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Association of the time that elapsed from last vaccination with protective effectiveness against foot-and-mouth disease in small ruminants

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Routine and emergency vaccination of small ruminants against foot-and-mouth disease (FMD) is mandatory in many endemic countries, yet data on the field effectiveness of the vaccines used is scarce. We conducted an investigation of a serotype O FMD outbreak that took place in a sheep and goat pen, and estimated the effectiveness of various routine vaccination statuses. We also evaluated the protection provided by colostrum administration and emergency vaccination. Animals which were routinely vaccinated twice were not clinically affected while disease incidence was observed among animals routinely vaccinated only once ($p = 0.004$ according to a two-sided Fisher's exact test). In groups vaccinated only once, there was a significant association between the average time that elapsed since last vaccination and the disease incidence (n = 5; Spearman correlation coefficient: $r_s = 1.0$, $p < 0.01$). In addition, non-vaccinated lambs fed colostrum from dams vaccinated more than 2 months before parturition had a mortality rate of 33%. Administration of emergency vaccination 2 days after the occurrence of the index case was the probable reason for the rapid blocking of the FMD spread within 6 days from its onset in the pen.

Keywords: colostrum, emergency vaccination, foot-and-mouth disease, maternal immunity, vaccine effectiveness

Introduction

Foot-and-mouth disease (FMD) is a highly contagious viral disease that affects domestic and wild cloven-hoofed animals [15], causing large economical losses [8]. Unlike cattle and pigs, infected sheep and goats usually have a relatively mild clinical presentation [2,14]. However, severe morbidity might occur [9]. Clinical signs include fever, anorexia, lassitude, lameness, and typical lesions on the foot, mouth, teats, vulva, prepuce, and rumen mucosa [2,9,14]. Per-acute death due to myocarditis or myocardial lesions might occur in affected young kids and lambs [9,14].

Outbreaks involving small ruminants are frequently reported. During some of these outbreaks, transmission of the virus between countries or farms was at least partly attributed to the involvement of small ruminants [2,5,7,13]. Yet, the overall significance of small ruminants in FMD virus (FMDV) transmission is still debatable. In several cases, a high sero-prevalence of FMD antibodies in small ruminant herds was found, suggesting circulation of the virus and indicating that these herds were potential reservoirs that might cause outbreak reoccurrence [2]. In other cases, however, low sero-prevalence of FMD antibodies in previously affected small ruminant herds was detected, which may suggest that transmission within these groups was self-limiting and hence these animals played a minor role in virus transmission [5,9,13].

Standard potency FMD vaccines [3 PD50 (50% protective dose)] are considered suitable for routine vaccination campaigns [17]. When using these vaccines, administration of booster vaccination followed by subsequent vaccination every 4 to 12 months is recommended [4,17]. However, in many countries small ruminants are rarely vaccinated more than once every year due to economical reasons [9]. In Israel, routine livestock vaccination is compulsory and small ruminants are

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vaccinated using a bivalent (O & A serotypes) vaccine with a PD50 greater than 6 (Aftopor; Merial, UK). The first vaccine dose is routinely administered to 2-month old lambs and kids of vaccinated dams as well as to 1-day old lambs and kids of non-vaccinated dams.

FMD outbreaks involving serotype O occur in Israel every other year on average [16]. Small ruminants are often affected during these outbreaks. In 2011, a large-scale outbreak caused by a Panasia strain of serotype O FMD virus occurred in Israel. This outbreak lasted 7 months (March ~ October). Thirty-three livestock farms were affected by the disease of which six (18%) contained small ruminants. According to the IVS protocol, emergency vaccination of all animals, quarantine measurements and disinfection measures on the affected farm and all other premises in a 10-km radius were applied during the outbreak. The vaccine used for emergency vaccination was the same as the bivalent routine vaccine (PD50 $\geq$ 6, Aftopor; MERIAL) and the strain subtypes included were O_1 Manisa, O 4625, O_1-3039, and A Iran 2005. The vaccine was prepared with purified, double-inactivated antigens and mineral oil as an adjuvant, and stored at 2 ~ 8°C according to the manufacturer's instructions. Vaccination was performed by the local district department of the IVS. The current study was conducted to provide epidemiological field data on the protective effectiveness of FMD vaccination during an outbreak of the disease among small ruminants.

Materials and Methods

Study population

During 2011, an FMD outbreak occurred in an intensive goat and sheep pen located in Moshav Sharona (coordinates: 32.726065, 35.47377) in the lower Galilee region within the northern district of Israel. The pen accommodated a non-grazing herd of Zanen-Alpine crossbred milking goats as well as Assaf and Booroola-Assaf crossbred milking sheep. During the outbreak, the sheep and goats were scattered in six sheds (sheds A-F in Fig. 1). Generally, the herd could be divided according to three vaccination statuses: 1. adult animals which included sheep, rams, and goats that were vaccinated at least twice with the last vaccine administered between 2.3 and 3.5 months prior to the outbreak, 2. ewe and ram lambs vaccinated only once with the last vaccine administered between 3 weeks and 4.8 months prior to the outbreak, and 3. kids and lambs (less than 3 months old) that were not vaccinated before the time of outbreak onset. More specific information on the vaccination status of each group is presented in Fig. 1 and Table 1. The pen was selected for this study as the intensive management system allowed good follow-up of the vaccination status and disease status of the different groups.

Pen management

The animals were divided into different groups according to their age and lactation status. Each group was located in a different shed. The sheds were surrounded by a rail fence (approximately 1 m high) and roads (approximately 5 m wide) separated the sheds (Fig. 1). Within each shed, the groups were separated by rail fences (approximately 1 m high) that allowed direct contact between animals from different groups (Fig. 1). Lactating dams were walked to the milking parlor twice daily, passing the other pen groups adjacently (Fig. 1). Breeding was performed by artificial insemination and locally raised rams. Offspring were fed by bottle with pooled herd colostrum that was pasteurized at 57°C for 30 min (a common practice for intensive goat and sheep herds in Israel). Colostrum was used immediately after pasteurization or kept frozen up to 1 month before use.

Data collection

Data were collected via an interview of the herdsman on June 20, 2011 and during a follow-up visit several months later. The collected data included location of the different groups within the pens, the number of animals within each group, species, breed, age, milking status, number and date of vaccines administrated prior to the outbreak, time of clinical signs onset in each group, and the extent of mortality and morbidity (*i.e.*, number of dead or sick animals respectively) as reported by the herdsman. Vaccination data received from the herdsman were confirmed by recorded data collected from the IVS.

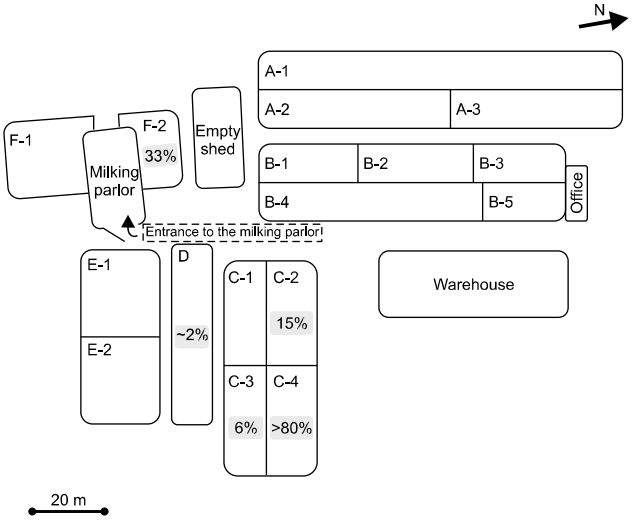

Fig. 1. Sketch of the sheep and goat pen affected by foot-and-mouth disease during 2011. Groups are represented by letters and numbers. Disease incidence (%) for the clinically affected groups is also shown. The groups' data, vaccination status, and disease incidence are presented in Table 1.

Table 1. Groups data (*i.e.*, species, gender, age range, and milking period), vaccination status (*i.e.*, the number of vaccines and time elapsed between the last vaccine and outbreak onset within the pen) and morbidity data (*i.e.*, date of the first clinical signs, direct contact with initial clinically affected group [C-4], time between emergency vaccine and clinical signs appearance, the number of animals in each group and of which the number of sick and dead animals, and disease incidence)

Group data				Vaccination		Morbidity				
Group	Species and gender	Age range	Milking period	Number of vaccines*	Months elapsed since last vaccination* (average)	Date of the first clinical signs	Direct contact with initial clinically affected group (C-4)	Time between emergency vaccine† and clinical signs appearance (days)	Number of animals (sick, dead)	Disease incidence (%)
A-1	Sheep ♀	1.5～7 yr	Lactating	≥ 2	2.3～3.5 (2.9)		No	−	220 (0, 0)	0
A-2	Sheep ♀	1～7 yr	Lactating	≥ 2	2.3～3.5 (2.9)		No	−	120 (0, 0)	0
A-3	Sheep ♀	1.5～7 yr	Dry	≥ 2	2.3～3.5 (2.9)		No	−	150 (0, 0)	0
B-1	Sheep ♀	1.5～7 yr	Lactating	≥ 2	2.3～3.5 (2.9)		No	−	80 (0, 0)	0
B-2	Sheep ♀	1.5～7 yr	Dry	≥ 2	2.3～3.5 (2.9)		No	−	40 (0, 0)	0
B-3	Sheep ♂	1～6 yr	−	≥ 2	3.5 (3.5)		No	−	30 (0, 0)	0
B-4	Goat ♀	1～5 yr	Lactating	≥ 2	3.5 (3.5)		No	−	120 (0, 0)	0
B-5	Goat ♀	≤ 2 mo	−	0	−		No	−	80 (0, 0)	0
C-1	Sheep ♀	～3 mo	−	1	0.7 (0.7)		Yes	−	80 (0, 0)	0
C-2	Sheep ♀	～5～6 mo	−	1	2.4～3.6 (3)	13 May 2011	Yes	0	200 (30, 0)	15
C-3	Sheep ♀	～4 mo	−	1	0.7～2.5 (1.6)	15 May 2011	Yes	2	50 (3, 0)	6
C-4	Sheep ♀	～7～8 mo	−	1	3.5～4.8 (4.15)	11 May 2011	Yes	−2	120 (> 100‡, 2)	> 80‡
D	Sheep ♂ + ♀	3～6 mo	−	1	0.7～2.4 (1.55)	17 May 2011	No	4	200 (< 5‡, 0)	～2‡
E-1	Sheep ♀	1.5～7 yr	Lactating	≥ 2	2.3～3.5 (2.9)		No	−	150 (0, 0)	0
E-2	Sheep ♀	1～7 yr	Lactating	≥ 2	2.3～3.5 (2.9)		No	−	80 (0, 0)	0
F-1	Sheep§ ♀ + ♂	1～2 mo	−	0	−		No	−	～200‡ (0, 0)	0
		1.5～7 yr	Dry	≥ 2	2.3～3.5 (2.9)		No	−	～20‡ (0, 0)	0
F-2	Sheep ♀ + ♂	1～10 d	−	0	−	11 May 2011	No	−2	150 (0, 50)	33

*Administered until outbreak onset within the pen. †Administered on May 13, 2011. ‡Estimation − exact numbers were not recorded. To calculate the groups' logit, we used 100 and five sick animals for group C-4 and D, respectively. §Several age groups within the same shed.

Diagnosis

On May 13, 2011 (2 days after the first clinical signs appeared in the pen), FMD was diagnosed by a private veterinarian and the IVS local district veterinarian. Clinical suspicion of FMD was supported by field postmortem autopsy of several per-acutely affected lambs in which distinct 'tiger heart' lesions were observed. Diagnosis was confirmed by isolation of the virus from a probang sample obtained from an ewe lamb in the FMD laboratory of the Kimron Veterinary Institute (KVI, Israel) on May 15. This isolate (World reference laboratory of FMD [WRLFMD] reference no. ISR/6/2011) was genotyped by the WRLFMD using the VP-1 sequence and was found to resemble viruses isolated from outbreaks occurring during 2011 in the northern part of Israel [10].

Clinical case definition

The minimal clinical case definition was abrupt onset of anorexia without any other apparent cause, with an onset of morbidity no earlier than May 11. In lambs (up to 10 days old) the only sign was per-acute death and thus this was the case definition.

Data analysis

Data were summarized in an Excel spreadsheet (Microsoft, USA). Cumulative incidence was calculated by dividing the estimated number of sick animals by the number of susceptible animals in each group. We performed Fisher's exact test to evaluate the association between the number of routine vaccines administered (*i.e.*, only one *vs.* two or more) and infection (*i.e.*, sick or healthy group). Groups that were vaccinated only once (*i.e.*, groups C-1, C-2, C-3, C-4, and D) differed in the average time elapsed from routine vaccination to outbreak onset (Table 1). Spearman correlation coefficient was calculated in order to test for an association between time from last routine vaccination and the logit of total morbidity among these groups. The logit of the incidence for Group C-1 was calculated by assigning a number of 0.5 animals affected (*i.e.*, in order to use the log function since this group had no clinical cases during the outbreak). Analyses were conducted using SPSS (ver. 21; IBM, USA). A p value < 0.05 was considered statistically significance.

Results

Outbreak outline

Overall, five out of 17 animal groups that were present in the pen at the time of outbreak onset were clinically affected. The first clinical case was detected on May 11, 2011 and the last clinical case was diagnosed on May 17, 2011 (Table 1). Affected groups included only animals that were either not vaccinated (*i.e.*, group F-2) or vaccinated only once (*i.e.*, groups C-2, C-3, C-4, and D). Clinical signs presented in ewe and ram lambs (3 months and older) vaccinated once included anorexia, bruxism, lameness, and lesions between the toes and in the mouth as the disease progressed. Two affected lambs died due to severe illness. In non-vaccinated lambs (up to 10 days old), the only sign was per-acute death. Location, characteristics, vaccination status, incidence, and onset of clinical signs for each group are presented in Fig. 1 and Table 1.

Detailed description of the outbreak

First clinical case of FMD in the pen occurred on May 11, 2011 in a group of 120 ewe lambs 7 to 8 months old (group C-4; Fig. 1) that had been vaccinated once between 3.5 and 4.8 months prior to the outbreak. Approximately 80% of the ewe lambs in the group were clinically affected during the outbreak and two of them died (Table 1). At the same time, an additional massive per-acute death and unusual diarrhea occurred in a group of 150 newborn lambs up to 10 days old. This group was located in a different shed (group F-2; Fig. 1). The lambs in this group were not vaccinated but were fed with a colostrum pool obtained from dams vaccinated against FMD 2.1 to 3.3 months prior to parturition. During the outbreak, incidence in this group reached 33% (50 out 150 lambs). Clinical cases among these animals were defined by per-acute mortality. Therefore, the incidence also represents mortality for this group. No other FMD-related mortality was observed in the pen (Table 1).

Emergency vaccine was administered on May 13 (2 days following outbreak onset) to all animals in the pen (Table 1). Meanwhile, the disease continued to spread among groups in the first affected shed. Group C-2 (Fig. 1) was the second to be affected in this shed. Clinical cases in this group were observed on May 13. This group included 200 ewe lambs 5 to 6 months old that were vaccinated between 2.4 and 3.6 months before the outbreak, and for the second time during the emergency vaccination of the entire pen on the day of the first clinical presentation in this group. Overall, 30 clinical cases (15%) occurred in the group during the outbreak (Table 1).

On May 15, the third group in the shed (group C-3; Fig. 1) was affected. This group included 50 ewe lambs approximately 4 months old that were vaccinated for the first time between 3 weeks and 2.5 months before the outbreak onset, and for the second time during the emergency vaccination of the entire pen 2 days prior to the first clinical presentation. Overall, three clinical cases (6%) occurred in this group during the outbreak (Table 1). No clinical signs were observed in group C-1 (Fig. 1), which included 80 ewe lambs approximately 3 months old that were vaccinated for the first time 3 weeks before the outbreak and for the second time during emergency vaccination of the herd (Table 1). Some sporadic cases with minor severity appeared 4 days after emergency vaccination in group D housed in a shed adjacent to the affected shed C (Fig. 1). This group included about 200 fattening ram and ewe lambs 3 to 6 months old that were vaccinated once between 3 weeks and 2.4 months before the outbreak, and for the second time during emergency vaccination of the herd 4 days prior the first clinical presentation (Table 1). There were no other clinical cases observed in the pen. On June 20, 2011, only a few ewe lambs that were severely ill during the outbreak still had small scars between the toes as a result of typical FMD lesions. A significant association between the number of routine vaccines administered (*i.e.*, only once *vs.* twice or more) and infection (*i.e.*, sick or healthy group) was observed ($p = 0.004$ according to a two-sided Fisher's exact test). Additionally, a significant association was observed between time elapsed from vaccination and the logit of the incidence among the groups vaccinated only once (n = 5; Spearman correlation coefficient: $r_s = 1.0$, $p < 0.01$).

Discussion

FMD has been investigated thoroughly for many years, yet research based on field data for FMD outbreaks among routinely vaccinated populations is limited. Most of the results presented in this study are in accordance with findings we recently published on protection provided by emergency

vaccination of cattle [6]. The outbreak investigation described in the current study lacks laboratory confirmation of the levels of anti-FMD antibodies in the different groups prior to the outbreak and the level of exposure to FMDV during the outbreak. Nevertheless, collected data on the incidence among groups with varying vaccination statuses and information on the pen management suggest that exposure to the virus was not limited to certain groups in the pen.

High incidence of FMD was observed in ewe lambs vaccinated once between 3.5 and 4.8 months prior to exposure, indicating a lack of vaccine-induced protection in this group as opposed to previous reports describing long-term protection provided by a single vaccination against serotype A [2]. Incidence was lower in the adjacent groups that had direct contact with the initially affected group (*i.e.*, group C-4). The incidence in groups of ewe and ram lambs (more than 3 months old) vaccinated only once was positively correlated with the average time that elapsed since the last vaccine administration. This association may suggest waning of immunity over time as was previously shown with routinely vaccinated cattle [6]. Although a strong association was found, it should be noted that only five groups were used for analyzing this association. Another explanation may be that the rapid administration of emergency vaccination 2 days after detection of the index case was effective in blocking the spread of the virus and thus reducing the incidence among the affected groups [7]. These groups were highly exposed to the virus due to the close proximity of these animals (*e.g.*, only a rail fence separated the groups within the same shed) and the long duration of pre-clinical virus excretion in sheep [7]. Clinical incidence was not observed in groups located in distant sheds. This may have been the result of the emergency vaccination administered to all animals within the pen 2 days after index case identification. However, lack of clinical incidence in these groups may also be a result of other causes. First, there could have been a lack of exposure to the virus among the non-affected groups. However, this is less probable for the lactating groups (*i.e.*, groups A-1, A-2, B-1, B-4, E-1, and E-2) since the milking parlor is located in close proximity to the affected group of lambs (*i.e.*, group F-2) and direct contact of lactating dams with affected lambs was possible while the dams walked into the milking parlor. Second, multiple vaccinations may have provided full protection against the virus since all of these groups were vaccinated at least twice with the last vaccine administered up to 3.5 months prior to exposure. Thus, two vaccinations were shown to provide better protection to sheep than that observed in our previous study of routinely vaccinated cattle [6]. Taking into account the fact that the calculated r_1 value of the vaccine used was 0.37 for the cattle outbreak [6], the same O serotypes were included in the vaccine used in the current study, and the virus responsible for the current outbreak (WRLFMD reference no. ISR/6/2011 [10]) was almost identical to those isolated in the cattle outbreak (WRLFMD reference no. ISR/11/2011 and ISR/13/2011 [11]), the same moderately low r_1 value was expected for this outbreak. Thus, the difference observed between cows and sheep vaccine effectiveness may be associated with the higher susceptibility of cows to develop morbidity due to infection by this virus [7]. Another explanation for the lack of clinical incidence in groups with multiple vaccinations might be that minor clinical signs of infection and morbidity in these mature sheep and goats were not noticed by the veterinarian or herdsman. However, this is unlikely since even minor clinical presentations would have influenced milk production, which was not observed during the outbreak despite high sensitivity to the disease occurrence.

High mortality was observed among the newborn lambs (up to 10 days old). This finding is in accordance with data for affected lambs and kids previously described in the literature [9,14]. These lambs were not vaccinated prior to the outbreak but were fed pasteurized colostrum. The colostrum pool was obtained from dams vaccinated at least twice with the last vaccine administered between 2.1 and 3.3 months prior to parturition. According to a previous report on lambs ingesting colostrum from dams vaccinated 2 months prior to parturition, the average half-life of maternal FMDV antibodies is 22.4 days post-parturition [3]. Although the levels of maternal antibodies in the colostrum and lambs during the outbreak were not measured, circumstantial epidemiological evidence and data from previous report [3] suggest that the high incidence observed in lambs in the described outbreak was due to consumption of colostrum with low levels of anti-FMD antibodies. The low antibody levels might have been a result of waning immunity among the dams that caused decreased secretion of anti-FMDV antibodies into the colostrum. Additionally, colostrum pasteurization might further reduce the antibodies levels. Pasteurization of colostrum is a common practice in commercial sheep and goat herd management, and is performed in order to prevent infection by several pathogens, primarily caprine arthritis and encephalitis virus (CAEV) in goats [1] and Maedi-Visna virus (MVV) in sheep [12]. Pasteurization decreases IgG levels to 37% and 35% compared to fresh colostrum from goats and sheep, respectively [1,12].

In conclusion, findings from the current field investigation suggest that a single vaccine administration during the first year of life, as practiced for small ruminants in many FMD endemic countries, provides only limited protection during an outbreak. This investigation was conducted in only one dairy goat and sheep pen. Thus, comparison of these results with data from future field investigations will provide more solid conclusions regarding the protective effectiveness of vaccination against FMD in small ruminants.

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Conflict of Interest

There is no conflict of interest.

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