Estimating the incidence of equine viral arteritis and the sensitivity of its surveillance in the French breeding stock

J.P. AMAT[1, 2*], T. VERGNE[3], J. TAPPREST[2], B. FERRY[4], A. HANS[5], P. HENDRIKX[6], B. DUFOUR[7], A. LEBLOND[1]

[1] National Institute for Agricultural Research (INRA), UR0346 Animal Epidemiology, F-63122 Saint-Genès-Champanelle, France (jean-philippe.amat@anses.fr, agnes.leblond@vetagro-sup.fr)

[2] French Agency for Food, Environmental and Occupational Health & Safety (ANSES), Dozulé Laboratory for Equine Diseases, Epidemiology and Pathological Anatomy Unit, F-14430 Goustranville, France (jackie.tapprest@anses.fr)

[3] Royal Veterinary College, Veterinary Epidemiology, Economics and Public Health Group, Hawkshead Lane, North Mymms, Hatfield AL9 7TA, London, United Kingdom (tvergne@rvc.ac.uk)

[4] French institute for horse and riding (IFCE), F-27310 Saint-Ouen-de-Thouberville, France (benedicte.ferry@ifce.fr)

[5] ANSES, Dozulé Laboratory for Equine Diseases, Virology Unit, F-14430 Goustranville, France (aymeric.hans@anses.fr)

[6] ANSES, Laboratory Affairs Department, 31 Avenue Tony Garnier, F-69007 Lyon, France (pascal.hendrikx@anses.fr)

[7] Veterinary School of Maisons-Alfort (ENVA), EpiMAI Unit ENVA-joint ANSES, 7 Avenue du Général de Gaulle, F-94704 Maisons-Alfort, France (bdufour@vet-alfort.fr)

* Author for correspondence: Dr. J.P. Amat, ANSES - INRA-EpiA UR0346, Laboratoire de pathologie équine de Dozulé, F-14430 Goustranville, France (Email: jean-philippe.amat@anses.fr, Office phone number: +33(0)2.31.79.79.58).

Highlights

- Suitable rules were proposed for identifying cases within sero-surveillance results
- Equine arteritis virus is circulating in French breeding stock
- 177 outbreaks were detected in the French breeding stock between 2006 and 2013
- Sensitivity of the French breeding stock surveillance estimated at 82% (CI_{95} 71-91)
- 15% of cases in French brood mares between 2006 and 2013 were probably re-infected

Abstract

Equine viral arteritis (EVA) may have serious economic impact on the equine industry. For this reason, it is monitored in many countries, especially in breeding stock, to avoid its spread during breeding activities. In France, surveillance is mainly based on serological tests, since mares are not vaccinated, but difficulties in interpreting certain series of results may impair the estimation of the number of outbreaks.

In this study, we propose specific rules for identifying seroconversion in order to estimate the number of outbreaks that were detected by the breeding stock surveillance component (BSSC) in France between 2006 and 2013. A consensus among multidisciplinary experts was reached to consider seroconversion as a change in antibody titer from negative to at least 32, or as an eight-fold or greater increase in antibody level. Using these rules, 239 cases and 177 outbreaks were identified. Subsequently, we calculated the BSSC's sensitivity as the ratio of the number of detected outbreaks to the total number of outbreaks that occurred in breeding stock (including unreported outbreaks) estimated using a capture-recapture model. The total number of outbreaks was estimated at 215 (95% credible interval 195-249) and the surveillance sensitivity at 82% ($CrI_{95\%}$ 71-91).

Our results confirm EVA circulation in French breeding stock, show that neutralizing antibodies can persist up to eight years in naturally infected mares and suggest that certain mares have been reinfected. This study shows that the sensitivity of the BSSC is relatively high and supports its relevance to prevent the disease spreading through mating.

Keywords: equine viral arteritis; seroconversion; breeding stock; surveillance; epidemiology; surveillance sensitivity; capture-recapture; Bayesian inference.

1. Introduction

Equine viral arteritis (EVA) is an equine respiratory and reproductive disease, caused by equine arteritis virus (EAV), which can lead among other clinical signs to abortions and neonatal deaths (Pronost et al., 2010; Timoney, 2011). EAV belongs to the *Arteriviridae* family, order *Nidovirales* and infects equids only. EAV is an enveloped virus with a positive single stranded RNA genome and has been originally described in 1953, after an abortion outbreak in the city of Bucyrus, Ohio. EAV is usually transmitted horizontally by aerosols or venereal contact, including frozen semen. Vertical transmission through infection *in utero* and occasional indirect transmission by fomites may also occur. Following infection up to 70% of stallions will carry the virus in their reproductive tract and will shed the virus in their semen. Those stallions are the reservoirs of EAV and can transmit the virus to mares during breeding, even in absence of clinical signs (Balasuriya et al., 2013; Timoney and McCollum, 1993).

Due to its economic impacts, EVA is one of the most frequently monitored equine diseases in many countries (Chirnside, 1992; Hans and Marcé, 2012; Newton et al., 1999). In France, the EVA surveillance system comprises several components, including the surveillance of breeding stock, i.e. brood mares and stallions (Amat et al., 2015). The aim of this surveillance component is to ensure that horses remain free of infection during breeding activities to avoid any spread of the disease either by airborne or venereal routes.

Over the last ten years, a few carrier stallions per year have been detected through assays based on virus detection in France (Hans and Marcé, 2012). More cases of infection, identified as seroconversions, have been identified by serological tests in mares, but their exact number remains unknown because there is no global data analysis. Moreover, some infected holdings remain undetected because the interpretation of some serological results may be difficult and because not all breeding horses are tested. Hence, the total number of EVA outbreaks—and thus EVA incidence—in the overall breeding stock population is not precisely known (Hans and Marcé, 2012).

Such incomplete detection of infected units is a recurrent issue in the field of disease surveillance. Capture-recapture methods are often advocated to address this matter because they give an estimation of the total number of infected units, whether or not they have been detected (Hook and Regal, 1995; Vergne et al., 2015). The sensitivity of the surveillance system or component, i.e. its ability to detect infected units, can then be estimated by the ratio of the number of infected units detected to the total number of infected units estimated.

Our first objective was to establish suitable rules for identifying seroconversion in order to estimate the number of EVA cases and outbreaks that were detected by the equine breeding stock surveillance component (BSSC) in France between 2006 and 2013. The second objective was to

estimate the sensitivity of this surveillance component, by estimating the total number of outbreaks that occurred in breeding stock during this period (including those that were not reported) using a capture-recapture model.

2. Material and methods

2.1. Breeding stock surveillance

The BSSC is managed by both public authorities, i.e. the French institute for horse and riding (IFCE), and equine industry, i.e. the studbooks (Amat et al., 2015). Depending on their breed and sex, certain breeding horses must be tested annually before the stud season, according to the regulations and the studbooks' code of practice. Surveillance is only mandatory for mares producing racehorse foals, i.e. Thoroughbred (TB), French Chaser (FC) and a part of Arabian horse (AR), Anglo-Arabian (AA) and French Saddle Horse (FS) mares. Surveillance is mandatory for around 20 breeds for the stallions used for natural mating, and for all breeds for the stallions used for semen collection, regardless of breed (IFCE, 2015a). There are around 40,000 breeding stock holdings in France, totaling some 8,000 stallions and 80,000 mares (IFCE, 2015b). Yet only around 7,000 holdings are monitored for EVA each year, with around 3,000 stallions and 10,000 mares actually tested. The 10,000 tested mares are located in around 6,000 of these holdings, spread over 2,000 communes. Between 2006 and 2013, the annual number of tested mares ranged from 9,777 to 10,867, while the annual number of communes with at least one tested mare ranged from 1,794 to 2,343. More than 40% of these communes are located in the three northwest regions of the country (Basse-Normandie, Pays-de-la-Loire and Bretagne) which keep the greatest number of holdings (B. Ferry, X. Dornier and S. Vinatier, personal communication).

A serological test is used for surveillance purposes. This viral neutralization test (VNT), performed on a blood sample, is the current standard test for EVA prescribed by the OIE, the World Organization for Animal Health (OIE, 2013). The VNT, considered as the gold standard test for EVA diagnosis, detects the presence of neutralizing antibodies which persist for several years after natural infection (Timoney and McCollum, 1993). Stallions are often vaccinated in France with an inactivated EVA vaccine (Artervac®, Zoetis Animal Health Inc., Kalamazoo, Michigan, USA) but mares are not (B. Ferry, personal communication). We were very confident in the assumption that mares are not vaccinated, based on our knowledge as well as the answers given by the breeders, professionals from racehorse industry and people used to check passport of brood mares before mating activities. Given that VNT may detect antibodies produced after both infection and vaccination and that only stallions may carry the virus more than a few weeks after infection, the status of brood mares for EAV is only based on VNT results, while, in the event of a positive VNT result in stallions, tests based on virus detection by virus isolation or reverse transcriptase-

polymerase chain reaction (RT-PCR) are performed on a semen sample in order to check for virus shedding and to confirm the case. All data related to breeding stock testing (including the test date, result, method, horse identification and location) are collected by the IFCE and held in 'SIRE', its national database.

2.2. Data

Data recorded in the SIRE database were used for analysis. We extracted data related to all breeding horses having at least one positive serological result using VNT between January 2006 and December 2013. These data included the horse identification number, location, dates and results of laboratory analyses (VNT) for EVA. We did not use data pertaining to stallions because the number of males with at least one positive result by VNT was very low (n=32) compared to mares (n=1,645) and some had uninterpretable results. The low number of seropositive stallions recorded in the SIRE database during this period is probably partly due to stringent health protection measures applied regarding stallions, which prevent them from infection (especially through airborne viral transmission), as well as the low number of movements and low level of mixing with other horses. Moreover, vaccinated -and some unvaccinated- stallions are monitored using tests based on virus detection and not VNT. Last, cases detected through epidemiological investigations (and not routinely by the BSSC) were not considered in this study.

Only 28 out of 8,934 VNT results recorded for mares were classified as uninterpretable by the laboratory and excluded from the analysis (0.3 per cent). The location was recorded as the commune of the holding, which is registered at the beginning of each year.

2.3. Case definition

For each year, we were interested in the detection of (new) cases. A case was defined as a mare with seroconversion, detected by the interpretation of several VNT results for the same mare.

Due to the difficulties in the interpretation of certain series of titers, a panel of four experts was specially gathered for this study to establish suitable rules for identifying seroconversion. The chosen experts were specialists in the disease and its laboratory diagnosis, as well as epidemiologists.

2.4. Outbreak definition

An outbreak was defined as a commune where at least one EVA case occurred within one year. We chose the commune because it is the smallest administrative unit provided by the SIRE database for the mares' location, given that no list of holdings was available. The commune is the smallest French administrative unit, with a median area of 10.7 km^2 and an interquartile range of [6.4:18.3]

km^2 (IGN, 2015). There are 36,552 communes in France (INSEE, 2011). For each mare, a commune is recorded in SIRE for each year within which at least one VNT is performed, so from one year to the other, several communes may appear for the same mare if it has been moved. If cases were identified in the same commune in different years, we considered that there were two separate outbreaks, i.e. two separate infected "communes-years".

Given that the breeding season starts in February each year, a VNT is usually performed in the first few months of the year (i.e. 82% of tests are performed before the end of April). We therefore assumed that if seroconversion is detected during a given year *y*, the infection occurred during the previous year, *y-1*. Hence, we took into account the location of year *y-1* when counting the number of new EVA cases in each outbreak. If the commune was not recorded for year *y-1*, we took into account years *y-2* or *y-3*. The dataset included the results of annual serological tests performed between January 2006 and December 2013. Identification of seroconversion needs comparison of several results. Thus, annual results of 2006 cannot be interpreted alone to identify cases but they allow to detect seroconversions between 2007 and 2013. Since we assumed that infection occurred the year preceding the identification of seroconversion, our data allowed us to detect infections that occurred between 2006 and 2012. Consequently, the number of outbreaks was estimated only for the seven-year period from 2006 to 2012.

2.5. Capture-recapture method

Once the distribution of EVA outbreaks that were observed or "captured" by the BSSC is known, the total number of outbreaks (or infected communes-years) N_{inf} can be estimated using a capture-recapture model. Capture-recapture models are often used to estimate the number of undetected units of interest by modeling multiple detections of each detected unit. In epidemiology, depending on the number of data sources, two capture-recapture approaches can be used: multilist and unilist approaches (Vergne et al., 2015). While multilist capture-recapture methods are relevant when infected units can be detected by several surveillance systems or components, unilist methods focus on the frequency of detection of infected units by a single source ("one list") of observations (Bronner et al., 2013; Chao et al., 2001; Del Rio Vilas and Böhning, 2008; Vergne et al., 2012). In this study, a unilist capture-recapture approach was used with the detection source being the surveillance component targeting the breeding stock. The frequency of detection of each outbreak was the number of infected horses detected in each outbreak by the BSSC. An outbreak could either be captured once or more (if one or more infected mares were detected) or not captured (if no infected mares were detected).

Let Y_i be the number of infected mares detected in outbreak *i*. We assumed that Y_i was distributed according to a binomial distribution of parameters n_i and π, with n_i being the number of mares that

were tested in each outbreak and π being the probability that a tested mare was identified as a case. Therefore, the number of tested mares n_i was included as a 'binomial denominator' corresponding to the maximum number of cases that could be detected in an outbreak (Cameron and Trivedi, 2013; Hothorn and Everitt, 2014). The number of tested mares was extracted from the SIRE database for each outbreak and ranged from 1 to 109, with a mean of 15.7 and a median of 7. A tested mare is detected as a case if it is infected *and* if its infection is detected. Therefore, the probability π that a tested mare was identified as a case was decomposed as the product of *Inc*, the incidence rate within an outbreak, and *SeR*, the sensitivity of the proposed rules (*SeR* accounts for the sensitivity of the test). Because the number of outbreaks where *zero* infected mares were detected is unknown, the observed distribution of the number of infected mares per outbreak follows a *zero-truncated* binomial distribution, that is:

$$P(Y_i = y_i | y_i > 0, n_i, Inc, SeR) = \frac{\frac{n_i!}{y_i!(n_i-y_i)!}(Inc*SeR)^{y_i}(1-Inc*SeR)^{n_i-y_i}}{1-(1-Inc*SeR)^{n_i}}, \quad (1)$$

with y_i being the number of infected mares detected in outbreak i and n_i the number of tested mares in outbreak i. Parameters *Inc* and *SeR* were estimated in a Bayesian framework using the WinBUGS software (Spiegelhalter et al., 2003). To specify the zero-truncated distribution we used the zero-trick, as proposed by Spiegelhalter et al., which is often used to specify sampling distributions that are not included in the list of standard distributions in WinBUGS (Spiegelhalter et al., 2003). To determine the prior distributions of *Inc* and *SeR*, an expert opinion elicitation was conducted based on the same expert panel as mentioned above. Using EpiTools software[1], priors were defined as beta distributions whose mode and 5th percentile were the average most likely and the smallest 5th percentile values determined from the panel elicitation. Priors for *Inc* and *SeR* were therefore beta distributions of parameters (4.6, 9.3) and (44.9, 11.3), respectively. To determine the posterior distributions, we ran three simulation chains of 10,000 iterations, and discarded the first 2,000 iterations of each chain to allow for burn-in of the chains. Chains were then thinned, taking every fifth sample to reduce autocorrelation amongst the samples. Convergence was assessed by checking the trace plots for all monitored parameters.

We then used the posterior distributions of *Inc* and *SeR* and the number of tested mares in the detected outbreaks to estimate the total number of outbreaks N_{inf} and the BSSC's sensitivity *SeB*. To do so, we assumed 1) that the number of cases amongst the tested mares in outbreak i (C_i) followed a zero-truncated binomial distribution of parameters n_i and *Inc*, since all brood mares were tested so that there was at least one infected mare tested in each outbreak i, and 2) that the number of detected cases in outbreak i (Y_i) followed a binomial distribution of parameters C_i and *SeR*. For each

[1] http://epitools.ausvet.com.au/

detected outbreak, we calculated analytically the probability Pr(Y_i=0) that no infected mare was detected in that outbreak. For each outbreak *i*, this probability is obtained by summing the probabilities that Y_i=0 for all possible values of C_i, that may range from 1 to n_i the number of tested mares in outbreak *i*:

$$\Pr(Y_i = 0) = \sum_{C_i=1}^{n_i} \Pr(Y_i = 0 \cap C_i = c_i) \quad (2)$$

Using the Bayes theorem, equation (2) becomes:

$$\Pr(Y_i = 0) = \sum_{C_i=1}^{n_i} \{\Pr(Y_i = 0 | C_i = c_i) * \Pr(C_i = c_i)\} \quad (3)$$

Using parameters *Inc*, *SeR* and n_i, the probability of not detecting the outbreak *i* can be calculated as follows:

$$\Pr(Y_i = 0) = \sum_{c_i=1}^{n_i} \{(1 - SeR)^{c_i} * \frac{\frac{n_i!}{c_i!*(n_i-c_i)!} * Inc^{c_i} * (1-Inc)^{n_i-c_i}}{1-(1-Inc)^{n_i}}\}, \quad (4)$$

with *Inc* the incidence rate within an outbreak, *SeR* the sensitivity of the proposed rules and n_i the number of tested mares in outbreak *i*. The probability Pr(Y_i=0) for each detected outbreak was calculated in WinBUGS using n_i and the posterior distribution of *SeR* and *Inc*. Assuming that the distributions of the number of tested mares in the detected and non-detected outbreaks are the same, the total number of EVA outbreaks was estimated using an extension of the Horvitz-Thompson estimator proposed by van der Heijden and colleagues (Van der Heijden et al., 2003):

$$\widehat{N_{inf}} = \sum_{i=1}^{N_{obs}} \frac{1}{1-\Pr(Y_i=0)}, \quad (5)$$

with N_{obs} being the number of detected outbreaks.

Then, the surveillance sensitivity (*SeB*) was defined as the proportion of outbreaks detected by the BSSC among all infected communes that kept at least one brood mare tested for EVA, i.e. used to produce racehorse foals. It was estimated by dividing the number of detected outbreaks (N_{obs}) by the estimate of the total number of EVA outbreaks that occurred between 2006 and 2012 ($\widehat{N_{inf}}$).

3. Results

3.1. Proposed rules for identification of seroconversion

The expert panel ascertained that changes in antibody titer in the same animal can be due to either recent infection/re-infection or other reasons, such as slight differences in laboratory practices. Since mares are not vaccinated in France, vaccination was not retained as a possible reason for titer increase for this study. Although a recent infection usually imply a much greater increase in antibody titer than for other reasons (Go et al., 2012), it is sometimes difficult to distinguish between these situations, and we did not find any accurate description in the scientific literature of the antibody titer curve over time in horses naturally infected with EVA.

First of all, the panel studied dozens of titer series taken from the dataset and two kinds of cases were distinguished. The first case is a change in antibody titer from negative to positive and the second, an increase in the titer for animals with previous positive results. In the first case, several profiles were identified (Fig. 1). Many mares exhibit negative initial results followed by positive ones only (see solid line 'mare 1' in Fig. 1). Thus, seroconversion is easily identified for such cases. Surprisingly, certain mares had negative results, then positive ones, then negative result(s) again, and even sometimes positive results yet again. In a few cases, a single negative result appears among a series of high titers and is likely an error, probably due to a data entry mistake (see 'mare 2' in Fig. 1). But in many cases, mares gave a mix of negative and low positive results with antibody titers ranging from four to eight (and even 16). This is likely to occur when a non-vaccinated mare has been infected not recently but many months or years ago (see 'mare 3' in Fig. 1). However, between these situations (mares 1 and 2 *versus* 3), a moderate rise in antibody titers may also occur, casting doubt about recent seroconversion.

In the second case, the expert panel tried to identify a sharp rise in the antibody titer of animals with positive initial results, which can be the consequence of reinfection (Balasuriya and MacLachlan, 2004). Among mares included in the 2006-2013 dataset with a positive initial result, several profiles were identified. For certain mares, a sharp rise in antibody titer was observed, for instance from 16 to 1024, consistent with recent reinfection (see 'mare 4' in Fig. 1). But in most cases, the first positive result is followed by lower, identical or slightly higher antibody titers (see 'mare 5' in Fig. 1). For these mares, the infection probably occurred several months/years ago and the antibody titer may decrease or vary around low ('mare 3') or high values ('mare 5'). Once more, moderate increases in antibody titers may sow doubt about recent reinfection. Interestingly, VNT results showed that neutralizing antibodies can persist up to eight years in naturally infected mares.

The panel then studied the possible reasons for fluctuations in the antibody titer that differs from a recent infection. According to the National Reference Laboratory (NRL) who regularly perform and organize proficiency tests with the French laboratory network approved by the Ministry of Agriculture, the antibody titer may vary a little in a horse infected many months or years ago and regularly tested. This variation usually ranges from one titer below to one titer higher than a central "true" value. In this way, titers from a horse with a constantly high antibody level is likely to vary along a four-fold scale, for instance from 64 to 256, and a horse with a constant but low antibody

level usually has titers ranging from “zero” (negative result) to eight. Greater variations appear unlikely.

For this research, seroconversion was finally defined by the panel as a change in antibody titer from negative to at least 32, or as an eight-fold or greater increase in antibody level in mares with previous positive results (from 16 to at least 128, for instance). Seroconversion was thus identified by comparing results from two or more successive years, given that mares can be not tested during one year and since detection of a sharp increase in antibody level can sometimes take two years as tests are annually performed. Indeed, the increase in antibody level can still be very weak when a mare is tested just after infection, thus the seroconversion could be definitely detected only the following year.

In order to investigate the sensitivity of our results to the seroconversion definition, we compared the estimated BSSC’s sensitivity with the estimate obtained by using another plausible definition, i.e. considering also the change in antibody titer from negative to 16 or 24 as a seroconversion. This alternative definition is more sensitive but less specific than the one used in this study.

3.2. Number of EVA cases and outbreaks detected by the BSSC

By applying the *ad hoc* rules established for identifying seroconversion, we observed 239 EVA cases in the 2006-2013 SIRE dataset that had been detected in brood mares by the BSSC (Fig. 2). The holding’s commune was not available for three of these mares, which were subsequently excluded.

Thoroughbred mares represent the largest proportion of EVA cases detected by the BSSC (Table 1). Of the 236 mares with a known location, all but one belonged to the five breeds previously quoted. The one exception was a riding horse of unknown breed.

For the 236 EVA cases with a known location, we counted the number of cases within each commune, considering each year separately. In respectively 85 and 14% of cases, the commune for years *y-1* and *y-2* was used, and we had to go back as far as year *y-3* for only less than 2% of cases (four mares). Finally, 177 outbreaks or infected communes-years were identified (Table 2). For most outbreaks, only one case was detected, while the maximum number of cases detected per outbreak was 30.

Using the alternative seroconversion definition mentioned above (including change in antibody titer from negative to at least 16, in addition to an eight-fold or greater increase in antibody level), we observed 304 cases in 235 outbreaks detected by the BSSC between 2006 and 2013.

3.3. Estimation of the total number of EVA outbreaks in breeding stock and of the BSSC's sensitivity

The posterior distributions of *Inc*, the incidence rate within the outbreaks, *SeR*, the sensitivity of the proposed rules for identifying seroconversion, N_{inf}, the total number of outbreaks and *SeB*, the BSSC's sensitivity, are summarized in Table 3 using their median and their 95% credible interval ($CrI_{95\%}$), which are the intervals containing 95% of the posterior values. According to the model, the total number of EVA outbreaks ($\widehat{N_{inf}}$) was estimated at 215 ($CrI_{95\%}$ 195-249) between 2006 and 2012, on average around 31 per year ($CrI_{95\%}$ 28-36). During this seven-year period, 177 outbreaks were detected by the surveillance system. Thus the overall sensitivity of the BSSC pertaining to communes having at least one mare used for the production of racehorse foals ($\widehat{SeB}$) was estimated at 82% ($CrI_{95\%}$ 71-91) (Table 3).

Using the alternative seroconversion definition, the total number of EVA outbreaks during the same period appeared to be greater at 287 ($CrI_{95\%}$ 260-334). However, the change in the posterior distribution of the BSSC' sensitivity was very limited, i.e. *SeB* was estimated at 82% ($CrI_{95\%}$ 70-90). Moreover, there were no significant changes in the posterior distributions of the incidence rate within the outbreaks (median at 3.5%, $CrI_{95\%}$ 2.7-4.6) and the sensitivity of the proposed rules for identifying seroconversion (median at 79%, $CrI_{95\%}$ 66-88).

4. Discussion

4.1. Rules defined for identifying seroconversion

For international trade of horses, the rules defined by the Terrestrial animal health code of the OIE (OIE, 2015) have to be strictly applied. However for the purpose of this study, we have decided to use the *ad hoc* rules previously defined in order to identify the number of seroconversions in the SIRE dataset since i) we did not find accurate information in the literature about the level of the serological response in naturally infected horses over several years and ii) the proposed rules seem appropriate to distinguish between recent cases and mares infected several years ago, regarding the SIRE dataset.

It has been shown that neutralizing antibodies are detected within 1-2 weeks following infection, peak at 2-4 months and persist for years thereafter (Balasuriya and MacLachlan, 2004), but we did not find any comprehensive description of the antibody titer curve several months or years after infection. As seen in the SIRE's dataset, it was difficult to find an obvious cut-off point to distinguish new cases. The rules defined in this study are probably imperfect and there was no "gold

standard" test available in order to check their relevance. The choice of not considering a change in antibody titer from negative to 4, 8 or 16 (as well as a four-fold increase) as a case may appear strict compared to rules usually applied for import/export, mating agreements (Anonyme, 2014) or prevalence studies (Chirnside, 1992; Newton et al., 1999). Nevertheless, natural and experimental infections usually appear to involve high antibody titers, although serum neutralizing antibody response may depend on the virus strain and duration of this response may differ following experimental infection compared to natural infection or vaccination (Go et al., 2012; MacLachlan et al., 1998; Summers-Lawyer et al., 2011; Timoney et al., 2007).

Although VNT seems the most reliable and relevant test for yearly surveillance of EVA in breeding stock, it is not always straightforward to identify true seroconversion. Slight variations in the antibody titer may lead to overestimate the number of cases, especially when antibody level is very low. Several laboratories have developed and evaluated enzyme-linked immunosorbent assays (ELISAs) and such serological tests could make it easier to identify seroconversion in the future (Balasuriya et al., 2013). Whichever serological test used, an important issue is to save the successive results from the same horse to be able to properly interpret future results. For epidemiological purposes, it is necessary to define *ad hoc* rules for identifying seroconversion, which may differ depending on the country, laboratory practices and test used. Some authors have also defined *ad hoc* algorithms for interpreting the antibody titer in the case of other infectious diseases (Premaratna et al., 2012). The rules defined in our study may be used for the overall epidemiological interpretation of data collected by other EVA surveillance components (such as testing before sales or movement and passive surveillance), while the application of the currently accepted guidelines for mating agreements and national and international trade of horses must be continued. Indeed, it is important to distinguish between epidemiological purposes, for instance estimating the annual incidence rate, and certification or policy purposes. For the latter purposes, it is prudent to continue considering that any four-fold or greater increase in VNT results or any change from negative to positive may be associated with a risk of spreading the disease (after infection or reinfection). This is especially relevant because the mating or import ban can generally be lifted in a few weeks, once a stable antibody titer—coinciding with viral clearance—has been obtained.

4.2. Statistical framework

For this study, a zero-truncated binomial distribution was preferred to a zero-truncated Poisson distribution to model the observed count data. Indeed, the Poisson distribution is a limiting case of the binomial distribution when a large number of trials or individuals is reached and the probability of success (i.e. probability that one tested animal is detected as being a case in our study) is small.

However, when the binomial numerator is close to the value of the denominator, which is often the case here, the binomial model is usually preferable (Hilbe, 2011). Moreover, the binomial model is a recommended approach when there are only a few outbreaks with more than one or two cases (Cameron and Trivedi, 2013), such as in the dataset we used.

There are assumptions inherent in fitting a zero-truncated binomial model. In particular, it is assumed that observations are independent from each other. In other words, all infected horses shall have the same probability of being detected (Dohoo et al., 2010). As is often the case regarding livestock, this assumption may be violated here because horses are usually maintained in groups and the probability of detecting a case may vary from one group to another due to several factors such as farming practices. Moreover, heterogeneity in the probability of detection also arises at the commune scale. A commune with many cases is much more likely to be detected than one with only a few infected mares. This phenomenon is named "abundance-induced heterogeneity" in ecology (MacKenzie et al., 2006). Here, it would depend on many factors, including the number of horses within the commune, infection pressure, delay between virus introduction and testing, type of operation, and farming practices—such as the level of mixing within the herd and with animals from other herds, allowing virus transmission by respiratory and/or venereal routes (including mating, meetings and contact in pastures with neighboring herds). The number of cases within an outbreak may also vary due to the proportion of mares with durable immunity, built up after previous infection. The higher this proportion, the lower is the probability to detect the outbreak. Consequently, everything else being equal, the probability of detection is lower for communes previously (and quite recently) infected compared to communes who have never been infected. This phenomenon was probably quite common in this study and can partly explain the low estimate of the incidence rate within outbreaks, because more than 1,400 mares with stable or decreasing antibody titers with no evidence of seroconversion were detected in our dataset. The interaction of all these factors is likely to lead to considerable heterogeneity in the probability of detecting infected horses and communes. It would have been useful to include all these parameters as covariates within our model to better handle the heterogeneity, but only the number of tested mares within the commune was available.

Non-parametric approaches can also be used when there is heterogeneity in count data. For instance, the Zelterman estimator (1988) and Chao's lower bound estimator may estimate the total number of outbreaks using only the total number of detected outbreaks and the number of outbreaks detected once or twice, i.e. with one or two cases detected (Chao, 1987; Del Rio Vilas and Böhning, 2008; Zelterman, 1988). However, because these approaches only use a small fraction of the available data (they only use the number of outbreaks detected once or twice and do not account for the number of tested mares in each outbreak), the parametric approach was preferred.

4.3. Number of cases and outbreaks detected by the BSSC

We estimated that 239 new EVA cases and 177 outbreaks were detected by the BSSC between 2006 and 2012 in brood mares. These numbers are not negligible and confirm that EVA was circulating in breeding stock during this period. It supports the relevance of EVA surveillance in breeding stock in order to identify cases and prevent the disease spreading through mating. This result could assist policy makers in their decisions on whether to extend active surveillance to other breeds and/or by other procedures, in addition to the other surveillance components implemented in France, i.e. compulsory and voluntary notification of suspicions, pre-sales surveillance and pre-export testing (Amat et al., 2015), in order to better estimate the incidence rate and better protect the equine population from EVA infection. Brood mares are not vaccinated and thus act as sentinels (Newton, 2007), while other unvaccinated horse populations may also be useful in signaling new subclinical infections.

Of the 239 cases, 35 mares (15%) had positive result(s) before showing a sharp increase in the antibody titer. Given that mares are not vaccinated in France, they were probably reinfected. To date, natural infection is generally recognized as resulting in durable immunity against reinfection with most if not all strains of the virus, but the possibility of reinfection has been assumed by certain authors (Balasuriya and MacLachlan, 2004). Moreover, 1,406 mares with at least one positive VNT result were detected during this period. These mares were probably infected before 2006. The high proportion of these “old” cases compared to the proportion of new cases may be surprising, but this situation highlights the disease’s presence in breeding stock for a long time, with a significant number of cases even before the first major occurrence of EVA recorded in France in 2007, characterized by numerous clinical cases and a few fatal cases in the north-western part of the country (Pronost et al., 2010). It was not possible to estimate the evolution of the incidence rate before and after 2006. Indeed, the VNT results for these 1,406 mares before 2006 as well as their dates of infection were not available.

Most detected cases were TB, followed by FS and AA (Table 1). These breeds, in the same order, are those with the highest numbers of mares tested each year. Indeed, they represent around 78%, 11% and 6% respectively of mares tested in 2012. For each of the five studied breeds, the average percentage of cases among tested mares was lower than 1% between 2006 and 2012. Nevertheless, the incidence rate for each breed was not estimated because it was not the aim of this study and it would have required specific calculation methods, given that our data must be considered to have been collected by a two-stage sampling process (sampling of the communes and then of the mares within the communes).

4.4. Estimation of annual EVA incidence and the BSSC's sensitivity

We estimated the incidence by keeping all the years together in order to improve the statistical robustness of our result. During this seven-year period, around 6,000 holdings with brood mares in 2,000 communes were tested for EVA each year due to mating activities. Thus, the yearly incidence rate at commune scale was estimated at around 1.6% for these 2,000 communes (or 0.1% considering all French communes). These estimates seem plausible when trying to compare them with other countries, although comparison is quite difficult for following reasons. Numerous studies have investigated the presence of EVA in various countries, but they have usually estimated serological point prevalence rather than annual incidence (i.e. the number of new cases or outbreaks), leading to higher values given that neutralizing antibodies persist several years. Moreover, sampling procedures applied in these studies were different. Some studies are based on surveys specifically carried out to assess prevalence (Braga et al., 2012; Chabchoub et al., 2002; Laabassi et al., 2014; NAHMS, 2000), while other studies have used actively collected samples in specific sub-populations or analyzed results from horses tested before mating, vaccination, import or export, or results from suspected cases (Chirnside, 1992; Newton et al., 1999). Furthermore, prevalence was usually investigated on an individual scale rather than holding or commune scale. EVA prevalence was estimated in several breeds, but farming practices vary largely from breed to breed and may influence both the risk of infection and prevalence. The most comparable results are the serological prevalence estimated in the same (racehorse) breeds and breeding stock. In the TB, individual prevalence has been estimated between 0.3% and 4.5% in the UK, Brazil, Algeria and the US during the last 20 years (Braga et al., 2012; Laabassi et al., 2014; NAHMS, 2000; Newton et al., 1999). In Arabian horses, prevalence has been estimated to range from 6.7% to 26% in Brazil, Algeria and Tunisia since 2000 (Braga et al., 2012; Chabchoub et al., 2002; Laabassi et al., 2014) (F. Laabassi, personal communication). Individual prevalence has been estimated at 5.6% ($CI_{95\%}$ 2.3-8.9) in breeding horses in the USA in 1998 (NAHMS, 2000). Lastly, one interesting result is the serological prevalence on a holding scale on breeding farms in the USA, estimated at 20.5% ($CI_{95\%}$ 5.8-35.2) in 1998 (NAHMS, 2000). All these results were calculated on the basis of horses without any history of vaccination against EVA. The higher prevalence found in breeding stock can be explained by a higher risk of infection due to the existence of two transmission routes: venereal and respiratory.

The estimated BSSC's sensitivity on a commune scale can be considered as quite high. However, the BSCC probably does suffer from a moderate lack of sensitivity. This could be due to the sampling process because only a fraction of the equine breeding stock is tested annually. This is why we focused our study on only the five most regularly tested breeds. Even for these five breeds, only around half of the brood mares are tested because the ones not used to produce racehorse foals

do not need to be tested. However, almost all TB and FC brood mares are tested every year and, for other breeds, the holdings producing racehorse foals usually do not hold brood mares producing non-racehorse foals (and thus not tested).

A slight lack of sensitivity in either the serological test or the data collection process may also be suspected. However, the VNT is very sensitive (OIE, 2013), and the quality of samples, notification procedures, data recording and reliability of the results have also been assessed as satisfactory (Amat et al., 2015).

The rules defined for identifying seroconversion may also have affected the results, but moderate modifications in these rules did not induce a substantial change. Indeed, the use of a more sensitive and less specific seroconversion definition (i.e. also considering a change in antibody titer from negative to 16 as a seroconversion) leads to no significant difference in the estimated BSSC's sensitivity.

Although the BSSC's sensitivity was estimated to be relatively high on a commune scale, it is likely to be as high on a holding scale, if not more. The result on a commune scale was obtained by dividing the number of detected infected communes by the estimated total number of infected communes between 2006 and 2012. The latter number is related to the probability π that one tested animal is detected as being a case, which is directly related to the number of tested mares in each unit, which is the commune. The commune was chosen because no more precise location was available, but each commune with brood mares tested for EVA contains three holdings on average. Consequently, using the commune scale may potentially lead to cases from several holdings being counted together, thus increasing the number of cases per unit (binomial numerator, C_i) compared to using the holding scale. However, the increase in the numerator is probably much smaller than the increase in the denominator (number of tested mares per unit, n_i). Indeed, the venereal route is probably the main route of infection of breeding stock holdings compared with the airborne one and there were only a few communes with more than one detected case (19/177), so the presence of several infected holdings within the same commune was probably unusual. On the other hand, the presence of one or more EVA-free holdings in the same commune as an infected holding is probably more common, especially in the major breeding stock farming areas where the number of holdings in certain communes can be quite high. The potential overestimation of the denominator is supported by the fact that the average number of tested mares within each tested commune (4.7) is around three times higher than the average number of mares in general breeding farms (1.7) (IFCE, 2015b). Moreover, even within the same holding, breeding horses are sometimes held in separate facilities without contact with each other, resulting in a low probability of the disease being transmitted from one batch of horses to another. Furthermore, all tested mares were counted in the denominator, including mares with stable or decreasing positive VNT results. As seen before these

mares had probably been infected several years ago and built up durable immunity, and their presence has probably artificially increased the number of susceptible mares.

Using a zero-truncated binomial model allowed us to take into account not only the detected cases and their clustering within communes, but also the number of tested mares in each commune. This enabled us to manage part of the heterogeneity in the probability of detecting cases and outbreaks. Including relevant covariates in the model (e.g. number of untested horses within the unit, number of risky contacts or primary function of the operation) could have potentially helped to account for the remaining observed heterogeneity. Unfortunately, such covariate information was not available and the small amount of data prevented the inclusion of several covariates. It would be useful to develop a model which correctly manages heterogeneity, but this is a difficult target to achieve. Taking heterogeneity into account vaguely or inexplicitly, such as using zero-truncated negative binomial or non-parametric models, may also be future avenues for research.

Conclusions

This study shows that the number of EVA cases and outbreaks is not negligible in the French breeding stock, and suggests that a proportion of brood mares have been reinfected, a situation which had not previously been documented to our knowledge. The estimate of the BSSC's sensitivity between 2006 and 2012 was at 82% ($CrI_{95\%}$ 71-91) which is relatively high. However, the sensitivity of EVA surveillance could be improved by a closer relationship between surveillance components and more detailed information about the horses' location, which is necessary to improve numerous kinds of epidemiological research. Expanding access to serological results collected in circumstances other than pre-mating surveillance (especially before sales or international trade) and using common rules for identifying seroconversion would improve future incidence investigations, particularly because they would pave the way for multilist studies.

Conflict of interest statement

None.

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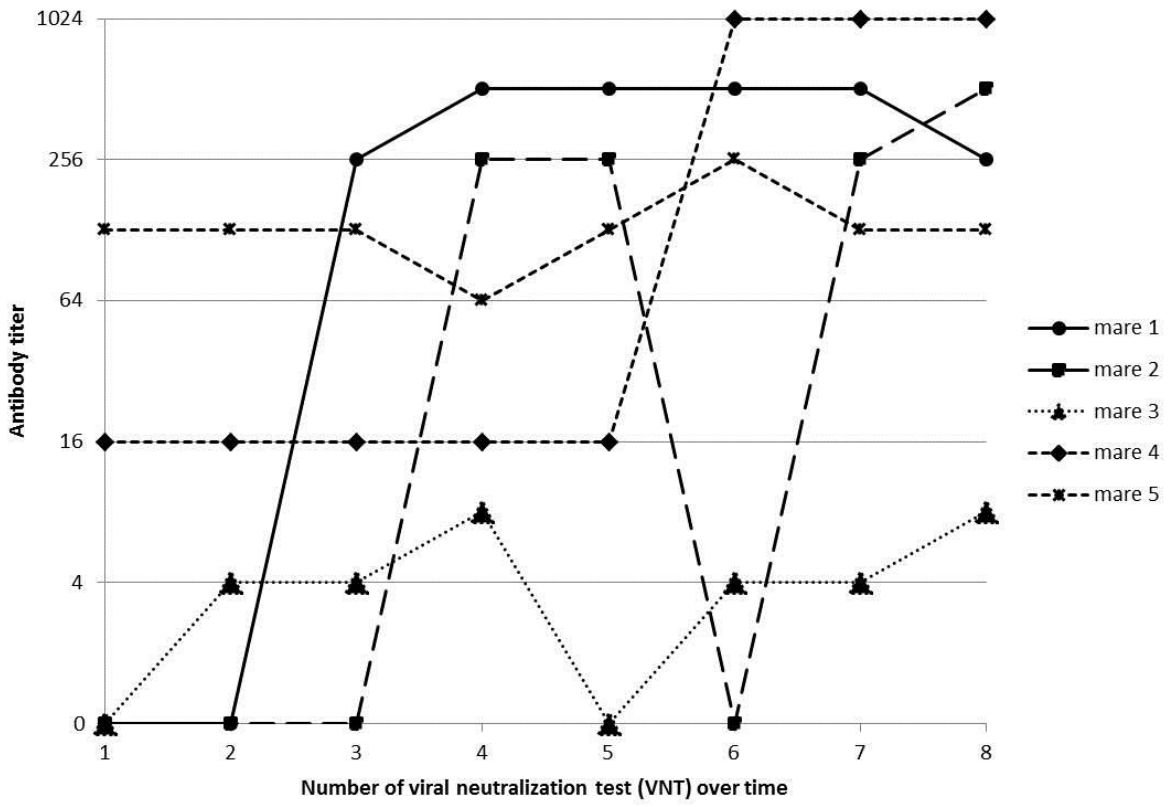

Fig. 1. Examples of equine viral arteritis antibody titer curves in five brood mares tested each year over an eight-year period.

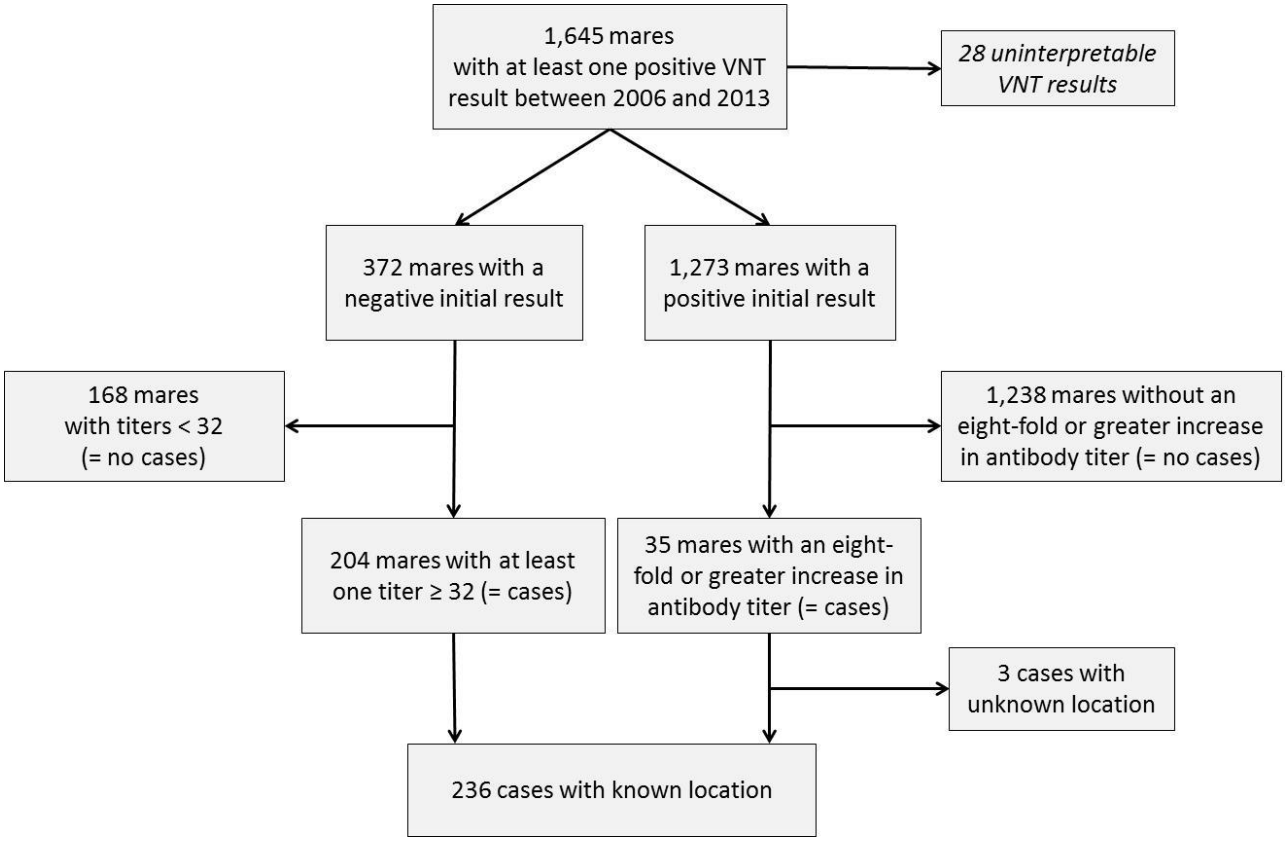

Fig. 2. Flow chart documenting the *ad hoc* rules used to identify equine viral arteritis cases among the brood mares tested between January 2006 and December 2013 in France using the viral neutralization test (VNT).

Table 1. Number of equine viral arteritis (EVA) cases with a known location detected in mares by the French breeding stock surveillance component (BSSC) between 2006 and 2013 for each breed.

Mare's breed	Thorough-bred	Arabian horse	Anglo-Arabian	French Chaser	French Saddle	Other (riding horse)	Total
Number of EVA cases with a known location detected by the BSSC	170	5	26	1	33	1	236

Table 2. Number of equine viral arteritis (EVA) cases detected in the outbreaks identified by the breeding stock surveillance component (BSSC) in French breeding stock between 2006 and 2013.

Number of EVA cases detected per outbreak	1	2	3	4	7	8	30	Total
Number of outbreaks identified using the seroconversion definition proposed by the panel	158	13	1	3	1	-	1	177
Number of outbreaks identified using the alternative seroconversion definition	209	18	3	3	-	1	1	235

Table 3. Incidence rate of equine viral arteritis (EVA) within outbreaks, sensitivity of the proposed rules for identifying seroconversion, total number of outbreaks in French breeding stock and sensitivity of the breeding stock surveillance component (BSSC) between 2006 and 2012 estimated using a zero-truncated binomial model.

Estimated parameter		Median	95% credible interval
$\widehat{Inc}$	Incidence rate within outbreaks (%)	4.9	3.8-6.4
$\widehat{SeR}$	Sensitivity of the proposed rules for identifying seroconversion (%)	79	66-88
$\widehat{N_{inf}}$	Total number of EVA outbreaks	215	195-249
$\widehat{SeB}$	BSSC's sensitivity (%)	82	71-91