The protective effectiveness of an inactivated bovine ephemeral fever virus vaccine

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A B S T R A C T

Bovine ephemeral fever (BEF) is an important viral disease of cattle. Despite the extensive use of inactivated vaccines for the prevention of BEF, a controlled study of their field effectiveness has never been performed. We conducted a large field effectiveness study of a BEF inactivated vaccine, during a large BEF outbreak. Neutralizing antibody titers measured in 385 heifers and calves 1 month after 2nd vaccination averaged 1:91.8 (CI95% = 76.6–110.0). The effectiveness study enrolled 2780 cows in nine herds. In two herds cows vaccinated twice, 1 year before the outbreak and once 2–3 months before outbreak onset were compared with non-vaccinated cows. Average vaccine effectiveness of three vaccine doses compared to no vaccination was 47% (CI95% = 34–57) in these herds. In two other herds cows vaccinated twice 1 year before the outbreak and twice again 2–3 months before outbreak were compared with cows vaccinated only twice 2–3 months prior to the outbreak. Average vaccine effectiveness of four doses compared to two doses was 49% (CI95% = 25–65) in these herds. In five herds cows vaccinated twice 2–3 months before outbreak onset were compared with non-vaccinated cows. This vaccination schedule was shown to be non-effective (average effectiveness of 2%, CI95% = −14–17). Milk production analysis on one of the affected herds, in which 56% vaccine effectiveness and an absolute reduction of 27% in morbidity were documented, revealed a net milk production loss of 175.9 kg/sick cow (CI95% = 127.9–223.9) and an average gain of 37 kg for each vaccinated cow (CI95% = −3.6–77.7). This study indicates that despite the fact that two vaccine doses of the tested inactivated vaccine elicited high titers of neutralizing antibodies, partial protection was induced only when at least 3 doses were administrated before natural challenge.

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1. Introduction

Bovine ephemeral fever (BEF) is an economically important viral disease of cattle and buffalo which occurs mostly in tropical and subtropical climates in Africa, Asia, the Middle East and Australia (Walker, 2005). Both live attenuated and inactivated vaccines are available for field
use, with the former shown to induce longer lasting immunity (Tzipory and Spradbrow, 1973, 1978; Van selow et al., 1995) than inactivated vaccines (Aziz-Boaron et al., 2013; Della-Porta and Snowdon, 1979; Inaba et al., 1973). However, attenuated vaccines are considered less safe. Possible risks include adverse clinical signs (Della-Porta and Snowdon, 1977), reversion to virulence, especially due to relatively high mutation rate of RNA viruses (Nak-Hyung Lee et al., 2012), as well as introduction of other contaminating viruses during the preparation process (Pastoret, 2010; Studer et al., 2002).

To date the only controlled field effectiveness study conducted for BEF vaccines was performed in Australia for a live virus vaccine, partially inactivated after mixture with the adjuvant Quil A. (Van selow et al., 1985, 1995). Administration of two doses of this vaccine resulted in high neutralizing antibody (NA) titers and 90% protective effectiveness, which lasted at least 12 months.

A MONTANIDE™ ISA 206 VG (water-in-oil-in-water) inactivated vaccine was developed in the Kimron veterinary institute in Israel (Aziz-Boaron et al., 2013). This vaccine was found to be safe and immunogenic, inducing a significant NA response (up to 1:256) following the second, third or fourth booster vaccination. However, a fast decline in NA titers was observed after 120 days (Aziz-Boaron et al., 2013). In addition, two out of 30 cows did not respond to vaccination and did not develop specific NA. This article describes the results of a large field study examining for the first time the effectiveness of various administration protocols of this inactivated vaccine.

2. Materials and methods

2.1. The vaccine

A MONTANIDE™ ISA 206 VG (water-in-oil-in-water) inactivated BEF virus (BEFV) vaccine was used in this study. BEFV isolated in Israel in 2000 (Yaqum-00) was used as the inactivated virus in this vaccine in a concentration of 10^4.5—10^5.5 TCID_{50}/ml. Further details on the preparation, immunogenicity and safety of this vaccine were recently described (Aziz-Boaron et al., 2013). All animals were vaccinated intra-muscularly (IM).

2.2. Serum neutralization test

BEFV NA were detected in the collected sera using the serum neutralization (SN) test as was recently described (Aziz-Boaron et al., 2013).

2.3. Ethics

The study took place in several dairy herds of high-producing Israeli Holstein cows located in the southern Coastal Plain region in Israel with the owner’s permission and cooperation. The study was approved by the Institutional Animal Care and Use Committee of the Ministry of Agriculture.

2.4. Study design

The study was divided to two stages. The first stage took place during the summer of 2007 aiming at monitoring the immune response following 1st and 2nd vaccination with the inactivated vaccine. In addition, it was aimed at revealing the percentage of cows not responding to vaccination in three different age groups. The studied population included 9 dairy herds located along the Jordan Valley, in which cattle bearing even numbers were vaccinated IM twice, 1 month apart with a 1 ml dose of vaccine. Cattle bearing non-even numbers were not vaccinated and served as a control group in order to monitor natural BEFV exposure during the study period. In each herd, blood samples were obtained from 10–15 vaccinated cows from three age groups: calves (6–12 months), pregnant heifers and primiparous heifers, according to the following schedule: before vaccination (n = 401), 1 month after 1st vaccination (n = 382) and 1 month after 2nd vaccination (n = 385). Blood samples were obtained from the non-vaccinated cattle (n = 298) 1 month after 2nd vaccination, parallel to the last sampling of the vaccinated cows. Sera were separated from whole blood and kept at −70°C until the performance of SN assay. The average antibody titer following each vaccination was calculated for each age group in each of the three samplings. The distribution of the non-responding (NA titer ≤1:1) and weak responsive (NA titer ≤1:8) cows according to age group was also analyzed.

The second stage was aimed at testing the effectiveness of the vaccine. The study population included cattle from 9 dairy herds located in the north and south of the Jordan Valley and the Jezerel Valley. In all herds, cattle were divided into two reference groups: Cows bearing an even number were referred to as the vaccine group and cows bearing an odd number were referred to as the reference group. Herds were vaccinated in several schedules. In some of the herds the reference group was vaccinated as well but always using a lower number of vaccine doses than the vaccine group. Vaccinated groups received two, three or four doses of 1 ml inactivated Israeli BEFV vaccine which was administrated IM during the summer (June-August) of 2007-2008 as follows: In herds 1–5, two vaccinations were administered to the vaccine group 1 month apart during 2008. In herds 6–7, two vaccinations were administered to the vaccine group, 1 month apart in 2007 and one vaccination in 2008. In Herds 8–9, two vaccinations were administered to the vaccine group 1 month apart in 2007 and two vaccinations 1 month apart in 2008. In herds 8–9, reference group was also vaccinated with two vaccinations 1 month apart in parallel to the vaccine group during 2008. Approximately 2 months after the 2008 vaccination (during September-October) a BEF outbreak had occurred in Israel. Clinical information on individual cow morbidity was collected by the herdsmen on a daily basis in herds 1–9, both by visualization of cows and by monitoring milk production. Computerized daily milk production data were collected automatically at the milking parlors by the Affifarm™ software (Afikim, Israel) and saved to computer files by the herd management software Noa™ (Israel Cattle Breeders Association, Israel).
Caesarea, Israel). A reduction of 30% or more in milk production in comparison to the previous day was documented by the herd management software and the herds were automatically informed, ensuring a uniform data collection for all cows. Every BEFV suspected cow was examined by the herd veterinarian who visited the farm at least twice weekly, to rule out any other non-arbo-viral disease which may have caused the reduction in milk production. Based on this information a probable BEF case was defined as any cow with an abrupt reduction in milk production (at least 30%) compared to the previous day with no signs of other disease.

In order to validate clinical observation with serology as well as to verify the immunogenic response induced by the vaccine, blood samples were collected from herd no.7, 19 days after the first index case was discovered (November 3rd 2008). A total of 49 healthy-vaccinated cows and 56 healthy-non-vaccinated cows were sampled. In addition a second sampling was conducted 104 days after the first index case was discovered (January 27 2009), and a total of 22 blood samples were collected from sick-vaccinated cows and 26 samples from sick-non-vaccinated cows. As vaccine was administered on July 1st, 2008, the time elapsed between vaccination and the first and second sampling dates was 125 days and 210 days, respectively. Sera were separated from whole blood and were kept at −70°C until the performance of SN assay. A measured NA titer of ≥1.8 in the non-vaccinated group was considered indicative for positive infection. The percentage of positive samples was calculated for each group.

2.5. Statistical analysis

Geometric mean titers (GMT) of BEF NA in the three groups in the first stages of the study were calculated for each blood sampling and were compared using General linear model containing the farm, age-group effects as well as their interaction if significant. If a significant interaction was found, the group comparison was performed separately for each farm. Percentage of cattle not responding to vaccination was also compared between the three groups at each sampling time. This comparison was performed by logistic regression, which included the farm and age-group effects as well as their interaction if significant. This analysis was performed using SPSS™ 21.0 for Windows™. Graphs presenting group differences in GMT and percentage of non-responders were drawn after summation of the data from all the 9 vaccinated herds.

Data from herds using the same vaccination schedule were combined by calculating the Relative-risk (and hence vaccine effectiveness), using the Mantel-Heanszel method. A p-value <0.05 was considered as indicating statistical significance in all tests. WINPEPI® statistical software (Abramson, 2004, 2011) was used for this part of the statistical analysis.

In order to calculate the loss of milk due to morbidity and to estimate the milk loss prevented by vaccination we chose the herd which showed the highest incidence and highest vaccine effectiveness (herd 6, Table 3). Computerized daily milk production data were saved automatically for the period before and after the outbreak. Data were truncated to include individual cow daily milk production from August 1st to December 31 and to include only milk data from cows that were no more than 200 days after calving (Days in milk–DIM). The loss of milk during morbidity was calculated using a generalized estimating equation (GEE) model using an auto regressive covariance matrix within each cow. The model included parity and a third degree polynome of days in milk. It also included the week in the year during which each milk production recording was recorded and a variable presenting morbidity. The same model was constructed for calculating prevention of milk loss due to vaccination with a variable signifying vaccination instead of morbidity. Interaction of this last variable (i.e. morbidity or vaccination) with the week of the year was inserted to both models. This interaction represents the average daily difference in milk production during each week as compared to the baseline difference between sick/non-sick or vaccinated/non-vaccinated cows and therefore was used to calculate milk loss associated with these specific conditions during the outbreak. This part of the analysis was performed using SPSS™ 21.0 for Windows™ (IBM). These milk production differences and their 95% confidence interval were drawn for each week, starting at August 1st, 2008 and ending on December 31st at the same year. In order to calculate milk loss for the period of outbreak, the values of milk difference were summed up for the period of onset of the massive part of the outbreak in herd 6 (Week 42) and ending two weeks after occurrence of the last case of the outbreak (week 49). In order to calculate confidence interval for this value, we performed a simulation through which a random value was picked from a normal distribution using the average and standard error of milk difference for each week. The values for weeks 42–49 were summed. This procedure was repeated 100,000 times and the standard deviation of the results was used as an estimation of the standard error of the average daily milk loss. CI95% as calculated by adding and subtracting this estimated standard error multiplied by 1.96 from the average. The average and CI95% limits were multiplied by the number of days included in the analysis (i.e. 8 weeks multiplied by 7 = 56 days). This part of the analysis was performed, using Excel™.

3. Results

3.1. NA response following vaccination

Significant rise in BEFV specific NA titers was observed, from a GMT of 1:1.02 (CI95% = 1–1.04) before vaccination to 1:4.2 (CI95% = 3.7–4.8) 1 month after 1st vaccination and up to 1:91.8 (CI95% = 76.6–110) 1 month after 2nd vaccination (Fig. 1). In the model describing GMT 1 month after second vaccination a significant effect was found for the farm (p < 0.001), group (p = 0.001) and the interaction between them (p < 0.001). As depicted in Table 1, significant difference was found between the GMT levels measured in the different farms for all groups. The GMT measured in the control group 1 month after 2nd vaccination of the vaccinated group was 1:1.07 (CI95% = 1.01–1.14). This
finding indicates that the herds were not exposed to natural infection during this stage of the study.

The highest percentage (17%) of non/weak responders was demonstrated in the calf group, followed by the pregnant heifers group (12%) and primiparous heifers (3%). The farm effect was found to be significantly associated with the percentage of non-responders ($p = 0.013$). No significant interaction was found between the age group and the farm effects. The percentage of weak/non-responders among primiparous heifers was significantly lower than among calves ($p = 0.001$) and pregnant heifers ($p = 0.013$) (Fig. 2). In total, an average of 11% of all tested animals (41/385) did not respond with satisfactory NA titers following two vaccinations.

3.2. Serological response following an exposure to vaccine or disease

In the sampled herd (herd 7) high titers were observed in the initial phase of the outbreak in the vaccinated healthy cows as compared to the non-vaccinated cows (Table 2). This indicates of high immunogenicity of the administered vaccine. A significant difference was observed in the percentage of seropositive cows and the average GMT between the samples obtained from sick cows after the outbreak and the samples collected from healthy cows during the beginning of the outbreak (Table 2). This confirms that the sick cows in the herd were exposed to BEFV. The significant difference in GMT between vaccinated and non-vaccinated cows indicates that vaccinated cows experienced a booster response due to exposure to the virus in the field.

3.3. Vaccine effectiveness

Table 3 depicts the morbidity and effectiveness calculated for each herd, categorized according to the vaccination status of the vaccinated and the reference groups. Cows vaccinated twice showed the same incidence of morbidity as non-vaccinated cows (herds 1–5). The average effectiveness in these herds was $2\%$ ($CI_{95\%} = -14$ to 17). However, the effectiveness of 3 vaccine doses compared to non-vaccinated group as measured in herds 6 and 7 was 56% and 39%, respectively. The effectiveness of 4 vaccine doses compared to two vaccine doses as measured in herds 8 and 9, was 53% and 46%, respectively. The average effectiveness displayed in herds vaccinated with 3–4 doses (herds 6–9) was 47.5% ($CI_{95\%} = 36–57$). No significant heterogeneity ($p$-value = 0.525 in Chi square test for heterogeneity) was found between the effectiveness measured in these herds despite the fact that the overall incidence of BEF varied significantly between the herds ($p$-value <0.0001).

3.4. Calculation of milk loss due to morbidity and save of milk loss by vaccination

Fig. 3 depicts the average daily milk difference between non-sick and sick cows (A) and between non-vaccinated and vaccinated cows from week 31 to week 52 as related to the outbreak occurrence (C). As depicted in Table 3, effectiveness of vaccine in this herd was 56% and resulted in an absolute difference of 27% in morbidity. The calculated average milk loss for the total outbreak period for herd 6 was 175.9 kg/sick cow ($CI_{95\%} = 127.9–223.9$) and the average gain for vaccinated cow was 37 kg/vaccinated cow ($CI_{95\%} = -3.6–77.7$).

4. Discussion

To the best of our knowledge this is the first study to evaluate the field effectiveness of an inactivated BEFV vaccine. The study findings indicate that administration of at least three doses of this vaccine provided an average effectiveness of 47.5% against natural challenge during a period of 3.5 months after last vaccination. Administration of 2 doses did not provide protection from challenge. This was indicated by the fact that cows vaccinated twice showed the same incidence of BEF morbidity as non-vaccinated cows. This conclusion was also supported by the observation that 3–4 vaccine doses provided the same effectiveness whether compared to non-vaccinated cows.


or to cows vaccinated twice. The findings were consistent in heavily affected herds and in herds which suffered from low incidence of BEF. Similar results were reported in a previous challenge study, where protection was observed only after administration of three doses of an inactivated vaccine (Della-Porta and Snowden, 1979).

The limited effectiveness of only 47.5% provided by 3–4 vaccinations may seem disappointing. This relatively low effectiveness cannot be explained by lack of similarity between the vaccine strain and the wild strain as these strains were found to be highly similar (Aziz-Boaron et al., 2012). Insufficient protection may be at least partly attributed to lack of development of BEFV specific NA in some of the vaccinated animals. However, as indicated by the 2007 serological survey, only 3% of the vaccinated primiparous heifers did not develop BEFV specific NA after 2 vaccinations. Similar to the results reported by (Della-Porta and Snowden, 1979) these findings may indicate that NA levels elicited by administration of inactivated vaccines are not fully correlated with protection from clinical disease.

Nevertheless, approximately fifty percent field effectiveness of inactivated vaccines is not an unusual finding as previously described for either BEFV inactivated vaccines (Bai et al., 1992; Wang et al., 2001) as well as for vaccines against other viruses such as foot and mouth disease virus (Dar et al.; Elnekave et al.). Moreover, the 50% protection provided by this and other inactivated vaccines may be highly cost effective when considering the direct saving of production losses associated with BEF. In herd 6 in which the attack rates in vaccinated and non-vaccinated cows were 21% and 48%, respectively, we calculated an average saving of 37 kg milk for each vaccinated cow. In this herd the average milk loss due to morbidity was 175.9 kg/sick cow. Division of these two figures by the absolute reduction in morbidity (27% and 100%, respectively) result in a theoretical saving of 1.37 and 1.76 kg milk production for each absolute reduction of morbidity by 1%, respectively. This may be used as the basis for calculation of the cost effectiveness of using vaccination given the risk of morbidity during a BEF outbreak. Vaccine effectiveness may be even higher considering the herd immunity it may provide, which may indirectly protect non-immunized cows due to reduction of virus spread.

The surprising finding that the administration of 2 doses did not provide protection from challenge may be attributed to a rapid waning of immunity. This hypothesis is supported by the finding of a previous immunogenicity study of this vaccine (Aziz-Boaron et al., 2013). In this study it was found that up to 4 months after vaccination there was a logarithmic decrease of NA titer, regardless of the number of vaccinations administered. However, after this period NA levels remained stable in cattle vaccinated 3–4 times, while they continued to decrease in cattle vaccinated only twice. In the current study, natural challenge occurred approximately 3 months after last vaccination. It may be that at this time period, immunity decreased to a non-protective level after 2 vaccinations but its level was still high enough in the cows vaccinated 3–4 times.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Calves</th>
<th>Heifers</th>
<th>Primiparous Heifers</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>119 (60–256)</td>
<td>104 (62–208)</td>
<td>194 (91–446)</td>
<td>0.47</td>
</tr>
<tr>
<td>B</td>
<td>239 (128–446)</td>
<td>39 (18–94)</td>
<td>128 (69–239)</td>
<td>0.03</td>
</tr>
<tr>
<td>C</td>
<td>274 (137–548)</td>
<td>279 (128–532)</td>
<td>256 (128–512)</td>
<td>0.31</td>
</tr>
</tbody>
</table>
One may attribute the lack of protection provided by 2 vaccinations to a possible lack of immunogenicity of the administered vaccine. However, the serological survey that was conducted shortly after vaccination in herd 7 indicated that the administration of two vaccine doses elicited high NA levels reaching an average titer of 1:64 to 1:128. It is generally believed that high titers of specific antibodies elicited after vaccination can be interpreted as correlated with protection (Plotkin; Sadoff and Wittes, 2007). This is the common belief regarding BEF vaccines as well, apart from exceptions in some individuals (Bai et al., 1992; Della-Porta and Snowdon, 1979; Snowdon, 1970; Theodoridis et al., 1973). However, lack of standardization for the SN of BEFV complicates the attempt to directly infer protection from NA titers. It is probable that similarly to other rhabdoviruses, like rabies, cell mediated immunity is also involved in protection (Della-Porta and Snowdon, 1979).

The serological survey performed in 2007 prior to the effectiveness study, revealed that the age groups differed significantly in the rate of non-responders, with the youngest group (calves) showing the highest rate (17%) and the oldest group (primiparous heifers) showing the lowest non-responding rate (3%). Lack of response to vaccination was described previously and was attributed mainly to genetic causes: For example lack of response to vaccination with hepatitis B inactivated vaccine was attributed to an inherited defect in monocyte metabolism which prevented the production of IL-2 during T-cell activation as a response to antigen contact (Meuer et al., 1989). Poor immunogenicity and vaccine failure to measles attenuated vaccine was also described, reaching up to 30% in children and has been associated with polymorphisms in the genes for key innate immune receptors, including measles cellular receptors and viral pattern recognition.

**Table 2**

Serological response following vaccination or natural challenge during an outbreak of bovine ephemeral fever (herd 7).

<table>
<thead>
<tr>
<th></th>
<th>Seropositive % (n/total)</th>
<th>p-value</th>
<th>Geometric mean titer (CI95%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non vaccinated</td>
<td>Vaccinated</td>
<td></td>
<td>Non vaccinated</td>
</tr>
<tr>
<td>Initial phase of outbreak</td>
<td>16 (9/56)</td>
<td>98 (48/49)</td>
<td>&lt;0.0001</td>
<td>1:1.7 (1.5–1.9)</td>
</tr>
<tr>
<td>Healthy One month after end of the outbreak</td>
<td>&lt;0.0001</td>
<td>100 (22/22)</td>
<td>0.002</td>
<td>1:16 (9.8–27)</td>
</tr>
<tr>
<td>Sick p-value</td>
<td>&lt;0.0001</td>
<td>0.2</td>
<td>0.002</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* Neutralizing antibody titer of ≥1:8 was considered as seropositive.

**Table 3**

Morbidity and vaccine effectiveness during an outbreak of bovine ephemeral fever in nine herds participating in the study.

<table>
<thead>
<tr>
<th>Farm No.</th>
<th>Time between last vaccination and outbreak onset (Days)</th>
<th>BEF confirmation by serology in non-vaccinated cows or by PCR</th>
<th>Vaccine group</th>
<th>Reference group</th>
<th>Effectiveness (Cl95%)</th>
<th>p-value</th>
<th>Average effectiveness (Cl95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2008 2007</td>
<td></td>
<td>Total vaccine doses</td>
<td>Morbidity % (x/n)</td>
<td>Total vaccine doses</td>
<td>Morbidity % (x/n)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>71</td>
<td>Yes</td>
<td>2 0 13 (14/108)</td>
<td>0 0 14 (15/108)</td>
<td>7 (–8 to 53)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>81</td>
<td>Yes</td>
<td>2 0 34 (39/115)</td>
<td>0 0 28 (32/115)</td>
<td>21 (–80 to 17)</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>79</td>
<td>Yes</td>
<td>2 0 55 (82/150)</td>
<td>0 0 58 (87/150)</td>
<td>6 (–15 to 230)</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>66</td>
<td>No</td>
<td>2 0 3 (4/118)</td>
<td>0 0 8 (9/118)</td>
<td>56 (–40 to 86)</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>75</td>
<td>No</td>
<td>2 0 22 (32/148)</td>
<td>0 0 22 (32/148)</td>
<td>0 (–54 to 35)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>106</td>
<td>Yes</td>
<td>1 2 21 (35/165)</td>
<td>0 0 48 (79/165)</td>
<td>56 (51–82)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>106</td>
<td>Yes</td>
<td>1 2 24 (49/202)</td>
<td>0 0 39 (88/223)</td>
<td>39 (18–54)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>108</td>
<td>No</td>
<td>2 2 9 (13/149)</td>
<td>0 2 19 (29/156)</td>
<td>53 (13–75)</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>91</td>
<td>Yes</td>
<td>2 2 20 (22/221)</td>
<td>0 2 19 (41/221)</td>
<td>46 (13–67)</td>
<td>0.014</td>
<td></td>
</tr>
</tbody>
</table>

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receptors such as CD46 and TLR8 (Clifford et al., 2012). Furthermore, immune memory responses to previously encountered pathogens can sometimes alter the immune response to an unrelated pathogen by a process known as heterologous immunity (Welsh and Selin, 2002). The age variation in the rate of non-responders might be attributed to different host factors affecting the homologous and heterologous immune response. Immune response to FMDV for instance, was found to be related to the age of the host (Samina et al., 1998). It is possible that previous exposure to other viruses improved the induced response to the viral antigen introduced by the vaccine, and thus the rate of non-responders among older experimental groups is lower than in younger groups. The finding of significantly higher rate of non-responders in younger animals is of less significance for BEF as the disease is primarily harmful.

Fig. 3. Weekly difference in average daily milk production (solid lines) with Cl95% (dotted lines) between (A) non-sick and sick cows and (B) between non-vaccinated and vaccinated cows. (C) Occurrence of new cases of BEFV in herd 6 during the 2008 BEF outbreak.

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for milking cows. This finding, however, may have further implications regarding the age of initial vaccination with inactivated vaccines in general. Thus, when implementing a vaccination program, adopting a differential dose strategy may be suggested according to age group in order to induce protection also among the younger animals.

Although the present article describes a large size effectiveness study, it is limited by the fact that animals were not diagnosed as infected by BEFV on an individual basis. Isolation of BEFV is restricted to the febrile phase of the disease and usually requires immediate delivery of the sample to the laboratory. Given the distance of these herds from the laboratory (200 km), virus isolation was difficult to perform. In addition, individual diagnosis by sero-conversion was of lower relevance in vaccinated animals which are sero-positive before infection. The high increase in antibody levels in herd 7 which served as a sentinel for infection in the region in addition to the virus isolation and PCR verification in many herds in the region (Aziz-Boaron et al. 2012) confirmed that BEFV was the cause of infection. In six of the nine herds which participated in this study infection by BEFV was demonstrated in the herds either by the detection of seropositive non-vaccinated cows or by BEFV identification by PCR. In herd 3 BEFV was also isolated. BEF typical epidemiologic pattern in all of the participating herds as well as the high consistency of the results indicate that all of these herds were infected by BEFV.

5. Conclusions

The results of the study have significant implications when vaccine schedule against BEF is planned. Since BEF is a disease with economic implications mostly in milking cows it may be recommended that initial vaccination will be performed twice at the age of 1 year and then repeated every year on August (in the northern hemisphere) as close as it can be to the outbreak season. The limited effectiveness of the presented inactivated vaccine may not be satisfactory at this point. Nevertheless, due to safety issues, inactivated vaccines are still preferred by many countries. Attempts to improve the current available inactivated vaccines possibly using a different adjuvant should be encouraged in order to ensure protection and reduce the losses caused by BEFV (Dar et al.; Vanselov et al., 1995).

Conflict of interest statement

The authors declare that no competing financial, or other, interests exist.

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