibodies developed by the vaccines are at values considered protective. Annual vaccination is better when compared to no vaccination or the use of only primary vaccination, when used. Observing the data, we can state that 10% of Portuguese Veterinarians do not follow the vaccination protocol recommended by FEP/FEI, this being one of the aspects to be improved at national level and that should be analysed by the competent authorities. In the remaining countries, only 3% of professionals do not use the vaccine protocol recommended by the FEI or regulatory body of the country.

The Portuguese Veterinarians who participated in the study did not show a preference between using annual or biannual vaccination protocols. In contrast, professionals from other countries showed a preference for the use of annual vaccination protocols.

Analysing the study data in detail, we observed that the number of Veterinarians who resorted to the use of laboratory tests does not correspond to the same number of professionals with more than five suspected cases of disease in the last two years. Only three out of the 50 professionals (6%) who participated in the study in Portugal resorted to the use of laboratory tests. This figure was higher in the other Member States (France, Ireland, the UK, and Sweden), with 65% of the professionals using laboratory tests. This difference can be explained by the fact that the declaration of the disease is compulsory in these countries, which is in line with the OIE guidelines.

There is, therefore, a set of measures to be taken in Portugal to improve the management of the disease.

Government Organisations and Health Authorities

- Inclusion in national legislation of what is stipulated by the OIE regarding the compulsory international declaration of EI.
- Creation of a surveillance and data collection program for EI that is constantly updated. This surveillance method should be in digital format and made available online so that researchers and veterinarians can access the data and

provide information relating to their clinical practice (anonymously to ensure data protection). This program should support the analysis of the direct and/or indirect economic impact of EI.

- Creation of a support fund for the disease and its research or study.

Professional Organisations

- Preparation of a guide for action by the Veterinary Medical Association with guidelines on the implementation of good practices in the study of cases, confirmation of diagnosis through laboratory tests, treatment, and vaccination protocol.
- Development of an awareness and training program for health professionals for the effective implementation of the guidelines for action, with positive reinforcement and recognition of all those who comply with the training program and demonstrate the adoption of good practices in their clinical activity.
- Development of an advisory program for horse owners and the owners and managers of equine facilities and equestrian centres, in order to promote vaccination practice for preventive control of the disease, to advise on the design of premises for the best management of the disease in the event of its occurrence and on the dissemination of the rules of action by all those normally involved on the premises. Inclusion of positive reinforcement and recognition of all those who adopt the recommendations.

Veterinarians

- Implementing EI vaccination practice in their clinical activity.
- Confirm suspected EI by laboratory testing, including a study of the infecting strain.

This study could be improved, namely regarding its international dissemination. The attitude of the Veterinarians in Portugal towards the study deserves to be praised, due to the willingness shown to share data on their clinical practice. Veterinarians in Ireland, on the other hand, also deserve to be recognised for their support, since although the request for dissemination of the study was made well before the date of its launch in Ireland, its dissemination coincided with the rapid spread of COVID-19 at international level and in the European area in particular. Despite this fact and the fact that Veterinarians in Ireland play a key role in local communities in situations of public health



crisis, they made time to participate in the study at a time when Ireland was in the midst of a pandemic. The study could be improved if the response time was extended with the aim of increasing the response rate, allowing for a more robust analysis.

### Conclusions

The use of laboratory tests for the confirmation of diagnosis is of paramount importance in the study of viral strains circulating in a given period of time, influencing the OIE on the strains to be approved for the use of vaccination. Data from the comparative qualitative study carried out point to the conclusion that in Portugal there is a level of adherence to vaccination and use of laboratory tests for diagnosis of EI, although it is low when compared to other European countries.

The study of the applicable national regulations shows that the obligation to declare the disease internationally has not been included in national legislation. This disease deserves greater attention from the Portuguese health authorities and from Veterinarians, and there is a clear opportunity to improve prevention and control of the disease, for example by increasing the number of reports of

suspected cases of the disease, the use of laboratory tests to confirm the diagnosis of EI, the use of vaccination protocols issued by the regulatory authorities and the declaration of the disease to the OIE.

As a final consideration, it should be mentioned that EI control is of increased importance in the case of public health risk, as the one we are currently experiencing, related to the SARS-COV-2 pandemic infection, COVID-19, since the possible interspecies transmission of EIV to humans may result in the subsequent development of viral respiratory infection, increasing the risk of development of concomitant severe disease. This may be a subject of future study if the necessary data can be collected.

**Conflict of Interests:** None to declare.

### Acknowledgments

This work was supported by National Funds provided by the FCT - Portuguese Foundation for Science and Technology, under the project UIDB/04033/2020.

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