Comparative Immunology, Microbiology and Infectious Diseases

Epidemiology of equine influenza in the Maghreb area

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Abstract:	Equine influenza (EI) is one of the most contagious respiratory infections in horses, donkeys and mules, caused by equine influenza A virus (EIV). It remains a disease with a strong economic stake for the equine industry. This review focuses on the epidemiological situation of EIV in the Maghreb area, which includes Algeria, Morocco and Tunisia. There is serological evidence for extensive circulation of EIV in the Maghreb area since the early 1970s, but reports of detailed investigation of outbreaks are scarce with no documented isolation or molecular characterisation of EIV from Tunisia. Isolates of EIV were obtained from outbreaks in Algeria in 1971/1972 and 2011. Similarly, in Morocco, isolates were obtained from outbreaks in 1997 and 2004. The viruses isolated in 2004 showed evidence of 'evolutionary stasis', with haemagglutinin and non-structural protein 1 sequences most similar to those of viruses isolated decades earlier. In conclusion, effective surveillance of equids in the Maghreb region, where there is potential for virus re-emergence, should be encouraged.
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Response to Reviewers:	Abstract Equine influenza (EI) is one of the most contagious respiratory infections in horses, donkeys and mules, caused by equine influenza A virus (EIV). It remains a disease with a strong economic stake for the equine industry. This review focuses on the epidemiological situation of EIV in the Maghreb area, which includes Algeria, Morocco and Tunisia. There is serological evidence for extensive circulation of EIV in the Maghreb area since the early 1970s, but reports of detailed investigation of outbreaks are scarce with no documented isolation or molecular characterisation of EIV from Tunisia. Isolates of EIV were obtained from outbreaks in Algeria in 1971/1972 and 2011. Similarly, in Morocco, isolates were obtained from outbreaks in 1997 and 2004. The viruses isolated in 2004 showed evidence of 'evolutionary stasis', with haemagglutinin and non-structural protein 1 sequences most similar to those of viruses isolated decades earlier. In conclusion, effective surveillance of equids in the Maghreb region, where there is potential for virus re-emergence, should be encouraged. Keywords: equine influenza; H3N8; epidemiology; Maghreb. Introduction Equine influenza (EI) is one of the most contagious respiratory infections of horses, donkeys, and mules [1]. Equine influenza is caused by viruses in the genus

Alphainfluenzavirus (influenza A virus, IAV) in the family Orthomyxoviridae [2]. Alphainfluenzavirus is classified according to the two surface glycoproteins HA and NA [3]. Currently, there are eighteen HA subtypes (H1H18) and eleven NA subtypes (N1N11) recognized [4]. Two subtypes of IAV have been responsible for causing equine influenza. The first, H7N7 (or equine 1) was isolated in Prague in 1956 for the first time [5]. The second, H3N8 (or equine 2) was isolated in 1963 in Miami from horses that were recently imported from Argentina [6, 7].

Equine influenza has a high morbidity, which can be 100% in susceptible groups of horses, but a low mortality [8]. The severity of clinical signs in equids is influenced by the viral strain and the immune status of the host [9]. Both H7N7 and H3N8 subtypes produce similar clinical manifestations, but in the case of H3N8 infection, they are more severe [10]. Fever up to 41°C is the first sign of equine influenza seen after an incubation period of 1 to 3 days, which can last between 4 and 5 days. This pyretic state is accompanied by dyspnoea, a persistent hard and dry cough and a nasal discharge that begins serous but can become mucopurulent due to secondary bacterial involvement (Figure 1) [11]. Donkeys and mules present the same clinical signs as horses; however, disease in donkeys is often more severe [12].

Equine influenza has a very short incubation period with very high viral shedding, especially during the first few days of infection, which can last up to 8 days [13]. The virus can spread very rapidly in a susceptible population. Transmission of EI occurs primarily between horses via the respiratory route through aerosol droplets released during coughing, which can infect horses over a distance of 32 m [14], on the one hand. On the other hand, animals can also be infected by indirect contact by contaminated fomites or personnel who do not respect hygiene rules [14].

Epizootics of equine influenza may lead to important economic losses to the equine industry worldwide, with important epizootics that stop horseracing, competitions, and sales. Indeed, in 2007 Australia experienced a major epizootic for the first time, which affected a large number of horses and caused a very significant economic loss [15].

The present review focuses on the epidemiological situation of EI in the Maghreb area (Figure 2). The horse industry is considered one of the most important sectors in the Maghreb countries; it plays an essential role in socio-economic development. According to the latest FAO statistics [16], there are approximately 2 million equids in the Maghreb area (Algeria, Morocco, and Tunisia), including 60% donkeys, 20% horses, and 20% mules (Table 1). In the Maghreb region, there are four main horse breeds: the Barb, the Arab-barb, the Arabian Thoroughbred, and the English Thoroughbred [17-19], the vast majority of these horses are commonly listed as the Barb or Arab-Barb breed; these breeds originate from the Maghreb [20].

Epidemiology

To date, equine influenza outbreaks have been reported all over the world, with the exception of New Zealand and Iceland [1]; EIV is enzootic in Europe and North America [21]. Previously, the H7N7 subtype was considered as the primary cause of equine influenza, but the last reported isolations of H7N7 virus from equids were in India in 1987 [22] and in Egypt in 1989 [23]. Over time, the H3N8 subtype became the major cause of EI. In the late 1980s, the H3N8 subtype was divided into two distinct lineages, Eurasian and American [24], and the latter lineage further was divided into three sub-lineages identified as Argentina, Kentucky, and Florida sub-lineages [25]. More recently, the Florida sub-lineage was subdivided into clade 1 (Fc1) and clade 2 (Fc2) [17], where Fc1 viruses were isolated in America and Europe, while Fc2 viruses have predominated in Europe [26], Africa [27] and Asia [28].

Morocco

During an outbreak in 1978/1979, H7N7 and H3N8 viruses co-circulated, with the presence of mixed infections in Morocco [35]. From 1990–1994, El Harrak et al. [36], showed a predominance of the H7N7 subtype with seroprevalence of approximately 16.9% compared to only 8.6% for the H3N8 subtype. Although this may indicate continued circulation of H7N7 viruses among Moroccan horses in the early 1990s, it may also be due to vaccination or presence of antibodies in older horses from previous infection. Furthermore, the haemagglutination inhibition (HI) test used to detect antibodies in this study can give false positive results due to non-specific inhibitors of agglutination in equine serum and kaolin, which is commonly used treatments to remove non-specific inhibitors, was previously shown to be ineffective when screening equine sera for antibodies to H7N7 virus [37]. However, El Harrak et al. [36] do not mention in the brief conference proceedings whether any treatment was used to remove non-specific inhibitors.

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and Essaouira. The virus was isolated for the first time in Morocco in December 1997 from a mule with acute respiratory signs [38]. The HA1 nucleotide sequence of the isolate, A/equine/Nador/1/97 (H3N8), had the highest similarity with A/equine/Rome/5/1991 (H3N8) and A/equine/Italy/1199/1992(H3N8), demonstrating that the virus belonged to the European lineage [39]. In June 2004, outbreaks of EI were reported in several parts of Morocco. Three equine species were affected, with 70 cases of morbidity and one donkey died [40]. The viruses responsible for this outbreak were A/equine/Essaouira/2/2004 (H3N8) and A/equine/Essaouira/3/2004 (H3N8), isolated from a horse and a donkey, respectively. Unexpectedly, both the HA [39] and NS [41] gene sequences clustered in the pre-divergent lineage (Figure 3), suggesting "frozen evolution". The simplest explanation for 'frozen evolution' is laboratory contamination, but there have been regular reports of the phenomenon occurring since the 1970s (Table 2) in which various authors have discounted the potential for this. If, as has been suggested (e.g. [42]), viruses circulating in populations of unvaccinated animals with little or no contact with other equids may exhibit 'evolutionary stasis', this emphasises that surveillance of these populations should not be neglected. Although its origins remain unclear, a human H1N1 subtype virus that emerged in 1977 and was most similar to viruses last isolated 20 years previously [43] caused a pandemic, mostly affecting younger people who would have no pre-existing immunity.

Interspecies transmission of influenza A viruses

Both subtypes of EIV that have been established in equids are thought to have resulted from direct interspecies transmission from birds. In 1989, an H3N8 influenza virus of avian origin and unrelated to the H3N8 virus endemic in horses caused a massive outbreak in horses and donkeys in the northeast of China [44]. The outbreak infected an estimated 20,000 horses with 400 deaths, but appeared to circulate for only a few years before dying out [45]. It is interesting that the H3N8 subtype apparently crossed species barriers into equine populations on at least two separate occasions. This may be because the H3N8 subtype requires less adaptation to the equine respiratory tract or because it is one of the predominant subtypes circulating in wild aquatic birds [46]. It is possible that migratory wild birds can reintroduce H3N8 or other subtypes of avian influenza virus (AIV) in the equine population of the Maghreb region and there is a risk that new equine influenza viruses can emerge in this region, especially as this region is located under the crossing points of migratory wild birds (Figure 2) [47]. Therefore, surveillance of AIV in migratory wild birds is very important to assess potential risks to the equine population caused by H3N8 or other AIV subtypes.

Until recently, it appeared that the equine adapted IAV did not readily transmit to other species. In January 2004, an American research team found that EIV H3N8 Florida lineage had been transmitted to dogs (racing greyhounds) [48]. In addition, it was demonstrated retrospectively that a closely related virus had been independently transmitted from horses to foxhounds in the UK [49]. In 2007, during the equine influenza epizootic in Australia, it was demonstrated that the H3N8 equine influenza virus was present in dogs living in close proximity to infected horses [50]. Recently, genetic characterization of canine influenza viruses (CIV) was carried out between 2018 and 2019 in Iraq. In this study, an H3N8 virus type was isolated from kennel dogs. The Iraqi H3N8 CIV isolated has been shown to be very similar to the H3N8 EIV isolated in the USA [51]. Surveillance for swine influenza virus carried out between 2004 and 2006 in China demonstrated of the first-time transmission of H3N8 equine influenza virus from horses to pigs [52]. In 2013, active surveillance of Bactrian camels in Mongolia led to the isolation of an H3N8 EIV in this population [53]. To date, there is no documented evidence of transmission of EIV from horses to dogs, or other species, in the Maghreb region.

Response to Reviewers

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Author Response 1st revision

Reviewer 1
Reviewer Comments:

This ms is a review of equine influenza outbreaks in northwest Africa. It is introduced by 2 pages of background on equine influenza virus and disease, which has been often reviewed elsewhere (many of the citations in this part are of previous reviews) and so might be of interest mainly to readers unfamiliar with the subject. Concerning the Maghreb, the information provided is useful insofar as equine influenza is a disease that is spread internationally and intercontinentally by performance or breeding horses transported by air. There is much information available from Europe and the Americas on EIV epidemiology and genetic evolution, but relatively little from locations such as the Maghreb that also contain large equine populations. Indeed, the ms mentions two issues that this reviewer wishes had been discussed in greater depth.

One was the continued seroprevalence of antibodies against the EIV H7N7 subtype into the 1990s in Morocco. There has not been a confirmed, recognized isolate of this subtype from horses since 1979, I believe, yet there have since been reports from Egypt, India, Mongolia, and Morocco of outbreaks attributed to it. If this subtype still survives in equines, that is important to know as it has been dropped from most EIV vaccines. Do the authors believe that seroprevalence is entirely associated with vaccination? (As they suggest regarding one study which, being in French, this reviewer was incapable of consulting.) One longs for further EIV surveillance in the Maghreb to clarify this situation.

The second is the finding of virus isolates showing 'frozen evolution' in that they are genetically more similar to strains from 40 years previous. Although the authors did not discuss this, the findings become even more interesting because the HA genes of the 'pre-divergence' Morocco strains, Essaouria/2/2004 and /3/2004, seem to not be particularly closely related to the previously known pre-divergence strains, Algiers/71 and Algiers/72. Also, a reference not discussed in this review (Boukharta et al, 2015) shows that the NS genes of Essaouria/2/2004 and /3/2004 are also 'pre-divergence.' Examples of frozen evolution have also been found in Europe and South America and are yet another stumbling block for the EIV vaccine

community: these examples have unknown provenance, seem to be unrelated, are typically blamed on 'laboratory escapes,' have never yet been shown to persist—but if one were to do so, current vaccines might not protect against it. Do the authors have any insights as to how isolates exhibiting frozen evolution might have arisen in Morocco?

The ms is clearly written and figures are adequate. There is one citation (that I noticed), in first sentence of the Morocco section, which is '[38]' and needs to be fixed.

Author Response:

Abstract

Equine influenza (EI) is one of the most contagious respiratory infections in horses, donkeys and mules, caused by equine influenza A virus (EIV). It remains a disease with a strong economic stake for the equine industry. This review focuses on the epidemiological situation of EIV in the Maghreb area, which includes Algeria, Morocco and Tunisia. There is serological evidence for extensive circulation of EIV in the Maghreb area since the early 1970s, but reports of detailed investigation of outbreaks are scarce with no documented isolation or molecular characterisation of EIV from Tunisia. Isolates of EIV were obtained from outbreaks in Algeria in 1971/1972 and 2011. Similarly, in Morocco, isolates were obtained from outbreaks in 1997 and 2004. The viruses isolated in 2004 showed evidence of 'evolutionary stasis', with haemagglutinin and non-structural protein 1 sequences most similar to those of viruses isolated decades earlier. In conclusion, effective surveillance of equids in the Maghreb region, where there is potential for virus re-emergence, should be encouraged.

Introduction

Equine influenza (EI) is one of the most contagious respiratory infections of horses, donkeys, and mules [1]. Equine influenza is caused by viruses in the genus *Alphainfluenzavirus* (influenza A virus, IAV) in the family *Orthomyxoviridae* [2]. *Alphainfluenzavirus* is classified according to the two surface glycoproteins HA and NA [3]. Currently, there are eighteen HA subtypes (H1–H18) and eleven NA subtypes (N1–N11) recognized [4]. Two subtypes of IAV have been responsible for causing equine influenza. The first, H7N7 (or equine 1) was isolated in Prague in 1956 for the first time [5]. The second, H3N8 (or equine 2) was isolated in 1963 in Miami from horses that were recently imported from Argentina [6, 7].

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Table 2. Examples of 'frozen evolution' of equine influenza A H3N8 viruses from the literature.

Isolate(s) [country]	Most similar isolate [country]	Notes	Reference
Tokyo/3/71 [JP], Brazil/78 [BR] and La Plata/88 [AR]	Miami/63 [US]	The HA genes of all three isolates differ from Miami/63 by only eight nucleotides. Laboratory contamination thought to be unlikely	Endo et al. [58]
Bhiwani/87 [IN]	Miami/63 [US]	Unvaccinated. Laboratory contamination thought to be unlikely; serological evidence for circulation of a Miami/63-like virus; Bhiwani/87, Tokyo/71, Brazil/78 and La Plata/88 share 4 nucleotide differences from Miami/63 suggesting a common source.	Gupta et al. [59]
Grosbois/93 [FR]	Aby/84 [SW]	Vaccination status not described	Manuguerra et al. [60]
Berlin/13/02 [DE], Berlin/14/02 [DE]	Brno/95 [CZ]	From unvaccinated horses in same stables Identical HA1 amino acid sequence	Börchers et al. [42]
Athens/2003 [GR], Athens 2007 [GR]	Newmarket/2/93 [UK]	Unvaccinated horses from the same stud farm. All HA amino acid sequences identical to Newmarket/2/93. NA sequences of 2003 and 2007 isolates identical and most similar to viruses from Spain in 2007 and 2009. However, the NA sequences were most like contemporary Fc2 viruses suggesting reassortment	Bountouri et al. [61]
Essaouira/2/2004 [MA], Essaouira/3/2004 [MA]	Miami/63 [US] Uruguay/64 [UY]	HA1 gene most similar to Miami/63 NS1 genes most similar to Uruguay/64	Boukharta et al. [39, 41]
Cheshire/1/06 [UK] Switzerland/P112/07 [CH]	Newmarket/1/93 [UK] Sussex/89 [UK]	Both HA and NS1 sequences of Cheshire/1/06 and Switzerland/P112/07 were most closely related to Newmarket/1/93 and Sussex/89, respectively	Bryant et al. [26]

Highlights

- Epidemiological situation of EIV in the Maghreb area
- Frozen evolution of EIV (H3N8) in the Maghreb area
- Interspecies transmission of equine influenza
- Vaccination against EIV in the Maghreb area

Review

Epidemiology of equine influenza in the Maghreb area

Hadda Kareche [a,*], Janet M Daly [b], Farouk Laabassi [a]

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Abstract

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Algeria

In 1971–1972, Algeria experienced an important EI (H3N8) outbreak, when horses from the Algerian–Moroccan border region and the northern provinces of the country were affected by the disease (Figure 3). The viruses responsible for this outbreak were A/equine/Algiers/6/71 (H3N8) and A/equine/Algiers/1/72 (H3N8) and clustered in the pre-divergent lineage (Figure 4) [29, 30].

Between May 2009 and January 2010, serum samples were collected from 297 unvaccinated horses in western and eastern Algeria [31]. Around 55% of the sera showed antibodies against H3N8 viruses.

Anti-H7N7 antibodies were found in sera from five horses that were imported from Europe and had certainly been vaccinated against equine influenza viruses with a vaccine containing H7N7 antigens. In this study, the seroprevalence for EI in the Algerian horse population varied with the age of the tested animals. Thus, more than 90% of equids over 16 years were seropositive compared to only 18.5% of animals under 3 years old. Moreover, susceptibility to EIV was significantly associated with the breed. Belonging to the Arabian Thoroughbred was a significant risk factor for viral infection, while other breeds (English Thoroughbred, Barb, Arabian-Barb, English-Barb, and English-Arabian) were clearly protected. Sex was not a risk factor for equine influenza infection.

Another outbreak of respiratory disease reported in Algeria was in 2005 [27], but neither blood samples nor nasal swabs from sick horses have been collected to confirm EI infection (F. Laabassi, personal communication).

In the second confirmed outbreak of the respiratory disease in Algeria in 2011, the disease occurred in a variety of locations and stud farms among Thoroughbred and non- Thoroughbred horse populations. Around 900 horses were affected during this outbreak, which led to race cancellation in the whole country for 2 months [32]. EI infection was confirmed by RT-qPCR in 11 nasopharyngeal swabs collected from sick horses. Ten virus isolates were identified as H3N8 by sequencing the haemagglutinin (HA) and neuraminidase (NA) genes and were named from A/equine/Tiaret/1/2011 (H3N8) to A/equine/Tiaret/10/2011 (H3N8). Alignment of the HA1 amino acid sequence confirmed that the viruses belonged to Clade 2 of the Florida sub-lineage in the American lineage (Figure 4). Moreover, the Tiaret 2011 (H3N8) viruses clustered with viruses circulating in the European horse population in 2010, 2011, and 2012. In this outbreak, morbidity again varied with the age of horses. Interestingly, morbidity increased sharply to reach 100% in horses aged between 18 months and 7 years, while in equids older than 8 years it was around 4.95%. This respiratory episode corroborates risks associated with seasonal mixing, in winter and summer. Viruses were identified from non-vaccinated horses only, thus, no evidence of equine influenza vaccine breakdown was seen [27].

Tunisia

According to the Maghreb scientific literature, to date, there is no documented isolation and molecular characterization of equine influenza virus from horses in Tunisia. According to Ellouze [33], the agent responsible for the first equine influenza epizootic in Tunisia during the period 1978–1979, was the subtype H7N7 with overall seropositivity of 65%. The highest EIV seropositivity (85%) was seen in horses. Conversely, a seroprevalence of 44% and 36% was observed for donkeys and mules, respectively. Ghram et al. [19] detected antibodies against EIV only in horses, between March and July 1991, while no serological trace of equine influenza virus was found in mules and donkeys. Furthermore, non- Thoroughbred horses (Barb and Arabian-Barb) have a high rate of EIV infection compared to Thoroughbred horses. In addition, it was noted that about 17% of male equids were positive for influenza, while only 8% of females. Tunisia experienced another outbreak of equine influenza in 1998, with morbidity varying from 50 to 100% [34]. The affected equine population was made up of approximately 70% horses, 12% donkeys, and 18% mules. It was shown by haemagglutination inhibition tests that the H3N8 subtype was responsible for the disease with antigenic characteristics similar to the strain circulating in Morocco. In contrast to what was found by Ghram et al. [19], donkeys and mules were also seropositive to EIV (H3N8) in this study.

Morocco

During an outbreak in 1978/1979, H7N7 and H3N8 viruses co-circulated, with the presence of mixed infections in Morocco [35]. From 1990–1994, El Harrak et al. [36], showed a predominance of the H7N7 subtype with seroprevalence of approximately 16.9% compared to only 8.6% for the H3N8 subtype. Although this may indicate continued circulation of H7N7 viruses among Moroccan horses in the early 1990s, it may also be due to vaccination or presence of antibodies in older horses from previous infection. Furthermore, the haemagglutination inhibition (HI) test used to detect antibodies in this study can give false positive results due to non-specific inhibitors of agglutination in equine serum and kaolin, which is commonly used treatments to remove non-specific inhibitors, was previously shown to be ineffective when screening equine

sera for antibodies to H7N7 virus [37]. However, El Harrak et al. [36] do not mention in the brief conference proceedings whether any treatment was used to remove non-specific inhibitors.

Two outbreaks were notified in Morocco during 1997 and 2004, respectively, in Nador and Essaouira. The virus was isolated for the first time in Morocco in December 1997 from a mule with acute respiratory signs [38]. The HA1 nucleotide sequence of the isolate, A/equine/Nador/1/97 (H3N8), had the highest similarity with A/equine/Rome/5/1991 (H3N8) and A/equine/Italy/1199/1992(H3N8), demonstrating that the virus belonged to the European lineage [39]. In June 2004, outbreaks of EI were reported in several parts of Morocco. Three equine species were affected, with 70 cases of morbidity and one donkey died [40]. The viruses responsible for this outbreak were A/equine/Essaouira/2/2004 (H3N8) and A/equine/Essaouira/3/2004 (H3N8), isolated from a horse and a donkey, respectively. Unexpectedly, both the HA [39] and NS [41] gene sequences clustered in the pre-divergent lineage (Figure 3), suggesting "frozen evolution". The simplest explanation for 'frozen evolution' is laboratory contamination, but there have been regular reports of the phenomenon occurring since the 1970s (Table 2) in which various authors have discounted the potential for this. If, as has been suggested (e.g. [42]), viruses circulating in populations of unvaccinated animals with little or no contact with other equids may exhibit 'evolutionary stasis', this emphasises that surveillance of these populations should not be neglected. Although its origins remain unclear, a human H1N1 subtype virus that emerged in 1977 and was most similar to viruses last isolated 20 years previously [43] caused a pandemic, mostly affecting younger people who would have no pre-existing immunity.

Interspecies transmission of influenza A viruses

Both subtypes of EIV that have been established in equids are thought to have resulted from direct interspecies transmission from birds. In 1989, an H3N8 influenza virus of avian origin and unrelated to the H3N8 virus endemic in horses caused a massive outbreak in horses and donkeys in the northeast of China [44]. The outbreak infected an estimated 20,000 horses with 400 deaths, but appeared to circulate for only a few years before dying out [45]. It is interesting that the H3N8 subtype apparently crossed

species barriers into equine populations on at least two separate occasions. This may be because the H3N8 subtype requires less adaptation to the equine respiratory tract or because it is one of the predominant subtypes circulating in wild aquatic birds [46]. It is possible that migratory wild birds can reintroduce H3N8 or other subtypes of avian influenza virus (AIV) in the equine population of the Maghreb region and there is a risk that new equine influenza viruses can emerge in this region, especially as this region is located under the crossing points of migratory wild birds (Figure 2) [47]. Therefore, surveillance of AIV in migratory wild birds is very important to assess potential risks to the equine population caused by H3N8 or other AIV subtypes.

Until recently, it appeared that the equine adapted IAV did not readily transmit to other species. In January 2004, an American research team found that EIV H3N8 Florida lineage had been transmitted to dogs (racing greyhounds) [48]. In addition, it was demonstrated retrospectively that a closely related virus had been independently transmitted from horses to foxhounds in the UK [49]. In 2007, during the equine influenza epizootic in Australia, it was demonstrated that the H3N8 equine influenza virus was present in dogs living in close proximity to infected horses [50]. Recently, genetic characterization of canine influenza viruses (CIV) was carried out between 2018 and 2019 in Iraq. In this study, an H3N8 virus type was isolated from kennel dogs. The Iraqi H3N8 CIV isolated has been shown to be very similar to the H3N8 EIV isolated in the USA [51]. Surveillance for swine influenza virus carried out between 2004 and 2006 in China demonstrated of the first-time transmission of H3N8 equine influenza virus from horses to pigs [52]. In 2013, active surveillance of Bactrian camels in Mongolia led to the isolation of an H3N8 EIV in this population [53]. To date, there is no documented evidence of transmission of EIV from horses to dogs, or other species, in the Maghreb region.

Vaccination

Vaccination remains the most effective means of preventing and controlling most equine infectious diseases [54]. There have been commercialized equine influenza vaccinations since the 1960s. Currently available vaccines include inactivated whole virus vaccines, ISCOM or ISCOMatrix, live attenuated, and live vector vaccines (reviewed in Paillot et al. [54]). The composition of equine

influenza vaccines is reviewed annually by the World Animal Health Organization (WAHO) expert surveillance panel on equine influenza vaccine composition. The recommendation made in 2020 was that vaccines contain FC1 (A/equine/South Africa/04/2003-like or A/equine/Ohio/2003-like) and FC2 (A/equine/Richmond/1/2007-like) viruses but it is not necessary to include an H7N7 virus or an H3N8 virus of the Eurasian lineage [55]. Despite the availability of vaccines, outbreaks of equine influenza continue to occur even in populations where most horses are fully vaccinated [56, 57]. To date there is limited information about types, viruses included, and the use of equine influenza vaccines in the Maghreb region.

Conclusion

The equine influenza virus subtype H7N7 does not seem to be present worldwide and the risk from this subtype has apparently gone, but if it did re-emerge, then most of the equine population would be naïve, therefore it could have a major impact. The equine influenza virus H3N8 subtype represents a threat not only to equine populations but also to other animal populations, including dogs, camels, and pigs, including in the Maghreb region. It is true that control measures play a crucial role in limiting and preventing this viral infection, but they are still insufficient. This is why it is necessary to put in place well-studied plans and well-organized structures to vaccinate the entire population without forgetting the updating of these new generation vaccines, which ensures an immunity closer to that induced by wild strains. Furthermore, effective surveillance is key for the early detection of the emergence or re-emergence of equine influenza viruses.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Table 1. Global populations of donkeys, horses and mules, in the Maghreb countries (Algeria, Tunisia, and Morocco) in 2020 [16].

Country	Total equids	Percentage		
		Donkeys	**Horses**	**Mules**
Algeria	149182	56.13	32.27	11.6
Morocco	1502000	61.58	12.72	25.77
Tunisia	382239	63.41	14.59	21.63
Total	2033421	60.37	19.85	19.67

Table 2. Examples of 'frozen evolution' of equine influenza A H3N8 viruses from the literature.

Isolate(s) [country]	Most similar isolate [country]	Notes	Reference
Tokyo/3/71 [JP], Brazil/78 [BR] and La Plata/88 [AR]	Miami/63 [US]	The HA genes of all three isolates differ from Miami/63 by only eight nucleotides. Laboratory contamination thought to be unlikely	Endo et al. [58]
Bhiwani/87 [IN]	Miami/63 [US]	Unvaccinated. Laboratory contamination thought to be unlikely; serological evidence for circulation of a Miami/63-like virus; Bhiwani/87, Tokyo/71, Brazil/78 and La Plata/88 share 4 nucleotide differences from Miami/63 suggesting a common source.	Gupta et al. [59]
Grosbois/93 [FR]	Aby/84 [SW]	Vaccination status not described	Manuguerra et al. [60]
Berlin/13/02 [DE], Berlin/14/02 [DE]	Brno/95 [CZ]	From unvaccinated horses in same stables Identical HA1 amino acid sequence	Börchers et al. [42]
Athens/2003 [GR], Athens 2007 [GR]	Newmarket/2/93 [UK]	Unvaccinated horses from the same stud farm. All HA amino acid sequences identical to Newmarket/2/93. NA sequences of 2003 and 2007 isolates identical and most similar to viruses from Spain in 2007 and 2009. However, the NA sequences were most like contemporary Fc2 viruses suggesting reassortment	Bountouri et al. [61]
Essaouira/2/2004 [MA], Essaouira/3/2004 [MA]	Miami/63 [US] Uruguay/64 [UY]	HA1 gene most similar to Miami/63 NS1 genes most similar to Uruguay/64	Boukharta et al. [39, 41]
Cheshire/1/06 [UK] Switzerland/P112/07 [CH]	Newmarket/1/93 [UK] Sussex/89 [UK]	Both HA and NS1 sequences of Cheshire/1/06 and Switzerland/P112/07 were most closely related to Newmarket/1/93 and Sussex/89, respectively	Bryant et al. [26]

AR = Argentina; BR = Brazil; CZ = Czechoslovakia, DE = Germany; GR = Greece; JP = Japan; UK = United Kingdom; US = United States

Figure legends

Fig. 1. Horse with a mucopurulent nasal discharge, possibly the result of equine influenza virus infection (El-Eulma, March 2021).

Fig. 2. Map representing the situation of the Maghreb region underneath the major flyways of wild birds.

Fig. 3. Map representing the major epizootics of equine influenza viruses in the Maghreb area (Algeria, Tunisia, and Morocco) from 1971 to 2011.

Fig. 4. Phylogenetic analysis of the HA1 nucleotide sequence for 26 equine influenza (H3N8) strains, including representative strains of the pre-divergent, Eurasian and American lineages, and Florida clade 1 (Fc1) and 2 (Fc2). The tree was created using the MEGA 11 using the Neighbor-Joining method. Percent bootstrap values are indicated on branches.

Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Epidemiology of equine influenza in the Maghreb area

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