Surveillance of equine infectious anaemia: two outbreaks detected in the South of France in 2014

Aymeric Hans (1) (aymeric.hans@anses.fr), Sophie Jean-Baptiste (2), Jean-Philippe Amat (3), Fabien Chevé (1), Gaël Amelot (1), Jean-Jacques Guyot (2), Françoise Dalgaz (2), Fanny Lecouturier (1), Aurélie Courcoul (4), Philippe Gay (5), Delphine Gaudaire (1) and Marie Grandcollot-Chabot (5)

(1) ANSES, Dozulé Laboratory for Equine Diseases, Virology Unit, Goustranville, France
(2) Departmental Directorate for Protection of the Population of the Gard, Nîmes, France
(3) ANSES, Dozulé Laboratory for Equine Diseases, Epidemiology and Pathological Anatomy Unit, Goustranville, France
(4) ANSES, Maisons-Alfort Laboratory for Animal Health, Epidemiology Unit, Maisons-Alfort, France
(5) Directorate General for Food, Animal Health Office, Paris, France

Abstract

In 2014, the French network of 12 laboratories approved by the Ministry of Agriculture to perform the serological diagnosis of equine infectious anaemia (EIA), completed over 15,500 tests using Agar Gel Immuno-Diffusion (AGID). Twelve of these tests were found positive for EIA and involved two horses kept in the Gard *département*, in two towns approximately 3 kilometres away from each other. The surveillance plan implemented following the declaration of these cases led to the testing of 205 horses in the Gard, in addition to the two positive equids. Serological analyses of the 205 horses all gave negative results. These data indicate that no transmission of the virus was detected neither between horses in the stables where the two infected horses were found, nor to horses that had more or less prolonged contact with them, nor to horses that were kept in the vicinity. On the other hand, phylogenetic analysis of the isolates collected from the two infected horses shows that there was no connection between the two cases reported in the Gard in 2014. Even though these two cases were found only a few kilometres from each other, molecular characterization of their viral isolates showed that they were different and had no common origins. These data confirmed the information collected during field surveys that showed no epidemiological link between the two cases. The second case of EIA declared in 2014 in the Gard was detected because the holding where it occurred was inside the 4 km surveillance zone set up following the discovery of the first case.

Keywords

Category 1 health hazard, Regulated disease, Equine infectious anaemia virus, Surveillance, Equids

Résumé

Surveillance de l'anémie infectieuse des équidés : deux foyers détectés dans le Sud de la France en 2014

En 2014, l'ensemble des laboratoires agréés par le ministère de l'Agriculture pour le diagnostic sérologique de l'anémie infectieuse des équidés (AIE), au nombre de douze, ont réalisé plus de 15 500 tests d'immuno-diffusion en gélose (IDG). Parmi ces analyses, douze ont été trouvées positives pour l'AIE et concernaient deux équidés stationnés dans le département du Gard, sur deux communes distantes de 3 kilomètres environ. La surveillance mise en place suite à la déclaration de ces foyers a conduit au dépistage de 205 équidés, en plus des deux équidés trouvés positifs. Les analyses sérologiques réalisées à partir de ces 205 équidés ont toutes présentés un résultat négatif. Ces données indiquent donc qu'il n'y a pas eu de transmission virale détectée aux équidés des structures où étaient hébergés les chevaux infectés ni à ceux identifiés comme ayant eu des contacts plus ou moins prolongés avec eux ou stationnant dans une zone géographique proche. D'autre part, l'analyse phylogénétique des isolats prélevés sur les deux équidés infectés montre que les deux foyers déclarés dans le département du Gard en 2014 sont indépendants. En effet, même si ces deux foyers ne sont distants que de quelques kilomètres, la caractérisation moléculaire des isolats viraux montre qu'ils sont différents et ne présentent donc aucune origine commune. Ces données confirment les informations recueillies au cours des enquêtes de terrain qui ne montraient aucun lien épidémiologique entre les deux foyers. Le second foyer d'AIE, déclaré en 2014 dans le département du Gard, a été trouvé car la structure se trouvait à l'intérieur du périmètre de surveillance de 4 kilomètres mis en place suite à la découverte du premier foyer.

Mots-clés

Danger sanitaire de 1ère catégorie, maladie réglementée, virus de l'anémie infectieuse des équidés, surveillance, équidés

Equine infectious anaemia (EIA) is caused by the Equine Infectious Anaemia virus (EIAV) belonging to the *Retroviridae* family, genus *Lentivirus*, which also includes Human Immunodeficiency Virus (HIV), Bovine and Feline Immunodeficiency Viruses (BIV and FIV) and the visna-maedi virus.

EIAV infects only Equidae (horses, donkeys, mules and zebras). Following infection, Equidae remain infected for life and are contagious for other Equidae even when there are no clinical signs (Issel *et al.*, 1982). The bloodborne virus is transmitted from one animal to another mainly by biting insects or iatrogenically through contaminated needles or surgical equipment (Foil *et al.*, 1983; Hawkins *et al.*, 1973). Insects – primarily horse flies and stable flies – are mechanical vectors; although the virus does not multiply within the insect, the infectious virus can remain in its mouthparts for several hours after a bite. The virus is disseminated most effectively by this type of mechanical vector-borne transmission when horses are gathered for equestrian events, since horse flies and stable flies often stop feeding to finish their meal on another host.

In France, EIA is currently classified as a Category 1 health hazard (Ministerial Order of 29 July 2013). The economic and health consequences of this disease can severely impact the horse industry since all positive Equidae must be slaughtered and exports of Equidae to certain third countries can be called into question.

An overview of the current EIA surveillance and control system is presented in the Box provided.

The serological test for EIAV recommended by the World Organisation for Animal Health (OIE) is the agar gel immunodiffusion assay (AGID) known as the Coggins test (Coggins and Norcross, 1970) according to French standard NF U47-002 (AFNOR, 2010). This test is required only for certain imports and exports of Equidae and, in the case of a stallion, as one of a battery of tests prior to reproduction (IFCE, 2010); it can also be requested by a buyer during vetting. This is why EIA outbreaks are often detected by a practising veterinarian following suggestive clinical signs in a client's horse. This initial suspicion may lead to the screening of other seropositive Equidae nearby or with an epidemiological link, whether ill or asymptomatic.

Objectives of the surveillance programme

To detect EIA in Equidae throughout France.

The population monitored

Domestic Equidae (horses, donkeys, mules and hinnies) nationwide.

Surveillance procedures

Outbreak surveillance

Clinical surveillance relies on owners, veterinarians and the network of laboratories accredited to perform serological analyses to detect EIA. It also relies on the results of autopsies. The Ministerial Order of 23 September 1992 defines a suspicious case as any Equidae showing marked signs of listlessness (typhoid-like state), anaemia or weight loss accompanied by fever. Any Equidae testing positive with an agar gel immunodiffusion assay (AGID, better known as the Coggins test) is considered to be infected.

RESPE, the French network for epidemiological surveillance of equine diseases, supported by a "sentinel" veterinary network, set up a "Piro-like" sub-network on 1 May 2014. Any sentinel veterinarian detecting an Equidae with fever associated with at least one other clinical sign on a predefined list (including loss of appetite, listlessness, loss of condition, oedema, petechiae, etc.) must take a blood sample to screen for four pathogens, including EIA virus.

Programmed surveillance

Programmed surveillance includes several different measures:

- Breeding stallions are mainly monitored systematically.
  - > All stallions used in artificial insemination programmes are tested on a regular basis. If the semen is to be sent to another European Union country, a negative Coggins result must be produced in the two weeks preceding the first collection. If it is for the national market, a negative Coggins result must be produced in the three months before the first collection during the first breeding season, then every three years before the breeding season.
  - > Stallions used naturally in certain breeds must also be tested in accordance with their stud book recommendations. A negative Coggins result must be produced in the three months prior to the first service then every three years. In 2014, this screening was mandatory for the following breeds: Thoroughbred, AQPS, French Trotter, Arab and DSA, Anglo-Arab and DSAA, French Saddle, Corsican horse, French Saddle Pony, New Forest, Haflinger, Welsh, Connemara, Merens and Shagya. This surveillance is coordinated by the French horse and riding institute, IFCE.
- All exported Equidae must be tested in accordance with the health requirements of the importing country. Imported Equidae must also be screened for EIA according to the exporting country, the type of importation (temporary, permanent or readmission after temporary export) and the type of use (slaughter or other). Screening is not mandatory for Equidae transported within the EU except for Equidae from Romania. This measure was introduced in 2010 (2010/346/EU) following several cases of EIA in the United Kingdom, Belgium and France in 2009 and 2010 among Equidae imported directly from Romania.

"Voluntary" surveillance

It is recommended to test for EIA whenever there is a change of ownership, particularly as the disease is considered a redhibitory defect. Tests of this kind can detect asymptomatic carriers which play an important role in spreading the disease because they act as reservoirs for the virus. A diagnosis must be established and actions to cancel the sale, when necessary, must be undertaken within 30 days of delivery. Several auction houses require any Equidae on sale to have had a negative Coggins result in the weeks preceding the sales.

Animal health rules

Any clinical suspicion or confirmation by the results of analysis by an accredited laboratory must be declared to the Departmental Directorate for Protection of the Population (DDecPP) and the Directorate General for Food (DGAL). Any clinical suspicion or positive test by an accredited laboratory must be sent to the National Reference Laboratory (NRL) for confirmation, which for EIA is ANSES's Dozulé Laboratory for Equine Diseases.

Should EIA be suspected, the veterinarian must isolate the animal and check its identity. The veterinarian immediately informs the DDecPP and takes a sample of serum, which is sent to an accredited laboratory for analysis along with comprehensive contextual data.

When EIA is confirmed, a declaration of infection (APDI) is issued, thus initiating health control measures. An epidemiological survey is led by the DDecPP, supported by the NRL. All the Equidae on the site of the outbreak are screened, as are all those considered at risk, i.e. animals generally within two kilometres of the outbreak and/or having been in direct contact with the infected Equidae. The site is visited by a mandated veterinarian who must list and identify all the Equidae present as needed. All equine movements in or out are prohibited. Buildings must be treated to eradicate all insects and thoroughly disinfected. All the Equidae on site must have a Coggins test and any positive animals are isolated and euthanised within 15 days. An epidemiological survey is conducted to find and test all the Equidae having been in contact with the infected animals. The Equidae present on the site of the outbreak are regularly tested (Coggins test). The health control measures are only lifted when all the Equidae have shown a negative result on two Coggins tests performed on two serum samples taken three months apart. The State financially contributes to veterinary visits in the event of a suspected or confirmed infection and to disinfection and insect control operations. It assumes EIA diagnostic costs and provides compensation to owners of horses slaughtered as part of an APDI.

Regulations

Outbreak surveillance and programmed surveillance in the event of an outbreak with health control measures

Ministerial Order of 23 September 1992 describing the sanitary measures for equine infectious anaemia.

Ministerial Order of 23 September 1992 determining the financial measures related to the health control measures for equine infectious anaemia.

Programmed surveillance of breeding stock

Ministerial Order of 4 November 2010 determining the conditions for health approval of Equidae semen collection centres and the animal health conditions for semen trade within the Community.

Council Directive 92/65/EEC of 13 July 1992 laying down animal health requirements governing trade in and imports into the Community of animals, semen, ova and embryos not subject to animal health requirements laid down in specific Community rules referred to in Annex A(I) to Directive 90/425/EEC.

Stud book regulations available on the IFCE website: http://www.haras-nationaux.fr/information/reglementation/races-et-stud-books.html.

Programmed surveillance of EU trade, imports and exports

Council Directive 2009/156/EC of 30 November 2009 on animal health conditions governing the movement and importations from third countries of Equidae.

Commission Decision of 6 January 2004 establishing the list of third countries and parts of territory thereof from which Member States authorise imports of live Equidae and semen, ova and embryos of the equine species and amending Decisions 93/195/EEC and 94/63/EC.

Commission Decision 92/260/EEC of 10 April 1992 on animal health conditions and veterinary certification for temporary admission of registered horses.

Commission Decision 93/195/EEC of 2 February 1993 on animal health conditions and veterinary certification for the re-entry of registered horses for racing, competition and cultural events.

Commission Decision 93/196/EEC of 5 February 1993 on animal health conditions and veterinary certification for imports of Equidae for slaughter.

Commission Decision 93/197/EEC of 5 February 1993 on animal health conditions and veterinary certification for imports of registered Equidae and Equidae for breeding and production.

Commission Decision of 18 June 2010 on protective measures with regard to equine infectious anaemia in Romania.

The animal health requirements of third countries are available at: https://teleprocedures.franceagrimer.fr/Expadon/.

Other

List of accredited laboratories for the detection of EIA: http://agriculture.gouv.fr/maladies-animales.

Health overview in 2014

In 2014, the network of twelve diagnostic laboratories accredited to perform serological analyses for EIA carried out 15,585 serological analyses; the breakdown of the reasons for undertaking these analysis (breeding tests (stallions), animal imports and exports, sales) is not known. Of these analyses, there were 5,021 involving mares, 4,516 involving stallions and 704 involving geldings. The sex of the Equidae for the 5,344 remaining analyses was not specified in the analysis request.

In 2014, three analyses undertaken by two different accredited laboratories were sent to the NRL (ANSES – Dozulé Laboratory for Equine Diseases) for confirmation. One of the three samples was declared negative by the NRL. The other two were confirmed as positive for EIA. These two samples tested positive in twelve analyses (they were tested several times by the accredited laboratory and the NRL) and came from two Equidae in the Gard *département*. The number of analyses carried out in 2014 was stable in relation to 2013, when 15,274 AGID analyses had been undertaken by the network of accredited laboratories. In 2013, two Equidae (donkeys) kept on Réunion Island (Hans *et al.*, 2014) were also found to be positive for EIA.

Reported outbreaks in the Gard *département*

The first EIA outbreak reported in October 2014 in the Gard *département* involved a stable of fifteen horses with different origins (including France, Netherlands, Spain and Portugal). The index case was a six-year-old Friesian stallion. This stallion was tested for EIA in the context of official mating controls. Its owner wanted to use it for mating to start breeding Friesian horses. This stallion was confirmed as positive for EIA by the NRL on 3 October 2014 and was euthanised on 14 October. In accordance with the regulations, this outbreak was placed under prefectural declaration of infection (APDI) and the fourteen remaining Equidae in the stable were tested for EIA. None were found positive. The investigation showed that the index case had come from the Netherlands with a mare of the same breed, found negative for EIA, and had been purchased at the age of around six months. Over the last five years, these two Equidae had been used for some rides, but according to the owner they had never left the Gard *département* and never showed any clinical signs.

Epidemiological investigations identified stables in the Gard with Equidae within four kilometres from this first outbreak; these stables were placed under prefectural monitoring order (APMS). The index case had been hosted in various pastures located within one kilometre of its town of residence. All of these pastures were considered part of the outbreak. A three-kilometre radius was thus established around this one-kilometre area. After the index case was euthanised and field investigations were undertaken, 205 Equidae were tested for EIA in this four-kilometre radius and another Equidae was found positive for EIA in a neighbouring town.

This second outbreak was detected thanks to the sero-epidemiological investigation launched further to the detection of the first outbreak located approximately four kilometres away. The second Equidae positive for EIA was a 21-year-old mare of unknown origin that had been kept in the town for twenty years. This Equidae was confirmed as positive for EIA by the NRL on 24 October 2014. None of the other seventeen Equidae belonging to the same stable were found positive for EIA.

In addition to identifying equestrian stables and Equidae with an epidemiological link to the reported outbreaks in order to establish appropriate surveillance measures, the aim of the epidemiological investigations was to determine whether there was an epidemiological link between the two 2014 outbreaks, and between these two 2014 outbreaks and those that had occurred in the Vaucluse and Gard *départements* in 2012 (these outbreaks were geographically close: they were located approximately 25 and 50 kilometres from the town of the first 2014 outbreak) and/or those occurring in the Var in 2009.

The information collected further to the field investigations did not indicate any epidemiological link between the two cases of EIA. No contact between these Equidae and/or these two stables could be demonstrated. Likewise, it was not possible to link these two outbreaks to those reported previously in the same region in 2009 in the Var or in 2012 in the Gard and Vaucluse.

Molecular epidemiology

To genotype the EIA strains isolated from the two Equidae testing positive for infection in 2014, the *gag* gene – about 1,400 nucleotides long – was sequenced. The two viral isolates were characterised from tissue samples (spleen, liver, mesenteric lymph nodes) taken from the two Equidae after euthanasia.

A phylogenetic analysis was undertaken for comparison and classification of the viral isolates in relation to those previously encountered in France and those described in the literature (Figure 1). This phylogenetic analysis, undertaken with MEGA 5.1 software, showed that the isolates characterised from the Friesian stallion and the mare of unknown origin in the Gard *département* in 2014 were different.

However, the viral isolate characterised from a sample from the index case was similar to those isolated in the Vaucluse in 2012. That said, the field investigations were unable to establish an epidemiological link between this Equidae and those tested in 2012 in the Vaucluse, in a town located approximately 25 kilometres north of that of the 2014 index case.

Likewise, the viral isolate characterised from the mare of unknown origin was similar to those isolated in 2009 in the Var *département* where sixteen horses were found to be infected. Once again, field investigations and visits found no epidemiological link between these two outbreaks. According to the owner, the mare of unknown origin was purchased approximately twenty years previously from a horse dealer based in the Vaucluse, who had indicated that she came from Romania. An analysis of the phylogenetic tree seems to support the assumption of a local infection of the Equidae, on French soil, and not an infection "imported" from Romania twenty years prior.

Financial overview

In 2014, the DDecPP of the Gard spent approximately €22,000 on the control of EIA. This amount does not take into account the time spent by staff involved in the implementation and monitoring of epidemiological investigations. While non-negligible, it remains limited compared to levels of spending for diseases of other species (tuberculosis, bluetongue, etc.). The low incidence of EIA, euthanasia of infected Equidae only, and the capping of compensation for the owners of euthanised animals explain this limited financial cost.

However, the person-time devoted to the management of EIA, including field interventions and the administrative management of records, is far from negligible.

Field work is particularly complicated due to a lack of reliable information regarding the location and identification of horses, the need to have up-to-date contact details for owners, and the fact that multiple parties are likely to be involved, since each owner is free to choose a veterinary practitioner. The administrative management of EIA is extremely cumbersome because investigations often involve several individuals (each one owning a small number of Equidae). As such, various prefectural monitoring orders (APMS) have to be written and then lifted. Furthermore, EIA management is a long-term task, since it consists in establishing and monitoring the results of two series of consecutive analyses undertaken three months apart.

In this case, the investigations and analyses involved 205 Equidae, half of which were held in three stables and the other half of which were spread out across 35 stables. The DDPP's staff spent over thirty full working days managing these two outbreaks.

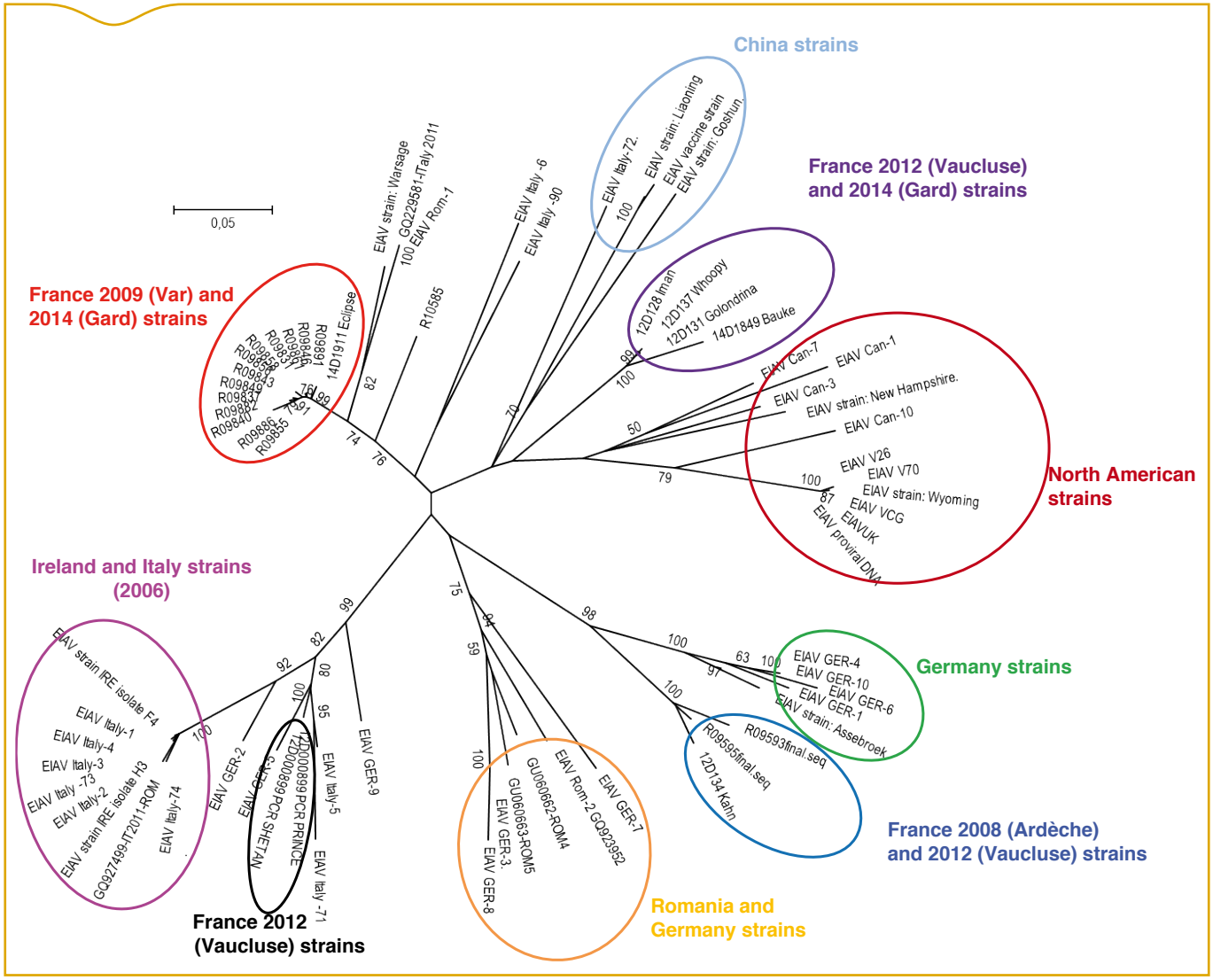

Figure 1. Phylogenetic analysis of nucleotide sequences of the *gag* gene in 69 Equine Infectious Anaemia virus isolates (1,400 nucleotides), including 24 isolated in France between 2007 and 2014

Discussion and conclusion

EIA is transmitted through the transfer of contaminated blood, either by biting insects (horse flies and stable flies) or iatrogenically (use of dirty syringes/needles, etc.). Epidemiological investigations show that, most of the time, spread of the virus within an equine population from an asymptomatic Equidae is low. Health risks and the risk of disease transmission are greater when there are infected Equidae showing clinical signs (together with high viraemia leading to maximum risk of viral transmission) and when a stable with hundreds of horses is involved, even though the virus's natural rate of transmission between animals fortunately remains fairly low.

Currently, only stallions used for artificial insemination have to be tested for EIA every three years, in addition to those used for natural mating in several breeds. There is no available information on the health status of the national equine population with regards to EIA. Information is obtained only when sporadic outbreaks occur. In this case, field investigations and mandatory testing shed light on the seroprevalence of EIA in one or more towns. Such investigations provide a small-scale snapshot of the situation but do not give an overview of the status of the French equine population, particularly in geographic areas with poor reporting and little or no identification of horses, and for sub-populations of Equidae that are very rarely monitored in the context of active surveillance programmes (mating, international trade, sales) such as recreational horses, heavier breeds, the meat industry, donkeys, etc.

Moreover, this is the first time since 2007 that viral isolates responsible for EIA outbreaks in France (Figure 1), separated in time, have very likely been epidemiologically related. The viruses characterised in 2014 in the Gard were very similar to those isolated in the Vaucluse and Var in 2012 and 2009 respectively. The data collected up to 2012 had supported unrelated sporadic outbreaks. Each new outbreak highlighted a new viral isolate, different from those of outbreaks in previous years. An analysis of the phylogenetic data obtained in 2014 shows that there are at least two separate viral isolates circulating in the equine population in the South of France. It should be noted that since 2008, reported EIA outbreaks had always been located in separate *départements*. The outbreak reported in 2008 was located in the Ardèche *département* (Rème *et al.*, 2009). The 2009 outbreak occurred in the Var (Hans *et al.*, 2010). In 2010, Dordogne, Gironde and Lot-et-Garonne were affected in addition to the North of France (cases "imported" from Romania) (Ponçon *et al.*, 2011). In 2012, outbreaks were discovered in the Vaucluse and Gard (Hans *et al.*, 2013). And in 2013, the only reported outbreak was located on Réunion Island in the Indian Ocean (Hans *et al.*, 2014). In 2014, this was the first time since 2008 that a second EIA outbreak was reported in the same *département* (the Gard), even though the two 2012 and 2014 outbreaks occurred approximately 50 kilometres from one another. In addition, the first 2014 outbreak was located only 25 kilometres from the one reported in the Vaucluse in 2012. This geographic proximity could explain the characterisation of two very similar isolates with the same origin. This seems to indicate that the infected Equidae detected in 2012 and 2014

may have had direct contacts resulting in viral transmission. However, the epidemiological investigations undertaken in the field did not find any direct contacts. These Equidae also could have been held in the same stables, causing them to become contaminated through contact with an infected animal. Likewise, the epidemiological investigations did not find any such stables. Furthermore, the 2014 index case was a five-and-a-half-year-old Equidae and the owner, who had had it for five years, indicated that this Equidae, purchased in the Netherlands, had never left the town. One assumption that could connect the 2012 outbreak reported in the Vaucluse to the first outbreak reported in the Gard in 2014 is that there was a common parking area in the South of France before the index case arrived in the Gard at the end of 2008. This possibility could not be confirmed due to a lack of reliable testimonials for a period covering the last five years.

No links were found between the second outbreak in the Gard in 2014 and the outbreak reported in the Var in 2009. However, the two stables in question were recreational stables whose horses are mainly purchased from horse traders in the South of France selling horses whose origin often cannot be verified. These horse dealers generally do not keep records showing their purchases and sales or the origin of the horses purchased/sold.

In conclusion, the EIA outbreaks reported over the last few years in France have primarily been located in the south-east of France and the infected Equidae have mainly been recreational horses. This population is not subject to any regulatory surveillance for EIA, unlike populations of breeding and sport Equidae. It is thus extremely difficult to assess the prevalence of the disease within this population. Nonetheless, it would seem that the EIA virus is circulating, albeit discreetly, in the equine population intended for recreational activities in the south-east of France.

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