Influential factors inducing suboptimal humoral response to vector-based influenza immunisation in Thoroughbred foals

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ABSTRACT

Context: Numerous equine influenza (EI) epizooties are reported worldwide. EI vaccination is the most efficient methods of prevention. However, not all horses develop protective immunity after immunisation, increasing the risk of infection and transmission.

Objectives: This field study aimed to understand the poor response to primary EI vaccination.

Study design: The EI antibody response was measured in 174 Thoroughbred foals set in 3 stud farms (SF#1 to SF#3) over a 2 years period. All foals were immunised with a commercial recombinant canarypox-based EI vaccine. Sera were tested by single radial haemolysis against the A/equine/Jouars/4/06 EIV strain (H3N8) at the time of the first vaccination (V1), 2 weeks and 3 months after the second immunisation (V2), 2 days and 3 months after the third immunisation (V3).

Results: The frequency of poor-responders (no detectable antibody titres) was surprisingly elevated after V2 (56.8%), increased to 81.7% at V2 + 3 months and reached 98.6% at V3. The frequency of poor-responder was still 19.2%, 3 months after V3. Two independent influential factors were identified. The short (V2 + 2 weeks) and mid-term (V2 + 3 months, V3 + 3 months) antibody levels were positively correlated to the age at V1 (p-value = 0.003, 0.031 and 0.0038, respectively). Presence of maternally-derived antibodies (MDA) at V1 was negatively correlated with antibody levels after V3 only (p-value = 0.0056). Given that SF#1 antibody response was below clinical protective levels at all-time points studied, the annual boost immunisation (V4) was brought forward by 7.0 ± 1.1 months. V1 was delayed by 7 weeks the following year, which significantly increased short- and mid-term antibody titres (p-value = 9.9e-07 and 2.31e-07, respectively).

Conclusion: The age and MDA at first immunisation with the canarypox-based IE vaccine play an independent role in the establishment of antibody levels. This study also highlights the benefit provided by serological surveillance to evaluate herd immunity and to implement corrective management/vaccination measures.

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1. Introduction

Equine influenza (EI) is considered one of the most important equine respiratory pathogen due to its high morbidity, contagious nature and potential economic losses associated with its epidemics [1]. Several countries around the world have experienced major epizooties in the past such as South Africa (1986 and 2003), India
(1987), Hong Kong (1992) and more recently Australia (2007) [2–6].

Vaccination against equine influenza virus (EIV) remains to this day one of the most effective methods to prevent or limit the impact of EI outbreaks [7,8]. Equine influenza vaccines are available commercially since the 1960s and are widely used worldwide. The effectiveness of EI vaccination to provide clinical and virological protection has been demonstrated repeatedly and the correlates of protection are well defined [9,10]. However, not all horses develop protective immunity after EI immunisation.

Suboptimal response to vaccination is a well-recognised phenomenon in which part of the vaccinated population fails to mount an adequate immune response and therefore remains susceptible to disease [11,12]. These horses, called low/poor vaccine responders, are partially protected and may develop a subclinical form of the disease. They can shed large quantities of infectious virus over significant periods of time [10] and can contribute to the spread of the disease [5,13,14]. Analysis of post-race samples indicated that up to 7.5% of Thoroughbred horses had no detectable levels of SRH antibodies, despite mandatory EI vaccination [15]. It is possible that the catastrophic outbreak of EI in Australia in 2007, during which over 76,000 horses were infected, was started by the importation of such a horse. Partial protection due to low vaccine response will not only reduce the overall herd immunity and lead to the spread of disease but also favour influenza virus antigenic drift that could lead to vaccine breakdown in the mid- to long-term [16].

Causes of poor response to EI immunisation remain largely unknown but are likely to be of diverse origins, including the host genetic background, the vaccine design, management conditions, health at the time of immunisation etc. This prospective observational cohort study aimed to evaluate the frequency of poor responder to EI vaccination in Thoroughbred foals. The specific objective was to measure the single radial haemolysis (SRH) antibody response, a correlate of protection against EI, during the primary field EI vaccination. All foals were immunised with a commercial EI-tetanus toxoid (TT) vaccine. Our results highlight an independent impact of age and maternally-derived antibodies (MDA) levels at the time of first immunisation against EI on short and mid-term antibody levels in Thoroughbred foals.

2. Materials and methods

2.1. Animals

2.1.1. Inclusion criteria

Thoroughbred foals receiving a primary course of EI vaccination (V1 to V3). Year #1 (July 2013 to October 2014): the study was carried out in a population of 117 unvaccinated Thoroughbred foals on 3 different private stud farms (SF#1 to SF#3) in Normandy (France). The age at the time of first vaccination (V1) ranged from 119 to 259 days for the first year (159.3 ± 27.6 days; 17–37 weeks). Year #2 (August 2014 to March 2015): the study was carried out on 81 unvaccinated Thoroughbred foals in SF#1 and SF#2. The age at V1 ranged from 142 to 249 days (189.8 ± 22.4; 20.3–35.6 weeks). SF#3 did not join the study for Year #2. The number of foals enrolled in the study was dependent of availability in the participating stud farms. All animal work received ethical approval from the LABEO Frank Duncombe ethical advisor and owner consent were obtained.

2.2. Vaccine and immunisation schedule

The EI vaccine choice and immunisation schedule were defined by the participating Veterinary Practitioners, as part of the field management of the horse population under their care. Year #1: a commercial recombinant canarypox-based EI-TT vaccine (ProteqFlu-Tt; Merial) was used. At the time of this study, it contains the EIV strains A/equine/Ohio/03 (H3N8; Florida Clade 1) and A/equine/Newmarket/2/93 (H3N8; European lineage) with tetanus toxoid. The commercial recombinant canarypox-based EI vaccine (ProteqFlu; Merial) was used in SF#2 at V3. Year #2: the recombinant canarypox-based EI-TT vaccine (ProteqFlu-Tt; Merial) was used by SF#1. SF#2 used a subunit EI vaccine (Equip FT; Zoetis) that contains the EIV strains A/equine/Newmarket/77 (H7N7), A/equine/Kentucky/98 (H3N8; American lineage), A/equine/Borlange/91 (H3N8; European lineage) with tetanus toxoid. Due to this change of EI vaccine between Year #1 and Year #2, SF#2 results (year #2) were not included in the study. The vaccines were administered by deep intramuscular injection in accordance with the vaccine manufacturer’s data sheet. The foals received 2 immunisations (V1 and V2), 4–6 weeks apart (30.96 ± 3.71 days for year #1 and 38.03 ± 5.21 days for year #2) and a third dose (V3), six months (177.65 ± 11.35 days for year #1 and 179.68 ± 1.71 days for year #2) after V2. SF#1 foals received a boost immunisation (V4) at 164.96 ± 3.01 days. This report follows the CONSORT 2010 guidelines (supplementary CONSORT check list and flow chart) [17,18].

2.3. Serum sample and serology

The sampling schedule was defined in collaboration with the participating Veterinary Practitioners in order to minimise the impact on usual veterinary and management procedures. Outcome measure: serum samples were collected at the time of the first vaccination (V1) to evaluate the presence of MDA (P1), two weeks after the second immunisation (V2) to measure the antibody response at the onset of immunity (P2); three months after V2 (P3) and 2 days after the third immunisation (V3; P4) to evaluate the immunity gap between V2 and V3, three months after V3 (P5) to identify individuals that failed to maintain their antibody response at protective levels (indicative of possible poor response to EI vaccination). Some P4 samples were missed and subsequently collected 1 week after V3. They will be referred as P4bis (P4’). The number of foals bled per sampling time points is detailed in Table 1 and the CONSORT flow diagram. Serum samples were stored at −20°C until analysis. Antibodies were measured by SRH assay against the EIV strain A/equine/Jours/4/06 (H3N8; Florida Clade 2), as previously described [19]. The A/equine/Jours/4/06 isolate is representative of the Florida Clade 2 strains circulating in France in recent years [20]. Control antisera from the European Directorate for the Quality of Medicines and Healthcare (EDQM) was included on each plate (A/equine/south Africa/4/03 Horse antisera BP reference Y000712; acceptable range defined for the A/equine/ Jours/4/06 antigen batch used = 178.1–217.7 mm²; average control titre = 193.4 ± 9.64; n = 65 plates/assays). The titres of SRH antibody were expressed as the area of haemolysis (mm²). An increase of at least 25 mm² or 50% in the area of the zone of haemolysis was regarded as significant. A poor responder was defined as a horse that had no detectable SRH antibody response.

2.4. Statistical analysis

Statistical analyses were performed with STATGRAPHICS Centurion XVI, version 16.1.12 (StatPoint Technologies, Inc). Where appropriate based on standard deviation, analyse of variance (ANOVA) or Kruskal–Wallis Test were used to test group and/or time points significant differences (with 95% confidence intervals). Where appropriate based on normality test for group distribution, Student’s t-test (S) or Wilcoxon signed rank test (W) were used to compare groups at specific time points. A two-tailed Fisher’s Exact...
The frequency of poor-responder was 56.8% at the peak of the cohort. The level of MDA against EI at the time of first immunisation was different between Stud Farms, with SF#2 > SF#1 and SF#3 at V2 + 3 months and V3 + 3 months respectively. 

The multiple regression analysis (all SF included, Fig. 3A) indicated that the age at first immunisation (V1) had a significant impact on the SRH antibody levels at V2 + 2 weeks (P2) and V2 + 3 months, and V3 + 2 weeks (P-value = 0.003, 0.031 and 0.0038, respectively). 

3.2. Influencing factors of the SRH antibody response 

The multiple regression analysis (all SF included, Fig. 3A) indicated that the age at first immunisation (V1) had a significant impact on the SRH antibody levels at V2 + 2 weeks, V2 + 3 months and V3 + 2 weeks (P-value = 0.003, 0.031 and 0.0038, respectively). 

The average age at V1 was statistically different between stud farms. Individual study farm analysis showed that the SRH antibody responses for SF#1 and SF#3 were lower than the one measured from SF#2, with a frequency of foals with no SRH antibody titres at V2 + 2 weeks (P2) well above 50% (65.9% and 56.7% in SF#1 and SF#3, respectively), as opposed to 34.8% for SF#2 (Table 1). SRH antibody levels induced by EI vaccination were significantly different between Stud Farms, with SF#2 > SF#1 but similar to SF#3 at V2 + 2 weeks (P-value = 0.031; ANOVA), and SF#2 > SF#1 and SF#3 at V2 + 3 months and V3 + 3 months (P-value = 0.0036 by Kruskal–Wallis and 0.0003 by ANOVA, respectively).

3.3. Serum samples from SF#3

SRH titre (all) 21.4 ± 36.7 50.6 ± 70.3 10.8 ± 26.2 2.4 ± 10.3 207.0 ± 10.9 347.0 ± 17.3 23 (100%) 115.4 ± 57.3 125.1 ± 52.0 20 (100%) (pos.) 63.5 ± 35.5 102.5 ± 74.3 13.0 ± 27.7 2.0 ± 8.4 115.4 ± 57.3 129.8 ± 46.8 20 (100%) (pos.) 53.4 ± 36.3 153.9 ± 58.9 67.1 ± 30.5 4.1 ± 12.9 115.7 ± 46.4 90.0 ± 27.7 20 (100%) (pos.) 192.5 ± 12.6 243.4 ± 13.6 312.8 ± 15.0 43.4 ± 38.9 43.4 ± 38.9 12 (3.6%) (pos.)
(V1, P1) was found to influence the SRH antibody response when measured at V3 + 3 months (p-value = 0.056). Nineteen foals out of 70 (27.14%) possessed detectable MDA levels at V1. The frequency per stud farm was 37.78% for SF#1 (16/45 foals; 29.4 ± 41.3 mm²), 4.76% for SF#2 (1/21 foals; 2.6 ± 12.0 mm²) and 50% for SF#3 (2/4 foals; 29.6 ± 36.8 mm²). MDA levels were significantly different between SF#1 and SF#2 only (p-value = 0.015; Kruskal–Wallis).

The polynomial regression (age at V1 (x) vs SRH titre (y)) indicated that an order of 1 should be considered for regression analysis (i.e. simple regression). Simple regression analyses (Fig. 3B) showed a positive correlation between SRH antibody levels and the age at first immunisation at all 3 time points analysed (V2 + 2 weeks, V2 + 3 months and V3 + 3 months) and a negative correlation between SRH antibody levels at V3 + 3 months and MDA levels at V1. Based on linear and best fit simple regression analyses (Supplementary Table 3), the average age to administered the first immunisation should be at 184 ± 18 days (6.1 ± 0.6 months) and 163 ± 9 days (5.4 ± 0.3 months) in order to reach the clinical protection threshold (SRH titres = 85–90 mm²) at V2 + 2 weeks and V3 + 3 months, respectively. The average age at V1 should be 245 ± 46 days (8.2 ± 1.5 months) and 236 ± 28 days (7.9 ± 0.9 months) to reach the virological protection threshold (i.e. 120–154 mm²) at V2 + 2 weeks and V3 + 3 months, respectively. The simple regression analysis indicated that the MDA level at V1 should be = 0 in order to reach the clinical protection threshold at V3 + 3 months.

3.3. Modification of the immunisation schedule

Due to the negative or low levels of SRH antibody measured in SF#1 at P5, the annual EI boost immunisation (V4) was advanced by 7.0 ± 1.1 months (V3 + 5.2 ± 1.1 months) for all 45 foals (year#1) in order to restore SRH antibody titres at protective levels. At the time of V4, the SRH antibody level was 43.4 ± 38.9 mm². The level reached 173.3 ± 39.2 mm² when measured 38 days later (Fig. 4A). The vaccination schedule in SF#1 was also modified for horses enrolled in the second year of the study. The age at first
immunisation was increased, from 143.9 ± 16.3 days during year#1, to 192.6 ± 11.2 days for year#2 (Fig. 4B). A slight increase in vaccination intervals was recorded between year#1 and year#2, with V1–V2 intervals of 30.8 ± 5.7 days and 35.4 ± 4.0 days, and V2–V3 intervals of 166.1 ± 6.4 and 179.9 ± 1.9 (for years#1 and#2, respectively). The year#2 SRH antibody level reached 102.5 ± 74.0 mm² when measured at V2 + 2 weeks, 13.0 ± 27.8 at V2 + 3 months and 125.1 ± 52.0 mm² at V3 + 3 months (Fig. 4C), which were significantly higher from year#1 levels in the same stud farm and measured at the same time points (\(p\)-value = 2.5e-07, 0.014 and 2.31e-07, respectively). The frequency of foals with no SRH titres was significantly reduced during year#2, with \(p\)-values = 0.0001, 0.0189 and 0.0007 for P2, P3 and P5, respectively). Incorporation of year#2 results in the multiple regression analysis confirmed that the age at first immunisation (V1) had a significant impact on the SRH antibody levels at V2 + 2 weeks, V2 + 3 months and V3 + 3 months (\(p\)-values < 0.0001, = 0.026 and = 0.0001, respectively) and the presence of MDA at V1 on the SRH antibody titres at V3 + 3 months (\(p\)-value = 0.0001). Simple regression analyses (Supplementary Table 4) indicated that the average age at first immunisation to reach the clinical protection threshold at V2 + 2 weeks and V3 + 3 months were 174 ± 13 days (5.8 ± 0.4 months) and 160 ± 4 days (5.3 ± 0.1 months), respectively. The average age at V1 to reach the virological protection threshold at V2 + 2 weeks and V3 + 3 months were 217 ± 23 days (7.2 ± 0.8 months) and 220 ± 24 (7.3 ± 0.8 months), respectively. The MDA level at V1 should be = 0 in order to reach the clinical protection threshold at V3 + 3 months.

4. Discussion

The main purpose of the study was to investigate poor response to EIV vaccination. The humoral immune response was measured in Thoroughbred foals from 3 different stud farms after primary EIV immunisation. Based on previous SRH antibody kinetics [21], the
sampling schedule was defined to favour identification of poor vaccine responder. The onset and peak of immunity induced by EI vaccination was only measured after V2 and is therefore under-represented in our study.

The overall patterns of antibody response were similar to those reported by Gildea et al. [22]. Results presented here indicate that age and MDA levels at first immunisation were determinant factors in the establishment of a lasting humoral response during the primary immunisation. Cullinane et al. demonstrated that foals who received their first immunisation at the age of 3 months had not obtained satisfactory levels of antibodies 1.5 months after the third immunisation [23]. Foals whose primary vaccination was administered at the age of 6 months developed significantly higher antibody levels when measured 1 month after the third immunisation. The involvement of MDA in the reduction of the humoral response, for the group of foals vaccinated at 3 months, was also demonstrated. The vaccine administered was a subunit EI vaccine or a whole inactivated EI vaccine [23]. These results were supported by the epidemiological analysis of the Newmarket EI outbreak in 2003, which clearly demonstrated that foals immunised for the first time between 6 and 18 months of age were at significant lower risk of infection than foals immunised prior to 6 months of age. A whole inactivated, hydroxide aluminium adjuvanted, EI vaccine was predominantly used in Newmarket prior to this outbreak [24]. Interference of MDA with the immune response to EI vaccines is well established [23,25] but the impact of such an interaction may depend on the type of vaccines used. Whole inactivated and/or sub-unit EI vaccines are known to be neutralised by MDA [26]. In our current study, the use of a canarypox-based vaccine should, in theory, circumvent this problem. This live recombinant vector vaccine has the particularity of being genetically stable, which allows the insertion of foreign DNA segment, such as the EIV hemagglutinin (HA), but the HA is not present on the surface of the vaccine vector (i.e. no direct interaction between MDA and the EI vaccine). A previous study has demonstrated that the use of the recombinant canarypox-based EI vaccine in 10–20 weeks old foals and in presence of MDA did not result in measurable SRH antibody levels after V1 but was able to prime the equine immune response in 7 out of 8 foals (87.5%), with significantly higher SRH antibody titres when measured 2 weeks after V2 [27]. Another study reported no or non-protective SRH antibody titres measured between V2 and V3 + 3 months in one weanling immunised with the canarypox-based EI vaccine in presence of MDA [22]. By comparison, only 9 out of 26 foals (34.6%) immunised in presence of MDA showed measurable SRH antibody titres 2 weeks post V2 in the current study. The multifactorial analysis revealed that MDA was not a significant influential factor at this specific time point but had a significant impact on the SRH antibody titres when measured 3 months post V3. MDA may possibly interfere with the establishment of the humoral response by targeting the host cells expressing the EIV HA during the immunisation process (i.e. host cells infected with the recombinant vectored EI vaccine; Fig. 5). Limited HA cell-expression prior to lysis by MDA binding and complement fixation may be sufficient to prime the immune response. However, the vaccine immunogenicity in vivo appears to be reduced, resulting in lower SRH antibody levels. Further investigation is warranted. The absence of direct interaction between MDA and the canarypox-based EI vaccine, when compared with whole inactivated or sub-unit EI vaccines, could explain a significant impact detected at V3 + 3 months only, when immunogenicity differences may be more exacerbated and/or detectable than at the peak of immunity (i.e. V2 + 2 weeks). A direct interaction between canarypox-specific MDA (for mares that received the canarypox-based EI vaccine) and/or a whole inactivated EI vaccine [23]. These results were supported by the epidemiological analysis of the Newmarket EI outbreak in 2003, which clearly demonstrated that foals immunised for the first time between 6 and 18 months of age were at significant lower risk of infection than foals immunised prior to 6 months of age. A whole inactivated, hydroxide aluminium adjuvanted, EI vaccine was predominantly used in Newmarket prior to this outbreak [24]. 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A previous study has demonstrated that the use of the recombinant canarypox-based EI vaccine in 10–20 weeks old foals and in presence of MDA did not result in measurable SRH antibody levels after V1 but was able to prime the equine immune response in 7 out of 8 foals (87.5%), with significantly higher SRH antibody titres when measured 2 weeks after V2 [27]. Another study reported no or non-protective SRH antibody titres measured between V2 and V3 + 3 months in one weanling immunised with the canarypox-based EI vaccine in presence of MDA [22]. By comparison, only 9 out of 26 foals (34.6%) immunised in presence of MDA showed measurable SRH antibody titres 2 weeks post V2 in the current study. The multifactorial analysis revealed that MDA was not a significant influential factor at this specific time point but had a significant impact on the SRH antibody titres when measured 3 months post V3. MDA may possibly interfere with the establishment of the humoral response by targeting the host cells expressing the EIV HA during the immunisation process (i.e. host cells infected with the recombinant vectored EI vaccine; Fig. 5). Limited HA cell-expression prior to lysis by MDA binding and complement fixation may be sufficient to prime the immune response. However, the vaccine immunogenicity in vivo appears to be reduced, resulting in lower SRH antibody levels. Further investigation is warranted. The absence of direct interaction between MDA and the canarypox-based EI vaccine, when compared with whole inactivated or sub-unit EI vaccines, could explain a significant impact detected at V3 + 3 months only, when immunogenicity differences may be more exacerbated and/or detectable than at the peak of immunity (i.e. V2 + 2 weeks). A direct interaction between canarypox-specific MDA (for mares that received the canarypox-based EI vaccine
during pregnancy) and the El vaccine administered to the foal could not be ruled out. However, pre-existing immunity to the canarypox-based vector does not impair efficiency of subsequent immunisation with the same or another canarypox-based vaccine [28], in contrast to vaccinia-based vector [29].

Several studies highlight the evolution of foal’s immunity during the first months of life. IL-4 [30] or IFNgamma [30,31] secretion by mitogen-activated T-lymphocytes was significantly lower in 3–4 months old foals when compared to 6 months foals or adults. Increased frequency of CD4⁺ CD25high FoxP3⁺ IL-10⁺ regulatory T-lymphocytes (Treg), which exhibit suppressive activity, was recently reported in foals up to 3.5/4 months of age when compared to yearlings and adult mares. Inhibition of Th1 and Th2 cells stimulation was suggested [32]. Immunisation of 4 months old foals in the presence of elevated regulatory mechanisms (cells and cytokines) may provide an explanation for the suboptimal vaccine immunogenicity observed in the current study. The importance of age, which also reflects the maturation of the immune response, could have been overlooked in previous epidemiological El vaccination studies due to the predominant impact of the presence of MDA at V1 in the context of whole inactivated and/or subunit El vaccine.

Most of the EIV strains circulating in France and the EU in recent years belong to the Florida Clade 2 sub-lineage [20,33–35], which justified the use of A/equine/Jouars/4/06 (Florida Clade 2) as the chosen SRH antigen in the current study. However, the El vaccine used at the time of the study did not contain a Florida Clade 2 representative strain but did contain the Florida Clade 1 A/equine/Ohio/03 strain. HA sequence comparison reveals 6 amino acid substitutions between the strains A/equine/Jouars/4/06 and A/equine/Ohio/03 (supplementary Table 1 and Fig. 1). The serological impact of heterogeneous EIV vaccine strains and SRH antigens has been previously observed after the first El immunization, but was usually non-detectable after the second immunization [21,36]. A selection of serums was analysed by SRH using the EIV A/equine/South Africa/4/03 strain as antigen (closely related to the El vaccine strain A/equine/Ohio/03) and compared with SRH titres obtained with the A/equine/Jouars/4/06 SRH antigen. Results showed a good

Fig. 4. Modification of the vaccination schedule in SF#1. (A) Early boost immunisation (V4; P6) and subsequent SRH antibody titre (P7). (B) Comparison of the age at first immunization (V1) in SF#1 between study year #1 and year #2. (C) Comparison of the SRH antibody titres measured in SF#1 foals at V2 + 2 weeks and V3 + 3 months between study year #1 and year #2. Statistical significant is indicated as bold text, with S = Student’s t-test and W = Wilcoxon signed rank test. Dotted lines represent thresholds for protection against clinical signs of El disease (>85 mm²) and EIV shedding (>154 mm²).

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The frequency of foals displaying low or negative SRH antibody titres in the first year following their primary course of EI immunisation is alarming. This poor response to vaccination could be classed as “circumstantial”. The associated susceptibility to EI infection is likely to be temporary and reversible if the vaccination schedule is adjusted, as illustrated in SF#1. These foals with low antibody titres should be differentiated from the “natural” or “fundamental” poor vaccine responders, whose inability to mount or maintain a satisfactory immune response may be linked to intrinsic reasons, such as genetic background [37].

Defining the age for first immunisation against EI is a difficult matter that probably requires a case by case analysis, taking into account the risk of contact with EIV. Foals immunisation at the minimum age (e.g. 4 months in this specific study) did not provide the optimal level of immunity. However, such vaccination strategy may be necessary in face of an imminent EI outbreak or contact risk when the period of susceptibility to EI infection need to be reduced. In conclusion, this study also highlights the benefits provided by serological surveillance in order to evaluate the protection level of a specific population, to identify poor vaccine responder and to implement corrective management/vaccination measures.

Conflict of interest and contribution

SF and DG report no conflict of interest. LL, RN, SP and RP have collaborated and published with all European veterinary vaccine manufacturers, including Merial who commercialises the EI vaccine used in this study. However, LL, RN, SP and RP have not acted as promotional speakers or writers on behalf of these companies. MF, XD’A and PB prescribe the EI vaccine used in this study in their quality of Veterinary Practitioners.

Contribution: SF, DG and RP have performed the sample analyses. LL, SP and RP have designed the study. MF, XD’A and PB have conducted the animal selection, enrolment and supervised sample collection. RN has provided support for the statistical analysis. SF, SP and RP prepared the manuscript.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2016.05.068.

References


