

# Equine viral arteritis in breeding stock: a quantitative estimation of the surveillance sensitivity

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## INTRODUCTION

Equine viral arteritis (EVA) is an equine respiratory and reproductive disease:

- caused by a virus of the *Arteriviridae* family which can lead to abortions and neonatal deaths,
- mainly transmitted horizontally by aerosols or venereal contact, including frozen semen,
- monitored in many countries in breeding stock to avoid its spread during breeding activities.

In France, the breeding stock surveillance (BSS) is mainly based on serological tests, but difficulties in interpreting certain series of results may impair the estimation of the number of outbreaks. Moreover, only a part of breeding horses are tested, depending on the studbooks' regulations.



Case of abortion due to EVA virus (Source: Anses Dozulé laboratory)

## OBJECTIVES

- ✓ Establish suitable rules for identifying seroconversion in order to estimate the number of EVA cases and outbreaks detected by the BSS between 2006 and 2013
- ✓ Estimate the sensitivity of the BSS, after having estimated the total number of outbreaks that occurred in breeding stock during this period (including undetected outbreaks) using a capture-recapture method

## MATERIALS AND METHOD

### BREEDING STOCK SURVEILLANCE (BSS)

French breeding stock surveillance is mandatory for:

- mares producing racehorse foals,
- around 20 breeds of stallions used for natural mating,
- all stallions used for semen collection.

A serological test (viral neutralization test, VNT) is used, which is the standard test for EVA prescribed by the OIE. All data are collected by the French institute for horse and riding (IFCE).

### DATA

We used data related to all breeding horses having at least one positive VNT result between January 2006 and December 2013:

- identification numbers,
- locations (town of the holding, recorded at the beginning of each year)
- dates and results of VNT, which are performed each year.

We did not use data pertaining to stallions because the number of males with positive VNT results was very low ( $n=32$ ) compared to mares ( $n=1,645$ ) and some had uninterpretable results.

### DEFINITIONS

'CASE' - 'A mare with seroconversion, detected by the interpretation of several VNT results'

Due to difficulties in the interpretation of certain series of titers, a panel of four experts was gathered to establish suitable rules for identifying seroconversion. The goal of the rules was to allow the estimation of the number of EVA cases detected by the BSS between 2006 and 2013.

'OUTBREAK' - 'A town where at least one EVA case occurred within one year' (an outbreak is thus a 'town-year' infected)

### CAPTURE-RECAPTURE MODEL

A unilist capture-recapture model was used to estimate the total number of outbreaks that occurred in breeding stock ( $N_{occ}$ ) between 2006 and 2013.  $N_{occ}$  was estimated using an extension of the Horvitz-Thompson estimator proposed by van der Heijden and colleagues in 2003:

$$\widehat{N}_{occ} = \sum_{i=1}^{N_{det}} \frac{1}{1 - \Pr(Y_i = 0)}$$

with  $N_{det}$  being the number of detected outbreaks,  $Y_i$  the number of detected cases within outbreak  $i$  and  $\Pr(Y_i = 0)$  the probability that no infected mare was detected in outbreak  $i$  (i.e. outbreak  $i$  is not detected).

$\Pr(Y_i = 0)$  depends on the sensitivity of the proposed rules ( $Se$ ) and on the real number of cases within the outbreak  $i$  ( $C_i$ , that ranges from 1 to  $n_i$ , the number of tested mares in outbreak  $i$ ), which depends on the incidence rate within an outbreak ( $ln$ ).

For each outbreak,  $\Pr(Y_i = 0)$  was calculated as the sum of the probabilities that  $Y_i = 0$  for all possible values of  $c_i$ :

$$\Pr(Y_i = 0) = \sum_{c_i=1}^{n_i} \left\{ (1 - Se)^{c_i} * \frac{\binom{n_i}{c_i} * l^{c_i} * (1-l)^{n_i-c_i}}{1 - (1-l)^{n_i}} \right\}$$

Sum for all possible values of  $c_i$       Probability that  $Y_i = 0$  knowing that  $C_i = c_i$ , assuming that  $Y_i$  follows a binomial distribution of parameters  $C_i$  and  $Se$       Probability that  $C_i = c_i$ , assuming that  $C_i$  follows a zero-truncated binomial distribution of parameters  $n_i$  and  $ln$

Then, the BSS's sensitivity was calculated as the ratio of the number of detected outbreaks ( $N_{det}$ ) by the estimate of the total number of outbreaks that occurred ( $N_{occ}$ ). The parameters were estimated in a Bayesian framework using the WinBUGS software. Priors of the parameters  $ln$  and  $Se$  were determined as beta distributions of parameters (4.6, 9.3) and (44.9, 11.3) respectively, using an expert opinion elicitation. We ran three simulation chains of 10,000 iterations, to determine the posterior distributions.

## RESULTS

### RULES FOR IDENTIFICATION OF SEROCONVERSION

Mares are not vaccinated in France. Therefore, an increase in antibody titer can be due to either infection/re-infection or other reasons, such as slight differences in laboratory practices.

In order to focus only on the mares which have a true seroconversion (such as mares 1 and 2 in Fig. 1), the panel tried to define rules able to exclude mares that were likely infected many months or years ago, i.e. with a mix of negative and low positive results (see mare 4) or with little variations in antibody titer (mare 5), also taking into account possible data entry mistakes (see 6<sup>th</sup> test of mare 3).

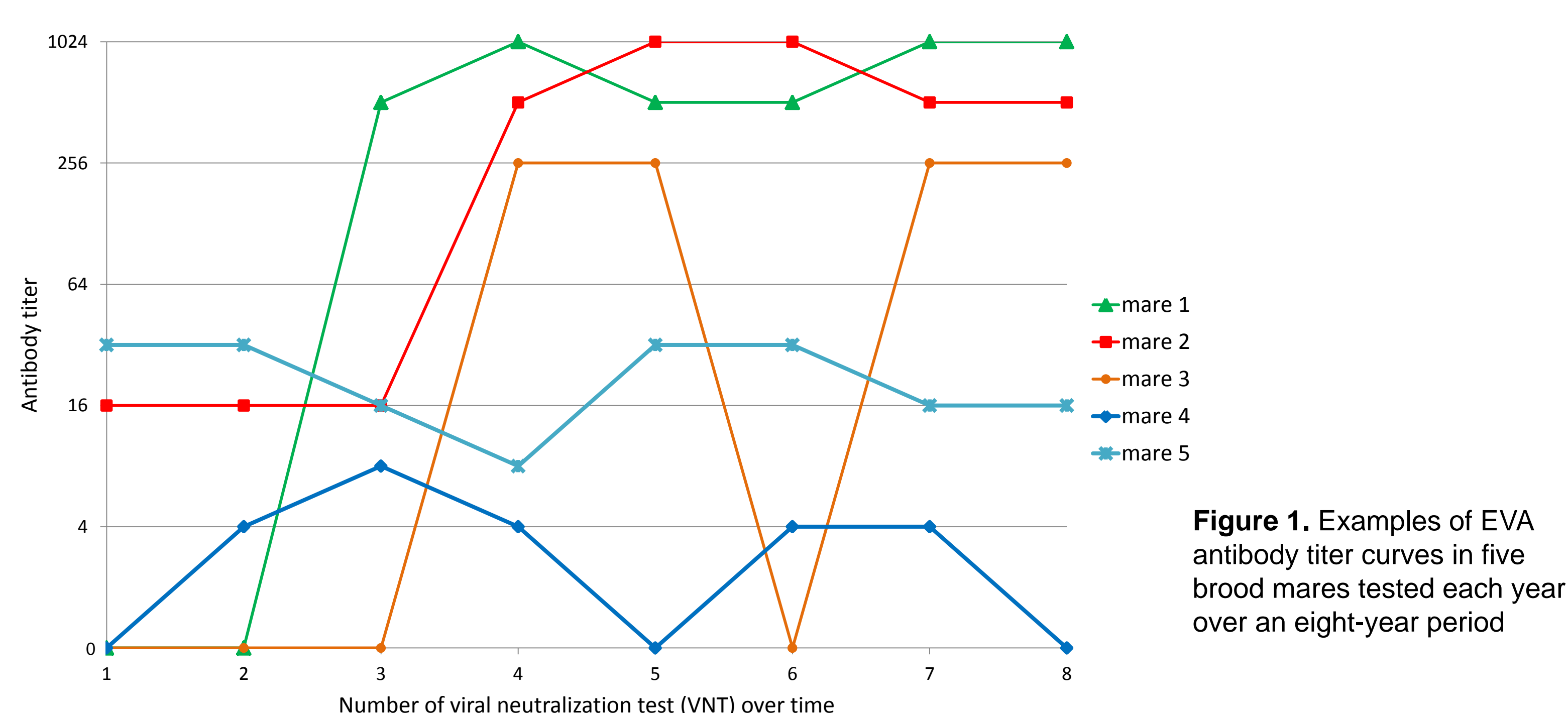


Figure 1. Examples of EVA antibody titer curves in five brood mares tested each year over an eight-year period

### NUMBER OF EVA CASES AND OUTBREAKS DETECTED BY THE BSS

By applying the proposed rules for identifying seroconversion, we observed 239 EVA cases detected in brood mares by the BSS between 2006 and 2013 (Fig. 2). The town was not available for three of these mares. Then we counted the number of cases in each town, considering each year separately, for the 236 cases with a known location: 177 outbreaks were identified (Table 1).

Figure 2. Flow chart documenting the proposed rules used to identify EVA cases among the brood mares tested between 2006 and 2013 in France using VNT

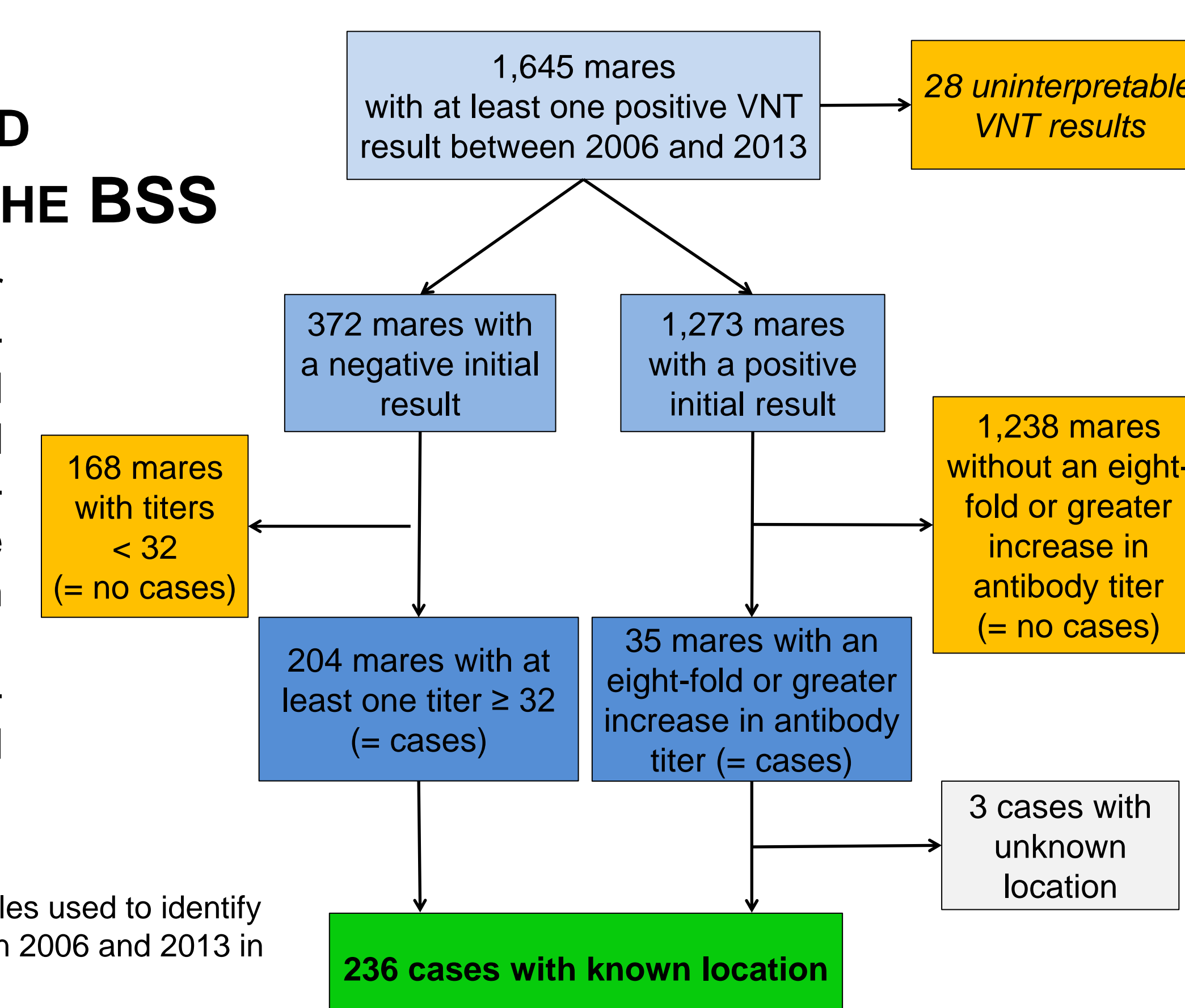


Table 1. Number of EVA cases detected in the outbreaks identified by the BSS between 2006 and 2013

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

### ESTIMATION OF TOTAL NUMBER OF OUTBREAKS AND BSS'S SENSITIVITY

According to the model, the total number of EVA outbreaks that occurred during this period was estimated at 215, on average around 30/year, while the BSS's sensitivity (i.e.  $N_{det}/N_{occ}$ ) was estimated at 82% (Table 2).

Table 2. Total number of EVA outbreaks and BSS's sensitivity estimated in French breeding stock between 2006 and 2013

Parameters	Median	95% credible interval (CrI <sub>95%</sub> )
Estimated number of EVA outbreaks that occurred during the 2006-2013 period	215	195-249
Estimated overall sensitivity of the BSS	82%	71%-91%

## DISCUSSION

**PROPOSED RULES** - Due to the lack of accurate information in the literature about the serological response in naturally infected horses over months and years, we needed to define *ad hoc* rules for identifying seroconversion. Although they are probably imperfect, the high antibody titers usually measured in horses naturally and experimentally infected seem to support the proposed rules for research purposes. These *ad hoc* rules may be used to analyze other EVA surveillance datasets based on serology, such as testing before sales or international trade.

**EVA INCIDENCE** - The number of cases and outbreaks detected by the BSS is not negligible and confirms EVA circulation in French breeding stock. Of the 239 cases, 35 mares (15%) had positive results before showing a sharp rise in antibody titer, suggesting that a proportion of mares have been reinfected, although natural infection is generally recognized as resulting in durable immunity. The total number of outbreaks estimated by our model seems plausible when trying to compare with other countries.

**SURVEILLANCE SENSITIVITY** - The BSS's sensitivity on a town scale seems relatively high. This result supports the relevance of EVA surveillance in breeding stock to prevent the disease spreading through mating.

## CONCLUSIONS

- ✓ The number of EVA cases and outbreaks is not negligible in the French breeding stock
- ✓ A proportion of brood mares have probably been reinfected (15% of cases), a situation which had not previously been documented to our knowledge
- ✓ The estimate of the BSS's sensitivity between 2006 and 2013 is relatively high, however it could be improved by a closer relationship between surveillance components and more detailed information about the horses' location
- ✓ 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

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