The field effectiveness of routine and emergency vaccination with an inactivated vaccine against foot and mouth disease

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\textbf{A R T I C L E   I N F O}

\begin{abstract}
High potent, inactivated foot and mouth disease (FMD) vaccines may be used in non endemic countries for emergency vaccination during outbreaks in order to prevent virus spread. In endemic countries either standard or high potency vaccines are used for routine vaccination. Despite their wide use there is a shortage of data on the field effectiveness of inactivated FMD vaccines. Epidemics of FMD caused by viruses of serotype O occur frequently in Israel, where a high potency (\textgreater{}6PD\textsubscript{50}) vaccine is used for both routine and emergency vaccination. We investigated an outbreak of FMD caused by a virus of serotype O, which took place during 2011 in a feedlot and an adjacent dairy herd. Post outbreak testing of antibodies against non-structural protein demonstrated that infection occurred in 96\% of the calves that received two doses of vaccine at least three months prior to the outbreak and more than 50\% showed clinical signs consistent with FMD. Replacement heifers that had been vaccinated 3–5 times with the last vaccination administered 7 months prior to the outbreak were all infected and 18\% showed clinical signs. Testing of cattle sera of the same vaccination status as the affected cattle demonstrated low neutralizing antibody (NA) titers against the field virus strain and an r\textsubscript{1} value of 0.37 compared to the vaccine strain. In contrast, cattle vaccinated only once but up to two weeks before the outbreak, were almost all protected from clinical disease and to a lesser extent, protected from FMD virus infection, despite low NA titers. We conclude that emergency vaccination was highly effective due to a mechanism not associated with NA, whereas routine vaccination with the same vaccine formulation provided only limited protection due to poor longevity of the elicited immunity and low matching with the field strain (despite an r\textsubscript{1} higher than 0.3).
\end{abstract}

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1. Introduction

Routine vaccination of cloven hoofed farm animals with inactivated foot and mouth disease virus (FMDV) vaccines is recommended in endemic countries as a measure of prevention of foot and mouth disease (FMD)[1]. In FMD free countries in which vaccination is not performed routinely, one of the strategies for outbreak control is to use emergency vaccination along with culling of infected animals [2,3]. According to the OIE FMD vaccines may be classified as either ‘standard’ or ‘higher’ potency vaccines. Standard potency vaccines are formulated to contain sufficient antigen and appropriate adjuvant to ensure that they meet the minimum potency level required (recommended as 3 PD\textsubscript{50} [50\% protective dose]). This kind of vaccine is usually suitable for use in routine vaccination campaigns. For vaccination in naïve populations to control FMD outbreaks, higher potency vaccines (\textgreater{}6 PD\textsubscript{50}) are recommended for their wider spectrum of immunity as well as their rapid onset of protection [2]. Such vaccines were shown to protect cattle from clinical infection even as short as 2 days and up to at least 21 days post immunization [4] with an average efficacy of 87\% [5]. However, the field effectiveness of such vaccines during an outbreak was hardly assessed. In addition, contradicting results were published with regards to the longevity of immune protection after one or several vaccinations. While some studies demonstrated long term immunity after one or more vaccinations [6–10], a model based on a field study found the half life of vaccine induced protection to be only 98 days [11].

Outbreaks of FMDV, mostly of serotype O occur almost every other year in Israel [12], in which vaccination of cloven hoofed farm animals is obligatory [13]. Cattle are vaccinated by a commercial high potency (\textgreater{}6PD\textsubscript{50}) vaccine. We present here the analysis of an outbreak, caused by a serotype O, Pan-Asia-2 FMDV, which
occurred in a feedlot and a dairy farm in the north of Israel. The information on the variability of vaccination statuses of the cattle in both farms enabled us to correlate incidence of disease with number of vaccinations and time elapsed from the last vaccination. In addition, emergency vaccination of only a portion of the animals enabled an assessment of its effectiveness for the prevention of clinical disease and viral infection.

2. Materials and methods

2.1. Study population

Ramat Magshimim farm is located on the southern part of the Golan Heights, Israel (32°50’46”N, 35°48’29”E). The feedlot and dairy farm were located within a common site, with only 30 meters separating between the two facilities (Fig. 1A). The site was surrounded by a fence. At the time of the outbreak onset there were two main types of calf groups in the feedlot: The first type comprised groups 1–18, which included 424 Israeli Holstein and mixed breed fattening calves aged 8–15 months (Table 1). These calves originated from Ramat Magshimim local beef herd and from three other dairy herds. All calves were vaccinated twice, with the last vaccine administered between 3 and 10 months prior to the outbreak onset (Table 1, Fig. 1A). The second type comprised group 19, which included 306 mixed breed fattening calves aged 2.5–8.5 months. These calves originated from several beef herds located in the north of Israel. They arrived in the feedlot in five batches, starting from 9 days before the outbreak onset. All were vaccinated only once, on the day of their arrival (Table 1, Fig. 1A).

The dairy farm accommodated 931 Israeli Holstein individuals, divided into 18 groups which were located in 5 sheds (groups A–R, Fig. 1A). There was no introduction of cattle from other localities into the dairy farm. Groups A–H, J, K (Fig. 1A, Table 2) included 611 cows and replacement heifers. These groups (excluding ten of the heifers in group J that were moved from group Q on June 13th) were vaccinated at least three times, with the last vaccine administered seven months before outbreak onset (Fig. 1A, Table 2). Groups I–Q (Fig. 1A, Table 2) included 261 replacement heifers and calves aged 3–22 months and group R included 59 suckling calves. These groups (excluding calves born after May 2nd) were vaccinated at least once, 14 days prior to outbreak onset in the dairy farm (Fig. 1A, Table 2).

2.2. Data collection

The information was collected as a part of an investigation of the FMD outbreak in 2011 at northern Israel. The herdsmen and the veterinarian were interviewed on arrival to the farms on June 16th, using preformed questionnaires. Follow up visits and phone interviews were conducted until November, 2011 (4 months after outbreak resolution). The collected data included location of the different groups within the farms, numbers of animals within each group, breed, age, origin and date of arrival to the farm, number and date of administration of vaccines prior to the outbreak, the time of clinical signs onset in each group and the extent of morbidity as was estimated by the herdsmen. Vaccination data were collected from the herd management software.

2.3. Clinical case definition

Morbidity in the feedlot was detected by the herdsman and defined as animal showing lameness with or without excessive salivation, and tongue lesions. In the dairy farm morbidity was detected by both the veterinarian and the herdsman and was defined as an animal showing typical tongue lesions with or without excessive

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Table 1

<table>
<thead>
<tr>
<th>Group data</th>
<th>Vaccination status and morbidity data in Ramat Magshimim feedlot during the FMD outbreak on 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Breed</td>
</tr>
<tr>
<td>A–H</td>
<td>BH</td>
</tr>
<tr>
<td>I–Q</td>
<td>BH</td>
</tr>
<tr>
<td>R</td>
<td>BH</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date</th>
<th>Vaccination</th>
<th>Clinical signs</th>
<th>Time since last vaccination (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-June-11</td>
<td>2</td>
<td>Lameness</td>
<td>20</td>
</tr>
<tr>
<td>11-June-11</td>
<td>2</td>
<td>Lameness</td>
<td>20</td>
</tr>
<tr>
<td>12-June-11</td>
<td>2</td>
<td>Lameness</td>
<td>20</td>
</tr>
</tbody>
</table>

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* BH – Israeli Holstein.
* Mix – mixed breed cattle.
* Administered until the outbreak onset within the feedlot.
* Not sampled.
salivation, lip smacking or lameness. Morbidity was reported as the percentage of clinically infected animals in each group. However, in order to take a conservative approach, whenever high morbidity was reported (i.e. the herdsman reported morbidity close to 100%) morbidity was defined as >50%.

2.4. Vaccination

Foot and mouth vaccination in Israel is performed in accordance with previous recommendations [14] with certain adjustments. The first FMDV vaccination is routinely administered to cattle between 2 and 6 months of age together with Brucella abortus live attenuated vaccine (two separate injections). This is followed by an FMDV booster vaccination 1–2 months later and a repeated annual vaccination for FMDV only. In the current study we referred to vaccination according to the above regime as ‘routine vaccination’. As opposed to this, we defined ‘emergency vaccination’ as any vaccination performed during or around the time of an outbreak (up to two weeks before outbreak onset). During such vaccination only FMDV vaccine was administered.

The same vaccine was used for both routine and emergency vaccination. Cattle were vaccinated with a trivalent (A, O, ASIA1) FMD vaccine with PD50 greater than 6 (Aftopox®, Merial, Pirbright, UK). Strains subtypes included were O1 Manisa, O-4625, O1-3039, A Iran 05, A-4165 and Asia1 Shamir (Batch no. 0-149, manufacture date 18.01.2011, expiry date 18.07.2012). The vaccine was prepared by the manufacturer with purified, double inactivated antigens and mineral oil as an adjuvant. In Israel, it was kept in 2–8 °C, according to the manufacturer instructions. Vaccination was performed by the local district department of the Israeli Veterinary Services by injection of the vaccine emulsion intramuscularly, as instructed by the manufacturer. Injection was performed using 0.75 in. 14–16 G needle mostly in the neck and sometimes on the hind.
Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccination</th>
<th>Morbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of animals</td>
<td>Number of vaccinations&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>A</td>
<td>≥30</td>
<td>108(0)</td>
</tr>
<tr>
<td>B</td>
<td>≥30</td>
<td>90(0)</td>
</tr>
<tr>
<td>C</td>
<td>≥30</td>
<td>24(0)</td>
</tr>
<tr>
<td>D</td>
<td>≥30</td>
<td>24(0)</td>
</tr>
<tr>
<td>E</td>
<td>23</td>
<td>25(0)</td>
</tr>
<tr>
<td>F</td>
<td>≥30</td>
<td>92(0)</td>
</tr>
<tr>
<td>G</td>
<td>≥30</td>
<td>83(0)</td>
</tr>
<tr>
<td>H</td>
<td>≥30</td>
<td>80(0)</td>
</tr>
<tr>
<td>J</td>
<td>12–26</td>
<td>59(0)</td>
</tr>
<tr>
<td>K</td>
<td>≥30</td>
<td>57(10)</td>
</tr>
<tr>
<td>L</td>
<td>3</td>
<td>28(1)</td>
</tr>
<tr>
<td>M</td>
<td>4</td>
<td>17(0)</td>
</tr>
<tr>
<td>N</td>
<td>4–7</td>
<td>14(0)</td>
</tr>
<tr>
<td>O</td>
<td>4–7</td>
<td>49(0)</td>
</tr>
<tr>
<td>P</td>
<td>7–9</td>
<td>45(0)</td>
</tr>
<tr>
<td>Q</td>
<td>9–12</td>
<td>43(0)</td>
</tr>
<tr>
<td>R</td>
<td>15–23</td>
<td>34(0)</td>
</tr>
<tr>
<td>S</td>
<td>≤2</td>
<td>59(0)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Administered until the outbreak onset within the dairy farm.
<sup>b</sup> Positive replacement heifer was moved on June 13th from group J.
<sup>c</sup> Positive cow was vaccinated 6 times.
<sup>d</sup> Not sampled

2.5. Post outbreak serological survey

Serum samples from the dairy farm and feedlot were collected on the 1st of August and the 7th of November 2011, respectively. One hundred and thirteen and 156 cattle heads were sampled from the dairy farm and feedlot, respectively. In the feedlot, animals were chosen randomly from each group. Samples from group 19 represent 3 out of the 5 batches that arrived in the feedlot from May 22nd to June 1st. In the dairy herd we first tested all the clinical cases and then from each group we chose a random sample from the cattle that did not show any clinical signs. Using the cow herd management program and the herdsmen records we were able to track the location of each individual at the onset of the outbreak in the dairy farm and feedlot.

These sera were tested for the presence of antibodies specific to FMD NSPs using PriCHECK® FMDV NS blocking ELISA. Tests were performed according to the guidelines provided by the manufacturer (http://www.prionics.com/diseases-solutions/foot-and-mouth-disease/PriCHECK_FMDV_NS/).

2.6. Serum neutralization (SN) study

Pre-outbreak sera were not available from the cattle in the affected farm. Therefore, in order to correlate the clinical outcome in the outbreak with the pre-exposure levels of neutralizing antibodies we tested sera collected from cows, heifers and calves which represented parallel vaccination status (i.e. number of vaccinations and time elapsed since last vaccine) to the same vaccine batch. All sera were tested twice by SN, which was performed as described previously by Golding et al. [15] using IB-RS-2 cells.

The first SN was performed with the vaccine strain O-4625 as the neutralized virus. This vaccine strain was chosen as in the initial tests it showed the highest r<sub>1</sub> value with the field strain. Another SN was performed with the field strain O ISR 11/11, which was isolated from this outbreak in Ramat Magshimim. The r<sub>1</sub> value was calculated by averaging the product of the division of the two SN titers for the samples of cattle that were vaccinated at least two times.

2.7. Data analysis

The collected data were summarized on a Microsoft Excel<sup>®</sup> data spread-sheet. Differences in rate of seropositivity to NSP and SN were tested for statistical significance by the Fisher exact test. Log of SN titers in the various groups were compared by the t-test. Analysis was performed by the WinPEPI<sup>TM</sup> statistical package.

3. Results

The first cases of FMD at Ramat Magshimim occurred on the 1st of June 2011 in two groups at the feedlot, which included 14 month old mixed breed beef cattle (groups 7 and 8; Fig. 1A, Fig. 2). During the next 20 days morbidity spread to other parts of the feedlot (Fig. 2). Both the veterinarian and the herdsmen reported high morbidity (>50%) in all feedlot groups except group 19 (Fig. 1A, Table 1). In group 19 only 3 out of 306 animals were clinically affected (<1%). Seventy out of 73 (96%) of the calves tested in groups 3–5 were serologically positive for anti NSP antibodies, while the rate of seropositivity in group 19 reached 55% (31/56) (Fig. 1B). The difference between group 19 and the other groups in the feedlot was statistically significant (P<0.0001). Calves from different sources within group 19 differed significantly in the rate of anti-NSP seropositivity, ranging from 16.7% to 82.8% (P<0.0001) (Fig. 3).

The first cases in the dairy farm occurred on June 16th (Fig. 2), in group J, which included 57 replacement heifers 18–26 month old of which 42, 12 and 3 were vaccinated 3, 4 and 5 times, respectively. All were vaccinated for the last time 7 months before the outbreak onset. This group was located at the west side of the shed, located in high proximity to the feedlot (Fig. 1A, Table 2). Ten out of 57 heifers showed clinical signs (18%). No clinical morbidity was observed in group 1 (adjacent to group J) and in groups L–Q which were located closer to the feedlot and included calves and heifers at an age range of 3–23 months. The cattle in these groups were vaccinated between 1 and 5 times (depending on their age), with the last dose administered 14 days prior to outbreak onset at the dairy farm, on June 2nd (Fig. 1A, 2; Table 2). Group R (suckling calves) was located north to these groups. Each calf older than 1 month
was vaccinated once on June 2nd. No morbidity or mortality was observed in this group either.

On June 17th, a day after the outbreak onset at the dairy farm, vaccinations were administered to all the remaining groups at the dairy farm (groups A–H, K and R, Fig. 1A), excluding suckling calves less than 1 month old (group R) and the replacement heifers from the clinically affected group (group J). Another clinical case occurred in one dry primiparous heifer from group K (Fig. 1A, 2; Table 2), which was vaccinated 5 times, with the fifth dose administered 3 days before the clinical signs. It is therefore probable that this heifer was infected prior to the last vaccination.

In the dairy farm, 26 out of 140 individuals samples had serological evidence for FMD infection by showing anti-NSP antibodies. Most of the positive samples were located in the shed adjacent to the feedlot (Fig. 1B).

SN results of the sera representing parallel vaccine status to the outbreak groups are depicted Table 3. These results indicate that one vaccination elicited only low neutralizing antibody (NA) titers despite being highly protective in this stage few days after vaccination. NA GMT to the vaccine strain after second vaccination was high two weeks after vaccination (GMT = 505), but dropped 3 and 7 months after vaccination to 127 and 95, respectively. NA GMT to the wild type was significantly lower (192, 28, 29 respectively). The $r_1$ value calculated for the compatibility of the wild field strain with the vaccine strain was 0.37. For the cows that were tested 7 months after vaccination, no association was found between NA levels and number of prior vaccinations (data not presented).

### 4. Discussion

Data from outbreak investigations, though less accurate than data elicited by clinical trials, are of high importance since they represent the actual effectiveness of vaccines in the field. In this study we have found that vaccination 7 months prior to natural challenge with a greater than 6PD50 inactivated vaccine did not provide adequate protection from infection, regardless of the number of prior administered vaccinations. This can be concluded from the fact that 100% of the tested heifers in group J in the herd were NSP antibody positive and 18% of the heifers in this group were clinically affected. The lack of morbidity and infection in groups A–H, which were farther from the feedlot and were in the same Vaccination status as group J is probably due to lack of exposure to the virus prior to the emergency vaccination and not due to immunity elicited by the vaccine. This is supported by the NSP results. High morbidity and anti-NSP antibody positivity observed in groups 1–18 in the feedlot imply that two vaccinations, with the last dose administered as close as 3 months before natural challenge did not provide protection from clinical or subclinical infection. The low SN titers against the wild type virus, measured in vaccinated cattle 3 and 7 months after vaccination provide the probable explanation for this lack of protectivity.

These results imply that vaccinating cattle as frequently as every 4 months as previously recommended for highly endemic regions [1,2] with a vaccine strain which matches only marginally with the wild type virus (i.e. $r_1$ = 0.3–0.4) may not be sufficient to confer protection from infection, due to rapid waning of immunity. Poor matching of the vaccine and field strains was already observed in Israel on 2007 [16]. Other explanations to the vaccine failure such as interference by the B. abortus vaccine is not probable because these vaccines are administered together only during the first vaccination against FMD. Low immunogenicity due to lack of adequate maintenance of the cold chain is also not probable. This is because routine vaccination (which was shown to have only limited protection) is performed in Israel during the winter when ambient temperatures are low while the highly protective emergency vaccination was performed during the hot summer.

In contrast to the low effectiveness of routine vaccination, when the same vaccine was administered up to 2 weeks before the outbreak it elicited an almost complete protection from clinical disease and some protection from infection by the virus. Many previous experiments demonstrated these findings for other FMD emergency vaccines administered in proximity to challenge with either homologous or heterologous viruses of the same serotype [4,5]. Such protection was also demonstrated when challenge was performed with viruses with $r_1$ values as low as 0.04–0.023 compared to the vaccine strain [17] and also after 6 months from vaccination when a highly potent (PD50 > 32) inactivated homologous vaccine was administered [7]. The high protection observed in our study.
shortly after one vaccination, despite low NA titers suggest that protection at this stage is probably attributed to other immunological mechanisms. Presence of opossuming antibodies [18], pre-challenge levels of IL-6 [19] and post challenge levels of IFN-γ [20].

As depicted in Fig. 3, an interesting finding is the significant difference in NSP seroprevalence between the three calf batches which arrived from different sources to the feedlot prior to the outbreak onset (group 19). These batches represent herds in which surveillance by the Israeli Veterinary Services was low. It is therefore possible that infection in these beef herds was unnoticed at the time they were sold and some were already NSP positive. When arriving to the feedlot, the calves were allocated to the different groups according to their age and not their source herd. It is therefore probable that the difference in the rate of NSP positivity represents prior exposure and not exposure due to a different location in the feedlot. This previous exposure to the virus may have contributed to protection from clinical infection. However, a significant portion of the calves were NSP negative, pointing of a probable protection by the vaccine, as the calves in these groups were vaccinated a few days prior to arrival and did not show clinical signs despite being in close contact with the infected calves.

5. Conclusions

In this article we showed that emergency vaccination with an inactivated vaccine with a potency of at least 6 PD50 and an r1 = 0.37 provided high effectiveness in preventing clinical disease despite low NA titers. However, low effectiveness was observed for the same vaccine when the time elapsed from vaccination to challenge was longer than few months. It is thus suggested that inactivated vaccines with similar potency will include viruses, which have higher matching with the field strain if used for routine vaccination, while for emergency vaccination matching between the strains is probably of less importance.

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Conflict of interest statement

All authors have no conflict of interest.

References


