גורמי הסיכון והדינמיקה של מחלת הפה והטלפיים בישראל

דוח מסכם למועצת החלב - תקציב 705-0057

*הערה: הדו"ח מפרט את תוצאות המחקרים שמומנו על ידי השירותים הווטרינרים ומועצת החלב מאחר והתוצאות משולבות זו בזו והמסקנות התקבלו על בסיס תוצאות כל העבודות.

ניתן לפרסם את תוצאות הדו"ח:

<u>תקציר</u>

<u>מבוא –</u>

מחלת הפה והטלפיים (פו"ט) הינה מחלה נגיפית מדבקת מאוד הפוגעת במיני חיות בר ומשק מסדרת מכפילי הפרסה. חשיבותה של מחלת הפה והטלפיים נובעות מהפגיעה שגורמת התחלואה הקלינית ובעיקר מההשלכות הכלכליות המשמעותיות שנובעות מהצעדים שננקטים למניעתה ולביעורה.

בישראל מדיניות שליטה במחלת הפו"ט כוללת חיסון כלל חיות המשק וניטור פאסיבי בזמן שגרה והטלת הסגרים סביב מוקדי תחלואה ומתן של חיסוני חירום בזמן התפרצויות. למדיניות שליטה זו השלכות כלכליות משמעותיות על המשקים אך למרות קיומה, התפרצויות מחלת הפה והטלפיים מתרחשות כמעט כל שנה ונגרמות בעיקר על ידי סרוטיפ O כאשר עיקר התחלואה היא בענפי הצאן, הבקר לבשר (בקב"ש) והפיטום. לעומת זאת בקר לחלב וחיות בר כמעט ואינם נפגעים. חדירות של נגיפי פו"ט ממדינות שכנות נמצאו בעבר כמקור להתפרצויות בישראל, אך האפשרות כי נגיעות אנדמית מהווה גם כן מקור להפצת הנגיף לא נבחנה מעולם. כמו כן ההבדלים שקיימים בצורות הממשק בין ענפי הגידול השונים עשויים להשפיע על מידת החשיפה הפוטנציאלית וההדבקה בנגיף וכן על מידת ההגנה המוקנית על ידי חיסונים. אף על פי כן מידת ההדבקה וגורמי הסיכון להדבקה בענפי הגידול השונים מעולם לא נבחנו באופן מסודר. מטרתה של עבודה זו היא לבחון האפידמיולוגיה של מחלת הפה והטלפיים בענפי הגידול השונים ולהסביר את הגורמים לשוני ברמת התחלואה ביניהם. מידע זה יאפשר ייעול של מדיניות השליטה הנהוגה כיום הן באופן שגרתי והן בזמן התפרצויות. באופן פרטני מטרות עבודה זו הן: (i) קביעת מועילות התרכיב בבקר ובצאן וכן בחינת דינמיקת הנוגדנים לאורך זמן בפרות שחוסנו בצורה שגרתית, (ii) קביעת גורמי הסיכון לתחלואה בפו"ט ו-(iii) קביעת גורמי הסיכון להדבקה וכן את מידת ההדבקה בענפי גידול שונים.

<u>שיטות העבודה –</u>

לצורך קביעת מועילות החיסונים וכן גורמי הסיכון לתחלואה נערכו תחקורים אפידמיולוגיים של התפרצויות מחלת הפה והטלפיים. התגובה החיסונית המושרית לאחר חיסון בפרות שחוסנו באופן שגרתי נבדקה בניסוי קליני שכלל 99 פרות

חלב ועגלות עם סטטוס חיסוני שונה (מספר החיסונים השגרתיים שניתנו עד לתחילת הניסוי) שנעקבו במשך שנתיים. סה"כ נאספו 988 דגימות ב-11 מועדים. הדגימות נבדקו בשיטת SNT (Serum neutralization test) כנגד זן התרכיב. על מנת לקבוע את המצאות ההדבקה ואת גורמי הסיכון להדבקה בוצעו מספר מחקרי חתך סרולוגיים: (I)צאן- נבדקו 2502 דגימות סרום מצאן בוגר וצעיר שנאספו בשנים 2011-2014 במדגם המייצג את כלל האוכלוסייה.(II) בקר- נבדקו 3 שלוחות באיזורי סיכון גבוה לתחלואה: (i) פרות חלב- נאספו 1582 דגימות סרום בשנים 2006 ,2009-2012; (ii) בקב"ש- נאספו 736 דגימות סרום בשנים 2006 ו-2014; (iii) עגלי פיטום- נאספו 256 דגימות במהלך השנים 2012-13. (iv) חיות בר – נאספו 209 דגימות בעיקר מחזירי בר וצבי ארץ ישראלי בשנים 2000, 2005-2013. בדיקת נוכחות נוגדנים לחלבונים לא מבניים (NSP) נבחנה על ידי קיט ELISA מסחרי (PrioCHECK®). רגישות בדיקה זו נבחנה במחקר נוסף שערכנו, בו עקבנו במשך 3 שנים אחרי פרות שנחשפו לנגיף בזמן התפרצות וביצענו בדיקות חוזרות להמצאות נוגדנים כנגד NSP. מממצאי המחקר עולה כי לשיטה זו רגישות גבוהה גם לאחר 3 שנים מזמן החשיפה. כלל נתוני המחקר סוכמו,מופו והנתונים נותחו במודלים סטיסטיים חד ורב גורמיים לזיהוי גורמי סיכון להדבקה.

תוצאות:

i. מועילות החיסונים ובחינת התגובה החיסונית המושרית לאחר חיסון שגרתי של פרות

- חיסון שגרתי הינו בעל מועילות מוגבלת בהגנה מפני תחלואה בצאן ובבקר.
- כאשר נעשה שימוש באותו תרכיב בעת חיסון 'חירום' (בזמן התפרצות) מועילותו היתה גבוהה מאוד בהקניית הגנה בפני תחלואה.
- בפרות שחוסנו עד 3 פעמים בעבר נראתה עלייה משמעותית בכייל בעקבות חיסון ובעקבותיה דעיכה מהירה בכייל. לעומת זאת, בפרות שחוסנו לפחות 4 פעמים בעבר, התגובה למתן חיסון נוסף היתה מינמלית. טיטר הנוגדנים בפרות אלו נשאר קבוע לאורך תקופת המעקב.

ii. בחינת גורמי הסיכון להתפרצויות פו"ט

- מחקירת ההתפרצות עלה כי עגלי בקב"ש צעירים, שהודבקו תת קלינית בפו"ט, גרמו בסבירות גבוהה להפצת הנגיף מעדרי הבקב"ש אל המפטמה.
- נוכחות של עגלים צעירים מגיל חצי שנה היוותה גורם סיכון מובהק לתחלואה בעדרי בקר לבשר.

iii. בחינת מידת ההדבקה בנגיף וגורמי הסיכון להדבקה בענפי גידול שונים ובחיות בר

- נגיעות אנדמית נמצאה רק בקרב צאן. רעיה ועדרים גדולים מ-500 ראש היו גורמים מגינים מהדבקה, בעוד שקרבה של פחות מ-5 ק"מ למוקדי התפרצות שבהן לא היתה תחלואה של צאן נמצאה כגורם סיכון מובהק להדבקה.
- ההמצאות הסרולוגית בבקב"ש היתה גבוהה (13.2%) עם אסוציאציה לגיל (>2.5 שנים) והדבקות קודמות. עיקר הדגימות החיוביות נמצאו בעדרים מרמת הגולן. לעומת זאת ההמצאות הסרולוגית בעגלי פיטום היתה אפסית.
- בפרות חלב ההמצאות הסרולוגית באיזורי הסיכון הגבוה היתה נמוכה (2.7%). קרבה למספר התפרצויות בטווח שאינו עולה על 3 ק"מ וכן קרבה של עד ל-5 ק"מ מהגבול נמצאו כגורמי סיכון מובהקים סטטיטית לסרו-חיוביות.

- בחיות בר ההמצאות הסרולוגית היתה גבוהה (7.7%). עיקר הדגימות החיוביות נאספו מחזירי בר בשנת 2007 שבה גם נצפתה תחלואה רבה של חיות בר במהלך התפרצות של מחלת הפו"ט. בשאר שנות הדיגום ההמצאות היתה אפסית.

דיון ומסקנות

מועילות התרכיב כחיסון חירום נמצאה כגבוהה מאוד בבקר ובצאן. מועילות החיסון השגרתי היתה לעומת זאת נמוכה יותר ונראה כי עיקר חשיבות חיסון זה היא בבלימת התפשטות התחלואה עד למתן חיסוני החירום. התגובה המינמלית למתן חיסון ורמות הנוגדנים הקבועות בפרות שחוסנו לפחות 5 פעמים, עשויים להעיד כי ניתן להפחית את תדירות החיסונים בפרות אלו.

נגיעות אנדמית נמצאה בקרב צאן, אך במצב הקיים בישראל בו כלל חיות המשק מחוסנות כנגד המחלה, נראה כי מידת ההשפעה של הצאן על הפצת והעברת המחלה היא מינורית. מידת ההדבקה הסרולוגית הגבוהה שנמצאה בבקב"ש היתה גבוהה. ממצא זה עולה בקנה אחד עם הארעות התחלואה הגבוהה בענף גידול זה ביחוד כאשר נוכחותם של עגלים צעירים מגיל חצי שנה בעדר מקטינה את מידת חסינות העדר ועל כן מגדילה את הסיכון לתחלואה. ממצאים אלו יחד עם ההדבקה של מפטמות כתוצאה ממעבר עגלים נגועים מעדרי הבקב"ש מעידים על חשיבותו של ענף זה בהתפשטות הנגיף בזמן התפרצויות. אף על פי כן הקורלציה בין ההדבקה בנגיף לבין גיל הבקב"ש והתפרצויות קודמות של פו"ט באותם משקים וכן ההמצאות האפסית בעגלי הפיטום שמגיעים למפטמות מעדרי הבקב"ש מעידים כי ככל הנראה לא קיימת סרקולציה מתמשכת של הנגיף בשלוחות אלו. ההמצאות הסרולוגית הנמוכה בקרב פרות החלב היא תוצר של אופי הממשק האינטנסיבי ברפתות שמפחית במידה רבה את הסיכון לחדירה של הנגיף אל הרפת בשילוב עם מדיניות החיסון יעילה בשגרה ובעיקר בזמן התפרצויות. העדר ההדבקה הסרולוגית של חיות הבר במרבית שנות האיסוף, למעט 2007, מעידה כי ככל הנראה אין לחיות בר תפקיד חשוב בסרקולציה המתמשכת של הנגיף בישראל, אך יתכן וחיות הבר מסייעות להתפשטות הוירוס בשנים מסוימות.

ממצאי מחקר זה מהווים בסיס להערכת העלות והתועלת של אסטרטגיות שליטה שונות במחלה על דינמיקת התחלואה בישראל. על ידי שימוש בנתונים שנאספו ניתן יהיה לגבש המלצות שיאפשרו למקבלי ההחלטות לבחור בדרך ההתמודדות היעילה ביותר עם מחלת הפו"ט בישראל. מסקנות מחקר זה יוכלו לשמש גם במדינות אחרות שבהן, בדומה לישראל, מתרחשות חדירות חוזרות ונשנות של הנגיף.

תוצאות המחקר מתוארות ב- 4 פרקים המכילים 9 פרסומים בעיתונות המדעית.

רשימת פרסומים שנבעו מהמחקר:

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Abstract

Introduction

Foot and Mouth disease (FMD) is a highly contagious viral disease affecting cloven hoofed species of wildlife and livestock. Beside the clinical implications of infection, the main importance of FMD is caused by the major economical consequences of the measurements taken in order to prevent and eliminate the disease.

In Israel, the control measures include: (i) routinely - passive surveillance and vaccination of all livestock, and (ii) during outbreaks - quarantines and emergency vaccinations. Despite the control measures, FMD outbreaks reoccur in Israel almost every year. Most of the outbreaks are caused by serotype O and the most affected livestock sectors are small ruminant farms, beef farms and feedlot farms, while dairy farms and wildlife are the least affected.

Introductions of FMD viruses from surrounding countries were demonstrated before, yet the possible contribution of endemic infections to the FMD outbreaks occurrence was never estimated. Additionally, differences in the management systems of the livestock sectors lead to differences in the level of possible exposure to the virus and vaccination coverage. Though these differences are expected to cause variability in the level of infection by FMD viruses, the prevalence of infection in these different sectors and the risk factors for infection in each of them were never estimated. This study is aimed at estimating the dynamics of FMD in different livestock sectors and explaining the differences in disease incidence between them. Such information is expected to enable evidence based resource allocation and an improvement in the efficiency of the current control regimen. Specifically, study objectives were to: (i) determine routine and emergency vaccination effectiveness and antibody dynamics, (ii) determine the risk factors for FMD outbreaks, (iii) determine the differences in virus dynamics among different livestock sectors and among wildlife and the possible risk factors for infection.

Materials and methods

Field investigations were conducted in order to determine vaccine effectiveness and risk factors for FMD outbreaks. A clinical trial was conducted in order to evaluate the neutralizing antibody (NA) response following vaccine administration. Ninety-nine cows and heifers of different vaccination statuses (i.e. the number of routine vaccines administered prior to the beginning of the trial) were followed for two consecutive years and 988 samples were collected in 11 collection times. SN (serum neutralization) assay was used in order to estimate the NA titers against the vaccine serotype.

In order to determine the prevalence and risk factors for infection, several cross sectional studies were conducted. (I) Small ruminants (SR) – 2502 sera were tested. Samples were collected during 2011-14 and represent the entire SR population. (II) Cattle located mainly in high risk areas were tested: (i) Dairy cattle – 1582 samples were collected during 2006, 2009-12; (ii) Beef cattle – 736 samples were collected during 2006 and 2014; (iii) Feedlot calves – 256 samples were collected during 2012-13; (iv) Wildlife – 209 samples were collected mostly from wild boar (*Sus scrofa*) and mountain gazelles (*Gazella gazella gazella*) during 2000, 2005-13. The presence of antibodies against non structural proteins (NSP), indicating FMD infection, was tested using a commercial ELISA kit (PrioCHECK®). Detection longevity of at least 3 years and high sensitivity were demonstrated in an additional study conducted as a part of this thesis. Specifically, cows infected by FMD during an outbreak were followed for three years and the presence of antibodies against NSP was repeatedly estimated.

Data were summed and mapped. Univariable and multivariable statistical models were fitted to data to determine risk factors for infection.

<u>Results</u>

i. Routine and emergency vaccination effectiveness and antibody dynamics:
   - Outbreak investigations revealed that routinely vaccinated SR (one vaccination prior to an FMD outbreak) and cattle (1-5 vaccinations prior to an FMD outbreak) were only partly protected from clinical and subclinical infection. The same vaccine was highly effective in providing protection from clinical infection when used up to 14 days before the outbreak (i.e. as emergency vaccine).
   - A significant increase in the NA titer after vaccine administration, followed by rapid decrease of the NA titer, was demonstrated in cows that were previously vaccinated up to three times. In contrast, only minimal NA response was found following the administration of vaccination to cows that were previously vaccinated at least 4 times. In these cows, high levels of the NA titers already existed and remained consistent throughout the follow-up period.

ii. Risk factors for FMD outbreaks:
   - Outbreak investigation revealed that young beef calves, sub clinically infected by FMD, were the probable source of FMD virus dissemination from beef herds into the feedlot during an FMD epidemic.
   - The presence of calves younger than 6 months was found as a significant risk factor for FMD outbreak occurrence in grazing beef herds.

iii. Differences in virus dynamics among different livestock sectors and among wildlife, and possible risk factors for infection:

- Endemic infection was found only in SR. Grazing SR herds and herds larger than 500 animals were at lower risk of infection, while proximity to outbreaks (≤5km) in which SR were not affected was positively associated with infection.
- High sero-prevalence was found in beef cattle (13.2%). This was positively associated with age (>2.5 years) and previous FMD outbreaks within the farm. Most of the sero-positive samples were found in herds grazing in the Golan Heights region. In contrast, a negligible sero-prevalence was found in feedlot calves.
- The overall sero-prevalence in dairy cattle located in high risk areas was low (2.7%). Proximity to multiple outbreaks (≤3km) and to borders (≤5km) was positively associated with infection.
- Sero-prevalence in wildlife was 7.7%, but this varied significantly among different species in different years. Most of the positive samples were collected from wild boar in 2007. In this year, a large FMD epidemic affected wildlife as well. In all other collection years the sero-prevalence among wildlife was negligible.

<u>Summary and conclusions</u>

In both cattle and SR, high effectiveness of emergency vaccination was found. However, incomplete protection was provided by the same vaccine when used routinely. These findings suggest that the role of routine vaccination is mainly at mitigating the infection spread until the administration of emergency vaccination. Therefore, considering the stable antibody levels in cows vaccinated at least 5 times along with minimal response to additional vaccine administration, reducing the frequency of vaccination in these cows may be cost effective. Endemic infection was found in SR, yet due to vaccination of all livestock against FMD, the risk of dissemination of virus from SR is minimal. High sero-prevalence of infection was found in beef cattle. This was in accordance with the high incidence in this livestock sector, especially in the presence of young calves, which reduces herd immunity. These findings, along with the demonstration of the feasibility of occasional virus transmission by calves delivered from beef herds to feedlots, indicates the importance of beef cattle sector in disease spread during FMD outbreaks. However, the positive association of FMD sero-prevalence with the existence of previous outbreaks within the beef farm and the negligible sero-prevalence found in young feedlot calves that, at least partially, originate from beef herds suggest that ongoing circulation of the virus between these two livestock sectors is less probable. Low sero-prevalence was found in dairy cattle. This was expected, considering the management system in these farms and the efficient control measures applied against FMD. The results of the study thus support the effectiveness of the current FMD control regimen in this sector. Finally, the absence of sero-positive samples of wildlife in all years, except 2007, indicates that ongoing circulation of the virus in these populations is less likely, though they may play a role in virus dissemination during some outbreaks.

The findings of this study provide the data needed for economists to conduct a cost-benefit analysis of different control strategies and their effects on FMD dynamics in Israel. Such an analysis would inform recommendations to help decision makers choose the best strategy for FMD control in Israel. The conclusions of this study could be expanded to other countries which face the same challenge as Israel (i.e. continuous incursions of FMD virus).

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1. Introduction

Foot and mouth disease (FMD) is a highly contagious disease affecting cloven hoofed (i.e. even-toed) ungulates worldwide (Grubman and Baxt, 2004), with major economical consequences (Knight-Jones and Rushton, 2013). The disease was included in the list of notifiable diseases of the World Organization of Animal Health (OIE) due to international spread of the virus and the significant levels of morbidity and mortality it causes (OIE, 2015).

1.1 FMD virus

The first documentation of the disease is from Italy in 1514 by Fracastorius, who reported a disease similar to FMD in cattle (Grubman and Baxt, 2004). The FMD virus was identified almost 400 years later, at the end of the 19th century by Loeffler and Frosch (Doel, 2003; Grubman and Baxt, 2004). The virus belongs to the *Aphthovirus* genus of the *Picornaviridae* family (Grubman and Baxt, 2004). Other important viruses included in this family are the following human pathogens: poliovirus, hepatitis A and coxsackievirus (Kerekatte et al., 1999). The last is one of the viruses causing Hand Foot and Mouth disease in humans (McMinn, 2002).

The FMD virus includes a single stranded positive sense RNA genome of about 8500 bases surrounded by a icosahedral (i.e. polyhedron with 20 faces) capsid (Sobrino et al., 2001; Grubman and Baxt, 2004) with a diameter of about 25-28 nm (Bachrach, 1968; Sobrino et al., 2001). The virus proteins can generally be divided into: (i) structural proteins (SP), which compose the viral capsid and include 4 proteins (VP1, VP2, VP3 and VP4) encoded by the genome P1 region; and (ii) non structural proteins (NSP), including 8 proteins that are essential for viral replication and are encoded by the genome L, P2 and P3 regions (Sobrino et al., 2001). Like other RNA viruses, the FMD viruses have a high mutation rate caused by the lack of an error correction mechanism during RNA replication (Drake and Holland, 1999; Domingo et al., 2003). This has led to the existence of seven distinguished FMD serotypes (A, O, C, ASIA-1 and SAT 1-3) and numerous, constantly evolving, subtypes (Parida, 2009). Most of the antigenic diversity of this virus is caused by variance in the P1 region as changes in the P2 and P3 are often lethal for the virus (Grubman and Baxt, 2004).

1.2 Infection and clinical signs

Different livestock ungulates are susceptible to infection by the FMD virus, including cattle, pigs, sheep, goats (Grubman and Baxt, 2004; Musser, 2004) and to some extent the Camelidae family (Musser, 2004; Wernery and Kaaden, 2004; Alexandersen et al., 2008; Larska et al., 2009). Many wildlife species are also susceptible to infection by the FMD virus (Grubman and Baxt, 2004; Musser, 2004), such as wild boar (*Sus scrofa*) (Alexandrov et al., 2013), deer species (Thomson et al., 2003), mountain gazelles (*Gazella gazella gazella*) (Shimshony, 1988) and African buffaloes (*Syncerus caffer*) (Vosloo et al., 1996). In general, the clinical signs are similar in all species, yet there is variation in severity and appearance between animals and between serotypes and strains (Thomson et al., 2003; Musser, 2004). For example, in the FMD outbreak in Taiwan in 1997 only pigs were affected while cattle, buffalo or goats remained intact (Yang et al., 1999). This was also demonstrated in an experimental study conducted following this outbreak (Grubman and Baxt, 2004).

Clinical signs in adult cattle include pyrexia (40°c); development of vesicles on the mouth, muzzle, feet and teats; hyper-salivation; smacking of the lips; nasal discharge; anorexia; lameness; drop of milk yield and mastitis (Kitching, 2002; Grubman and Baxt, 2004; Musser, 2004). In young calves, infection often results in per-acute death due to myocarditis with typical 'tiger heart' lesions (Kitching, 2002; Musser, 2004). Clinical signs in adult small ruminants (SR) are usually less prominent, yet high mortality of infected young animals may occur (Kitching and Hughes, 2002; Musser, 2004).

Infection of animals that do not lead to clinical presentation is possible in different species such as cattle, sheep and goats (Sutmoller and Casas, 2002). These sub-clinically infected animals pose a major threat as they might enhance the spread of the virus between farms (Mansley et al., 2011) and between countries (Bouma et al., 2003).

Infected animals from which the virus can still be isolated 28 days following infection are referred as 'carriers' (Salt, 1993). These are regarded as posing low or no threat of infection, as there is no evidence of infections caused by persistently infected animals (Sutmoller and Casas, 2002), except for African buffaloes (Vosloo et al., 1996).

Following infection, the virus localizes in the primary replication sites such as the dorsal soft palate, nasopharynx and lungs (Pacheco et al., 2010). This is followed by viremia and spread of the virus to secondary replication sites, in which it replicates and causes typical blisters characterizing FMD clinical disease (Zhu et al., 2013). Several factors associated with the affected tissues were suggested as possible mechanisms for the virus tropism, such as: (i) high availability and accessibility of the αVβ6 receptor, which is a primary FMD virus receptor in animals, (ii) lower type I interferon signaling expression, which results in reduced antiviral protection in these tissues, and (iii) low expression of fibronectin (ligand of the αVβ6 receptor), attributed to high turnover of the extracellular matrix (ECM), which results in better accessibility of FMD receptors to the virus infecting the cells (Zhu et al., 2013). A recent experimental study in cattle used a Bayesian framework and estimated that the average incubation period (i.e. time from exposure to the onset of clinical signs) is 4.1 days (95% credible interval = 2.9 - 5.9 days) followed by an average infectious period of 1.7 days (0.3 - 4.8 days). The average latent period (i.e. the time elapsed since an animal is infected to becoming infectious) lasts 4.6 days (3.1 - 7.2 days). Therefore infected cattle are not infectious before presenting clinical signs (Charleston et al., 2011). In SR, a latent period shorter than the incubation period results in an average viral dissemination period of 2.7 days (95% confidence interval ($CI_{95\%}$) = 2.3 – 3.1 days) before the appearance of clinical signs (Hughes et al., 2002).

1.3 Transmission

Disease transmission between an infected animal to a nearby non infected animal by airway particles (i.e. aerosol) is the most common mechanism for ruminant infection. Pigs are mainly infected by ingestion of contaminated animal products such as meat or offal as waste food. Other paths for viral transmission include mechanic transmission of the virus by contaminated milk or animal transportation trucks, and rarely transmission by wind (Donaldson and Alexandersen, 2002). The ability to spread the virus and to infect other animals varies between species and virus serotypes. For example, the amount of airborne virus excreted by infected pigs is remarkably higher than sheep or cattle, suggesting that infected pigs pose higher risk of infection to surrounding cattle or sheep (Donaldson and Alexandersen, 2002).

1.4 Epidemiology

Some countries are free from FMD, while others are affected (Vosloo, 2013). The different FMD serotypes are not distributed equally worldwide (Knowles and Samuel, 2003); there are certain pools of serotypes characterizing different areas in the world (Vosloo, 2013). Accordingly, additional characterization of FMD virus subtypes is based on their geographic origin, defining different topotypes as the virus subtypes affecting different areas of the world based on limited genetic variance between them (Knowles and Samuel, 2003).

Due to the high infectivity of the FMD virus and frequent trade between countries, introduction of FMD virus to previously 'FMD free' countries, or introduction of new FMD serotypes and subtypes to areas in the world not yet affected by them, is highly possible. A well known example is the introduction of FMD serotype O subtype Pan-Asia to Great Britain in 2001, which led to the occurrence of an epidemic involving 2,026 outbreaks of FMD. Contaminated food, illegally imported from Asia, then given to pigs as waste food was suspected as the source of infection (Mansley et al., 2011). More than 6.5 million animals were destroyed as part of the outbreak control measures (Mansley et al., 2011) and losses to agriculture and the food chain were estimated at more than 3 billion British pounds (Thompson et al., 2002). An additional example is the invasion of the SAT-2 serotype, common in Africa (Rweyemamu et al., 2008), into the Gaza strip in the Middle East region in 2012 (Valdazo-Gonzalez et al., 2012a), which surprisingly resulted in infection of only three unvaccinated fattening calves (http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=11875).

According to OIE definitions, a country or zone can be generally defined as either: (i) free from FMD without vaccination, (ii) free from FMD with vaccination, or (iii) infected by FMD (i.e. endemic). Limitations on the international trade of livestock and livestock products are applied according to these definitions (OIE, 2015).

The prevalence of FMD in different livestock species has been estimated in many endemic countries, such as Ethiopia (Megersa et al., 2009; Ayelet et al., 2012), Cambodia (Tum et al., 2015), India (Ranabijuli et al., 2010) and Bhutan (Dukpa et al., 2011). Additionally, the prevalence of FMD in wildlife species following FMD epidemics in livestock have been estimated in different countries, such as Bulgaria (Alexandrov et al., 2013), The Netherlands (Elbers et al., 2003) and Zimbabwe (Hargreaves et al., 2004). Even though some studies have suggested transmission of FMD from infected wildlife to livestock (Hargreaves et al., 2004; Alexandrov et al., 2013), there is no evidence that wild animals serve as long term hosts for FMD viruses, expect for endemic infection of African Buffaloes (*Syncerus caffer*) with SAT serotypes in sub-Saharan Africa (Thomson et al., 2003).

1.5 Control

Measures applied to control FMD generally include surveillance, vaccinations, quarantines, stamping-out and restrictions on importation of animals and animal products (OIE, 2015). Stamping-out of infected animals is restricted mostly to countries or zones free from FMD (with or without vaccination), as it is a necessary measure in order to regain 'free' status in a short period after an FMD outbreak (OIE, 2015). Stamping-out is not performed in endemic countries, as most countries are not able to sustain the costs of such a campaign, which was estimated at about 2.6 billion British pounds (including the slaughter and disposal of carcasses, cleaning and disinfection of premises and administration costs) during the 2001 FMD outbreak in Great Britain (Thompson et al., 2002). Additionally, it is not possible to distinguish between former and recent infections. Lastly, culling can't be justified in the existence of repeated incursions of the virus. Countries which are free from FMD can use emergency vaccines (described below) in the face of an outbreak, as was described in the Netherlands (Bouma et al., 2003). In countries free with vaccination and in some endemic countries, standard vaccines (described below) of livestock are administered routinely. Routine vaccination can either be used alone, as in Kenya (Lyons et al., 2015), or in combination with emergency vaccination, as in Israel. In other endemic countries, such as

Ethiopia (Ayelet et al., 2012), India (Ranabijuli et al., 2010) and Cambodia (Tum et al., 2015), vaccination of livestock against FMD is not applied or only partially applied.

Commercial production of inactivated antigen vaccines against FMD began with the semi-industrial production by Waldmann, and later on by Frenkel in the mid 20th century (Doel, 2003; Lombard et al., 2007). Currently these inactivated antigen vaccines are the most commonly used (Rodriguez and Grubman, 2009). According to OIE recommendations (OIE, 2012), the production process of these vaccines should include: (i) inactivation of the antigens of one or more serotypes or subtypes common in the region, (ii) purification of the vaccines from non structural proteins, which will allow differentiation between vaccinated and infected animals (DIVA) using serological methods, and (iii) oil or aluminum hydroxide and saponin adjuvants in order to stimulate the antibody response. Rodriguez et al. (2009) describe a few disadvantages of the currently available inactivated vaccines, as follows. (i) Vaccine production requires the use of a live virus, such that spillover the virus from the lab might lead to an FMD outbreak, as in Great Britain in 2007 (HSE, 2007). (ii) High antigenic diversity, as a result of the high mutation rate, might result in low matching between the vaccine and field strains (i.e. r1 value; described below) (OIE, 2012), resulting in inadequate protection. (iii) Frequent repeated vaccine administrations are required to maintain protection (Doel, 2003). Trying to overcome some of the disadvantages of inactivated vaccines, other types of vaccines have been developed, such as recombinant protein vaccines, empty capsid vaccines and live attenuated vaccines. Yet, currently, only the inactivated vaccines are suitable for commercial production (Rodriguez and Grubman, 2009).

According to the OIE terrestrial manual, FMD vaccines are classified as either 'standard' or 'higher' potency vaccines. The protective dose 50% (PD50) is one method used to determine efficacy of the vaccine, based on protection acquired in vaccinated calves experimentally exposed to FMD virus. The PD50 is calculated according to efficacy trial results (OIE, 2012).

It can be interpreted as the dose that protects 50% of the animals. Vaccine potency is then reported as the amount of PD50 contained in a single vaccine dose. 'Standard' potency vaccine should contain sufficient antigen and adjuvant to reach the minimal potency required (i.e. vaccine with 3 PD50). This kind of vaccine is usually suitable for routine vaccination. Higher potency vaccines (i.e. vaccine with PD50≥6) are recommended for FMD outbreak control in naïve populations (i.e. emergency vaccine) due to their wider spectrum of immunity as well as their rapid onset of protection (OIE, 2012).

Studies on the efficacy of high potency vaccines in livestock species demonstrate rapid clinical protection, acquired as short as 2 days and up to 6 months following vaccination (Cox and Barnett, 2009; Cox et al., 2010). In a meta-analysis conducted by Halasa et al. (2011) the average efficacy after experimental challenge of emergency vaccines was estimated at 87%. However, the field effectiveness of emergency vaccines was never properly addressed, especially when used in repeatedly vaccinated populations of animals, such as those that exist in endemic countries. Additionally, only few studies with contradicting results have estimated the long term protection acquired after repeated (routine) vaccination of cattle (Terpstra et al., 1990; Dekker and Terpstra, 1996; Woolhouse et al., 1996; Lyons et al., 2015).

1.6 Diagnosis

1.6.1 Viral identification

The methods and procedures required for FMD diagnosis are beyond the scope of this work and are detailed in the OIE terrestrial manual (OIE, 2012). Generally, virus isolation; immunological methods, such as ELISA test; and molecular methods, such as reverse transcription polymerase chain reaction (RT-PCR), can be used for viral identification. The use of these methods is especially important in order to differentiate between FMD and other vesicular diseases resulting in similar clinical signs, such as vesicular stomatitis, vesicular exanthema, blue tongue infection of sheep, and swine vesicular disease (Radostits et al., 2006).

1.6.2 Serology

Serological tests, such as virus neutralization test (VNT) and ELISA (detailed in the OIE terrestrial manual; (OIE, 2012)), are used in endemic countries in order to: (i) evaluate the NA response against SP elicited by vaccination, and (ii) determine FMD infection by the presence of antibodies against NSP.

I. Estimation of the NA response elicited by vaccination

The levels of NA against SP of specific serotype and subtype are often evaluated using VNT. Interpretation of VNT results vary between laboratories. A cutoff titer for detection of infection in non vaccinated animals was set to a titer of 1:45 (OIE, 2012). Yet, a general estimation of the NA cutoff titer required for protection from infection was not published.

II. Determining FMD infection

As NSPs are common to all serotypes and subtypes (detailed above), the presence of antibodies against NSP will indicate any FMD infection. This is often evaluated using ELISA methods (OIE, 2012). Additionally, as the vaccines are purified from NSP (detailed above), detection of anti-NSP antibodies can be also used to demonstrate infection in vaccinated animals.

1.7 FMD epidemiology in Israel

Israel is located in the Middle-East region, an area endemic to FMD (Aidaros, 2002), characterized by infections of serotypes A, O and ASIA-1 (Rweyemamu et al., 2008; Vosloo, 2013). FMD outbreaks reoccur in Israel every other year (Stram et al., 2011). The exact situation of surrounding countries is unfortunately less clear. Due to the geopolitical complexity, FMD outbreaks in the surrounding countries are scarcely reported and there is uncertainty concerning the actual situation (i.e. how many outbreaks occur and which serotypes are involved). Several studies used molecular tools in order to demonstrate incursions of the virus into Israel from surrounding countries (Stram et al., 1995; Alkhamis et al., 2009; Stram et al., 2011). Yet, the prevalence of the disease in Israel was never estimated and the role of different livestock sectors and wildlife in the epidemiology of the disease is uncertain.

Israel's livestock industry is composed mostly of cattle and SR bred throughout the whole country. Pigs are only bred in 4 secluded locations due to religious restrictions. Since 1984, Israel was affected by 24 FMD epidemics including 264 outbreaks. Most outbreaks involved serotype O (87.1% of the outbreaks) and to a lesser extent serotypes A (9.5%) and Asia-1 (3.4%). SR farms were affected most frequently (36.4% of the outbreaks) followed by beef farms (33%), feedlot farms (13.3%) and mixed farms (i.e. including SR and cattle or cattle from different sectors) (8.7%). Dairy farms (4.9%) and wildlife (2.3%) were the least affected. Pigs were not affected by FMD outbreaks until recently when a FMD outbreak of serotype O was reported in one farm in the western Galilee region.

FMD outbreaks reoccur in Israel despite the control measures applied routinely and during outbreaks. Measures applied routinely include: (i) Vaccination of all livestock using a commercial vaccine purified from NSP that have a PD50 greater than 6 (Aftopor, MERIAL, Pirbright, UK). Sheep are vaccinated with a bivalent vaccine (including serotypes A & O) and cattle and pigs are vaccinated with a trivalent vaccine (including serotypes A, O & ASIA-1) (for detailed information on vaccination regime (in Hebrew): http://www.vetserv.moag.gov.il/NR/rdonlyres/1F2917BB-2ACD-48BF-BF55-987D62074D9A/0/hisun_pot_20142015.pdf). (ii) Passive surveillance for detection of clinical signs of FMD is conducted by the Israeli veterinary services (IVS) personnel, private veterinarians and the farm owners. (iii) Import restrictions on animals and animal products. During outbreaks, measures applied include: (i) quarantines of the affected farm and all surrounding farms located in a radius of up to 10 km. Animal movement is prohibited for a period of up to six months, milk is transported to local dairy factories that don't export milk and disinfection of vehicles and personnel is required. (ii) Emergency vaccination is applied to all livestock within the quarantine area. Stamping-out policy is not applied in Israel.

Three separate management systems of cattle exist in Israel: (I) Dairy farms are intensively managed and kept in closed facilities, with almost no introduction of animals to the farm (i.e. locally bred replacement heifers and artificial insemination) and no grazing. All animals are marked (i.e. burn number and ear tag) and herd management software is used for individual follow up. (II) Feedlot farms are mostly semi-intensively managed. Calves are kept in closed facilities with no grazing, yet there is frequent introduction of imported calves (from Europe and Australia), calves from grazing beef herds and from dairy farms into the feedlots. All calves are marked (i.e. ear tags), yet individual follow up is not conducted. (III) Beef farms – these extensively managed grazing herds are bred mostly at the northern part of Israel, especially at the Golan Heights region. All animals are marked, yet individual follow up is only partially conducted. The differences between the cattle management systems are reflected in two main aspects: (i) the probability of exposure to FMD virus – grazing beef herds are at a higher risk of exposure to the virus, (ii) vaccination coverage – intensively managed dairy farms are vaccinated rapidly and efficiently, both on a routine basis and during outbreaks. In feedlot farms and even more in beef farms, due to management difficulties, vaccine administration is less rapid and efficient, especially at the time of outbreak occurrence.

Three different management systems exists in SR farms in Israel: (i) Intensively managed, not grazing large herds of sheep or goats. (ii) Semi-intensively managed, mostly large herds of sheep or goats, which are authorized to graze. The grazing permit is administered by the government and the herd owners are required to have detailed financial documentation of the herd accounting as well as of vaccine administration. (iii) Extensively managed small to moderate herds of sheep and goats. Some are reared in the backyard as 'family herds', which can be often found grazing illegally. These three management systems differ in: (i) Possible exposure to FMD virus – grazing herds encounter other herds of SR and beef cattle, as well as wildlife, and are therefore in greater risk of exposure to the virus. Additionally, illegal movements of SR between farms are common in extensively managed farms. (ii) Vaccination coverage – farms managed intensively or semi-intensively are regulated and monitored by the owners and authorities more often. Therefore, vaccination coverage in these farms will be higher as a part of the routine vaccination campaign and as a part of emergency vaccine administration in the face of an outbreak.

Cloven hoofed wildlife species in Israel include mainly wild boars (*Sus scrofa lybicus*) and mountain gazelles. Large populations of these species are located in the northern part of Israel. Additionally, small populations of water buffaloes (*Bubalus bubalis*) and Persian fallow deer (*Dama dama mesopotamica*), which are re-introduced into the wild, are located in restricted locations in Israel. Previous infections of mountain gazelles in Israel were described (Shimshony, 1988; Berkowitz et al., 2010), but endemic infection and the possible role of these species in the FMD dynamics in Israel were never addressed.

1.8 Summary

FMD outbreaks reoccur in Israel despite enormous efforts invested in disease control both on a routine basis and in the face of outbreaks. Several studies demonstrated that viruses are introduced into Israel from surrounding countries (Stram et al., 1995; Alkhamis et al., 2009; Stram et al., 2011) and wildlife species crossing the borders were suggested to have a role in the disease dissemination. Throughout the last three decades the disease incidence varied between different livestock sectors. Differences between different management systems in the livestock sectors and their assumed implications on exposure and vaccination provided (at least partially) explanation for this variance. However, the prevalence of infection in the different sectors and the risk factors for infection were never estimated as well as the effectiveness of the vaccine used. Estimating these will provide a better understanding of the complex dynamics of FMD in Israel. More specifically, it will allow addressing two main questions regarding the disease dynamics in Israel: (i) Do repeated introductions of the virus from surrounding countries provide the only explanation for the disease re-occurrence? Or is it possible that endemic infection of different livestock sectors or wildlife contribute to these repeated outbreaks occurrence? (ii) Is the current control regimen in Israel and especially the vaccination, effective in preventing infection?

1.9 General study hypothesis

This study hypothesis was that FMD dynamics in the different livestock sectors are the result of differences in exposure to FMD and vaccine effectiveness.

Specific study hypotheses:

1. Routine vaccination is only partially effective in disease prevention, due to inferior immunologic response in young animals which are vaccinated only few times.

Immunologic response and resultant protection provided is expected to increase with the number of vaccination administered.

2. There are differences in the virus dynamics **within** the different sectors, due to certain risk factors.

3. There are differences in the virus dynamics **between** the different sectors, during both endemic and epidemic periods.

2. Study objectives

Study objectives were set according to the specific study hypotheses:

I. Determine routine and emergency vaccination effectiveness and antibody dynamics. This objective is addressed in two separate subchapters:

   a. Determine the field effectiveness of the vaccine for acquisition of protection from infection in cattle (**subchapter 1a**) and SR (**subchapter 1b**)

   b. Determine the NA response following vaccine administration to repeatedly vaccinated dairy cattle in different vaccination statuses (i.e. the number of vaccines administered routinely) (**subchapter 2**)

II. Determine the risk factors for FMD outbreaks:

   Determine the risk factors for FMD outbreak occurrence in beef cattle (**subchapter 3**)

III. Determine the differences in virus dynamics among different livestock sectors and among wildlife and the possible risk factors for infection. This objective is addressed in 3 subchapters:

   a. Evaluation of the serological method used to estimate sero-prevalence of infection in vaccinated animals (**subchapter 4a**)

   b. Determine the risk factors for infection and the sero-prevalence of FMD among different livestock (**subchapters 4b and 4c**)

   c. Determine the risk factors for infection and the sero-prevalence of FMD among different wildlife species (**subchapter 4d**)

3. Results

3.1. Chapter 1

3.1.1. Subchapter 1a:

“The field effectiveness of routine and emergency vaccination with an inactivated vaccine against foot and mouth disease”: **See appendix article 1.**

3.1.2. Subchapter 1b:

“Association of the time that elapsed from last vaccination with protective effectiveness against foot-and-mouth disease in small ruminants”: **See appendix article 2.**

3.2. Chapter 2

3.1.1. Subchapter 2a:

“The serological response against foot and mouth disease virus elicited by repeated vaccination of dairy cattle”. **See appendix article 3.**

3.1.2. Subchapter 2b:

" The long term effect of age and maternally derived antibodies against foot and mouth disease on the serological response following vaccination in young dairy calves.". **See appendix article 4.**

3.3. Chapter 3

“Risk factors for foot and mouth disease outbreaks in grazing beef cattle herds” . **See appendix article 5.**

3.4. Chapter 4

3.4.1. Subchapter 4a:

“The longevity of anti NSP antibodies and the sensitivity of a 3ABC ELISA – A 3 years follow up of repeatedly vaccinated dairy cattle infected by foot and mouth disease virus”.

See appendix article 6.

3.4.2. Subchapter 4b:

“Prevalence and risk factors for Foot and Mouth Disease infection in small ruminants in Israel”. **See appendix article 7.**

3.4.3. Subchapter 4c:

“Prevalence and risk factors for Foot and Mouth Disease infection in Cattle in Israel”. **See appendix article 8.**

3.4.4. Subchapter 4d:

“Sero-prevalence of foot and mouth disease in susceptible wildlife in Israel”. **See appendix article 9.**

4. Discussion

In this study we estimated for the first time the contribution of vaccine effectiveness, and FMD infection in different livestock sectors and wildlife, on the dynamics of FMD in Israel.

4.1 Determination of routine and emergency vaccination effectiveness and antibody dynamics

The differences regarding vaccination coverage in various management systems of livestock species and sectors (previously described) can generally explain the differences in disease occurrence among these species and sectors in Israel. Yet this cannot explain the re-occurrence of FMD outbreaks in Israel almost every year (based on data published in the IVS yearly reports and the WAHID interface), especially when the affected herds were vaccinated repeatedly against circulating FMD serotypes.

4.1.1 Determination of the field effectiveness of vaccine in acquiring protection from infection in cattle (**subchapter 1a**) and SR (**subchapter 1b**)

Estimation of the protection acquired through vaccination can be either based on experimental studies or field data. Vaccine efficacy can be estimated following experimental infection of vaccinated cattle. However, due to the high costs required to conduct these studies, they are mostly restricted to a short period of follow-up after vaccination of naïve animals, such as calves (Cox et al., 2005; Cox et al., 2007; Brehm et al., 2008) or lambs (Cox et al., 1999; Parida et al., 2008). Additionally, due to the high infectiousness of the virus, special facilities are required (i.e. containment level for Group 4 pathogens) to conduct these trials (OIE, 2012). Vaccine effectiveness can instead be estimated following natural exposure of vaccinated animals to FMD. In Israel, vaccine efficacy cannot be evaluated through experimental studies due to economical considerations. However re-occurrence of FMD outbreaks in vaccinated herds enables us to estimate vaccine effectiveness in repeatedly vaccinated animals of different vaccination statuses (i.e. the number of vaccines administered prior to exposure and the time elapsed between last vaccine administration and the outbreak occurrence).

4.1.1.1 Cattle (**subchapter 1a**)

In a field investigation of an FMD outbreak in a feedlot and adjacent dairy farm, we found that feedlot calves that were routinely vaccinated twice, with the last vaccine administered at least 3 months before the outbreak, were not fully protected from both clinical and sub-clinical infection (96% presented antibodies against NSP and more than 50% presented clinical signs during the outbreak). This was also demonstrated in replacement heifers at the adjacent dairy farm. These were vaccinated 3-5 times with the last vaccine administered 7 months before the outbreak. All heifers were infected (100% presented antibodies against NSP) and 18% presented clinical signs of FMD. Similar findings indicating failure of repeated vaccination to provide protection from infection was also demonstrated in a field investigation conducted in Saudi Arabia (Woolhouse et al., 1996) and in Kenya (Lyons et al., 2015).

We estimated the NA titers in different groups prior to the outbreak occurrence based on serum samples obtained from cows and heifers from farms not affected during the FMD epidemic. These animals had the same vaccination statuses (i.e. the number of vaccines administered and the time elapsed since last vaccine administration) as the cows and heifers in the affected farms. Matching between the vaccine and the field strains is estimated by calculating the r1 value $= \frac{\text{antibody titer elicited against the field strain}}{\text{antibody titer elicited against the vaccine strain}}$ (OIE, 2012). When the

NA titers are estimated by VNT, values above 0.3 are indicative of sufficient matching between the field and the vaccine strains, which will allow protection from heterologous infection (OIE, 2012). However, in our study, a vaccine r1 value of 0.37 with the field strain was not sufficient and a high percentage of the animals were infected and presented clinical signs. Terpstra et al. (1990) experimentally exposed cattle, which were routinely vaccinated 3 times, to heterologous A serotype one year following the last vaccine administration. Even though the r1 value calculated was 0.25, a high percentage of the cattle was protected from clinical manifestations of FMD. Several differences between the two studies may explain the contradicting results. (i) The commercial vaccine used in Israel is produced in a baby hamster kidney (BHK) cell line (Doel, 2003), while the Dutch vaccine was locally produced using the Frenkel method (Terpstra et al., 1990). In the latter, epithelium obtained from the tongues of recently slaughtered healthy cattle are maintained in vitro (in a suspension) and used as a cell line for the virus production (Doel, 2003). (ii) The vaccine and the infection serotypes and subtypes in the two studies were different. Differences in the virulence between serotypes and subtypes may affect the ability to cause infection and may lead to variance in the severity of clinical manifestation (Grubman and Baxt, 2004). (iii) Additional factors varying between the vaccines used in these studies, such as the vaccine PD50 and the adjuvant used, may have influenced the response to vaccination.

In contrast to the low effectiveness of the routine vaccination, the same vaccine had high effectiveness in prevention of infection and clinical presentation when it was administered up to 14 days before the outbreak (i.e. as an emergency vaccine). The high effectiveness of the emergency vaccination was obtained regardless of the number of FMD vaccines administered before and NA titers at the time of exposure. High efficacy of emergency vaccines was also presented in several experimental studies. Cattle vaccinated between 10 and 21 days before the outbreak with high potency vaccines (PD50 ranges between 2 and 32), were highly protected from clinical presentation of FMD and from infection (Cox et al., 2005; Cox et al., 2007; Brehm et al., 2008). High protection using the emergency vaccination was obtained even when the r1 values ranged between 0.023 and 0.04 (Brehm et al., 2008). Positive association between the NA titers against FMD and protection from infection was demonstrated in animals vaccinated 21 days before exposure to the virus (Brehm et al., 2008). Yet, the rapid protection acquired by emergency vaccination cannot be explained only by serological response. Several mechanisms were proposed in order to explain this rapid protection, such as presence of opsonising antibodies (Lannes et al., 2012), pre-challenge levels of IL-6 (Cox et al., 2011) and post challenge levels of IFN-γ (Parida et al., 2006).

4.1.1.2 Small ruminants (**subchapter 1b**)

Vaccination of SR in general and especially routine vaccination was scarcely addressed in the peer reviewed literature.

The role of SR in disease transmission is debatable. In some cases SR were suspected as reservoirs of FMD (Barnett and Cox, 1999), while in other studies low sero-prevalence of FMD in previously affected herds suggested that FMD transmission among these herds was self-limiting (Donaldson, 2000; Kitching and Hughes, 2002; Mansley et al., 2011). This was also supported by limited transmission of FMD from infected SR in experimental studies (Orsel et al., 2007; Bravo de Rueda et al., 2014).

Several studies estimated the efficacy of emergency vaccines in SR (Cox et al., 1999; Parida et al., 2008) in developed countries which are free from the disease. However, there is lack of knowledge regarding the effectiveness of vaccines administered routinely to SR in countries endemic to FMD (existing data is primarily based on anecdotal observations). We conducted an epidemiological investigation in a well managed intensive farm of dairy SR, which allowed us to estimate the vaccine effectiveness in SR. We found that SR vaccinated at least twice, with the last vaccine administered about 3 months before the outbreak, were not clinically affected by FMD. The high effectiveness of routine vaccination found in SR compared to the low effectiveness found in cattle (subchapter 1a) was despite the similar matching between the vaccine and the field strains (r1 value) in both outbreaks. This can be a result of the difference in the susceptibility to clinical infection between cattle and SR (Grubman and Baxt, 2004; Musser, 2004). In contrast to SR vaccinated twice, in SR that were vaccinated only once, negative associations between the time elapsed since last vaccination and protection from clinical manifestation of FMD were found. This may be the result of rapid wane of immunity over time, such as was described in cattle (subchapter 1a, (Woolhouse et al., 1996)). Finally, similar to cattle (subchapter 1a), we found a high effectiveness of emergency vaccination of SR in blocking the outbreak spread within the farm. This finding was in accordance with previous reports on the high efficacy of such vaccines as recently as 4 days before exposure (Cox et al., 1999; Parida et al., 2008).

4.1.2 Determination of the NA response following vaccine administration to repeatedly vaccinated dairy cattle with different vaccination statuses (i.e. the number of vaccines administered routinely) (**subchapter 2**)

The incomplete effectiveness of routine vaccination in both SR and cattle, which was observed in our studies (subchapters 1a,b), raised several questions: (i) Can a decay in the NA titer levels following vaccination explain the reduction in vaccine effectiveness over time? (ii) Does the NA response following vaccination vary among animals of different vaccination statuses? These questions are highly relevant to endemic countries who routinely vaccinate against FMD with commercially available vaccines.

Contradicting findings regarding the longevity of the NA titers elicited by vaccination are documented in the literature. Several studies demonstrated high NA titers in repeatedly vaccinated cattle three (Terpstra et al., 1990) and four years (Dekker and Terpstra, 1996) following the use of a locally manufactured vaccine. In contrast, a rapid decrease of NA titers 3 months following the use of commercial vaccine was suggested by Woolhouse et al. (1996).

We were able to address the previous questions in an experimental setting. We demonstrated that the dynamics of NA titers following vaccination varied between 'low vaccination' (i.e. up to three vaccines administered routinely before the trial) and 'high vaccination' groups (i.e. at least four vaccines administered routinely before the trial). The NA titer increase following vaccination was negatively associated with the number of prior vaccines administered. In the 'high vaccination' groups, the vaccine administration during the trial's first year resulted in almost no change of the NA titers. On the other hand, in the 'low vaccination' groups, a marked increase, followed by rapid decrease of the NA titers, was observed. However, because the 'high vaccination' group's initial NA titers (i.e. on day 0) were higher, the titers remained high until the end of the study. A general estimation of the NA titer cutoff required for protection from infection was not published by the OIE. It was recommended to establish a correlation between NA titers evaluated in the laboratory and protection from infection of vaccinated animals, based on potency test results using the relevant vaccine and target species (OIE, 2012). However, in countries like Israel in which potency trials are not conducted, an approximation of the cutoff value required for protection can be roughly estimated based on published studies. In these studies, the cutoff titers acquired for protection were evaluated as ranging between 1.3 and 2.086 (titers in log 10) against O serotypes (Barnett et al., 2003; Dekker A., 2008; Goris et al., 2008). Based on these studies we arbitrarily chose a titer of

6.64 in log 2 (2 in log10) as the cutoff titer indicating protection. High percentage (78%) of the 'high vaccination' cows remained with NA titer above the cutoff titer at the end of the first year compared to lower percentages (ranges between 14 and 60%) in the 'low vaccination' groups.

Consistent NA titer levels were demonstrated during the study period among the 'high vaccination' groups. Therefore, Doel's (2003) recommendation to repeat vaccination every four months in endemic countries, may be justified only because it increases the probability of vaccinating shortly before an outbreak occurrence (i.e. as an emergency vaccine). However, such a frequent vaccination regimen in Israel cannot be conducted due to financial restrictions. The current vaccination regimen in Israel includes, in addition to routine vaccination, a rapid administration of emergency vaccine in the face of an outbreak. In these settings, routine vaccination is aimed only to mitigate the outbreak spread until the administration of emergency vaccine. Therefore, reducing the frequency of routine vaccinations in cows that were already routinely vaccinated at least 5 times may be considered.

4.2. Determination of the risk factors for the occurrence of FMD outbreaks

4.2.1 Determination of the risk factors for FMD outbreak occurrence in beef cattle (**subchapter 3**)

The risk factors for FMD outbreaks in grazing beef cattle were determined in a case control study conducted in herds that were grazing during the 2011 FMD epidemic in the Golan Heights region. Previously, Stram et al. (1995; 2011) and Alkhamis et al. (2009) demonstrated that FMD viruses are introduced into Israel from the surrounding countries.

Grazing beef herds are bred mostly in the northern part of Israel, especially in the Golan Heights region. While grazing next to the borders, these herds are therefore at higher risk of exposure to the virus, especially in herds that are grazing in the north-west part of the Golan Heights (i.e the "Chebaa farms" area). In this area, due to unresolved claims on the location of the international border with Lebanon, there are unfenced areas and livestock and wildlife can cross the border freely. Under these circumstances, infected grazing herds may play a significant role in the introduction of FMD viruses into Israel and further spread of the disease.

A significant positive association was found between the presence of calves younger than six months old and outbreak occurrence within grazing beef herds. The presence of the young calves may increase the herd density, a factor which was previously demonstrated as a risk factor for FMD in Argentine (Ward and Perez, 2004). Additionally, higher susceptibility of these young calves to FMD may have resulted from lack of immunity, as part of the calves groups were vaccinated only once. Administration of only one vaccination against FMD was able to promote only moderate elevation in the NA titers (Sadir et al., 1988), as was also demonstrated by the initial (i.e. day 0) low NA titers in the vaccine trial (subchapter 2). In addition, not all calves groups were vaccinated at the time of the outbreak and it is possible that due to the normal decay of the maternally derived antibodies (MDA) (Nicholls et al., 1984) they were not protected from infection. The presence of these calves reduced the herd immunity and enabled the disease introduction and spread within the herd.

4.2.2 Possible route of FMD dissemination (**subchapter 1a**)

We found that sub-clinically infected calves that arrived from beef herds in the Golan Heights region into a local feedlot were the probable source for FMD introduction into the feedlot.

Sub-clinical infection of animals with FMD and their potential role in the disease transmission were previously described in the literature (Kitching, 2002; Sutmoller and Casas, 2002). This finding highlight an important possible route for FMD transmission during FMD outbreaks in Israel, as beef cattle are often affected during FMD epidemics in Israel (based on data published on the IVS yearly reports and the WAHID interface).

4.3 Determination of the differences in virus dynamics among different livestock sectors and among wildlife and the possible risk factors for infection

4.3.1 Evaluation of the method used to estimate sero-prevalence of infection in vaccinated animals (**subchapter 4a**)

In order to estimate FMD infection we used an ELISA test for evaluation of the presence of the 3ABC NSP proteins (Sorensen et al., 1998). This test (PrioCHECK® FMDV NS blocking ELISA) was evaluated by Brocchi et al. (2006) and found to have high specificity and sensitivity compared to other currently available tests. ELISA tests are extensively used as a part of endemic and post epidemic surveillance programs (Chung et al., 2003; Chen et al., 2011; Ayelet et al., 2012; Dukpa et al., 2012). The presence of antibodies against NSP was reported in several studies conducted with as few as 5 animals that were followed up to 742 days using commercial and 'in house' ELISA tests (Mackay et al., 1998; Malirat et al., 1998; Moonen et al., 2004; Brocchi et al., 2006; Robiolo et al., 2006). Yet, the longevity of antibodies against NSP in vaccinated animals infected by FMD and the sensitivity of the commercial ELISA test in detection of antibodies in repeatedly vaccinated animals were rarely evaluated.

We found that the test sensitivity 45 days after the outbreak was 100% compared with a sensitivity of only 68.1% 28-100 days post exposure during an outbreak, previously reported by Brocchi et al. (2006). Despite a decrease in the percent of inhibition (PI) values between years 2011 and 2014, we found that test sensitivity remained high even 1118 days after the outbreak. This sensitivity varied between 85.7% and 100% depending on the way equivocal results were treated.

4.3.2 Determination of the risk factors for infection and the sero-prevalence of FMD

Using the ELISA test kits we were able to estimate for the first time the sero-prevalence of FMD and the risk factors for infection among different livestock sectors and wildlife in Israel.

Different sampling methods were used in different livestock sectors and wildlife. In our study SR and wildlife samples pose better representation of the entire population while in the different cattle sectors only the population at high risk is represented as a risk based sampling strategy was preferred.

4.3.2.1 Determination of the risk factors for infection and the sero-prevalence of FMD among different livestock sectors (**subchapters 4b and 4c**)

Small ruminants (**subchapter 4b**)

Moderately low sero-prevalence of 3.7% (95% confidence interval ($CI_{95\%}$) = 3.0 - 4.5%) was found in adult animals and significantly lower prevalence of 1.0% ($CI_{95\%}$ = 0.1 - 3.6%) was found in young animals. The adult SR positive samples were scattered all over Israel with only two small infection clusters. As the survey represents the entire population, the wide distribution of positive samples indicates endemic infection of SR with ongoing circulation of the virus, which is also reflected in the infection of the young animals. Several studies in endemic countries found prevalence varying between 11.9% and 38% in Bhutan, Uganda, Morocco and India (Barnett and Cox, 1999; Balinda et al., 2009; Ranabijuli et al., 2010; Dukpa et al., 2011; Rout et al., 2014). The lower sero-prevalence obtained in Israel is probably the result of the control measures applied and the intensive vaccination regimen of livestock in Israel. In contrast to other countries, SR in Israel are vaccinated routinely every year and additionally during outbreaks (i.e. emergency vaccination). High effectiveness of routine vaccination was demonstrated in SR vaccinated multiple times (subchapter 1b) and emergency vaccination was highly effective in disease prevention (subchapter 1b, (Cox et al., 1999)). Additionally, several studies demonstrated that the risk of infection from SR, especially when vaccinated, is low (Kitching and Hughes, 2002; Orsel et al., 2007; Parida et al., 2008; Bravo de Rueda et al., 2014).

Location in proximity of up to 5km to an FMD outbreak that didn't affect SR was associated with a 15 times higher risk for FMD infection. Contact between animals is the main route for SR infection (Donaldson and Alexandersen, 2002). Mechanical transmission (i.e. by vehicles or personnel moving between farms) and transmission by air may pose alternative routes of infection. Yet, the later is less significant due to the small respiratory volume of sheep, which make it less susceptible to this kind of infection (Kitching and Hughes, 2002). Therefore it seems probable that these infections are the result of quarantine breech. Illegal movements of SR occur frequently, mainly in extensively managed herds in which livestock are often used as a payment method. It is suggested that SR herd owners were more alert to the risk of FMD infection when the nearby outbreak affected SR. Therefore, a better enforcement of the quarantine was applied as well as better cooperation with the IVS in the administration of emergency vaccines.

Grazing and herd size larger than 500 animals were negatively associated with sero-positivity. Both factors characterize farms which are semi-intensively and intensively managed. These farms are supervised more regularly by the authorities and the owners are more aware of the importance of bio-security and the risks of introduction of diseases into the farm. Therefore, there is better compliance with routine vaccination and it is possible to rapidly administer emergency vaccination during an outbreak. Additionally, as these farms are better regulated, illegal introduction of animals to the farms is less common.

Cattle (**subchapter 4c**)

Sero-prevalence's of 0.4% ($CI_{95\%}$ = 0 - 2.2%), 13.2% ($CI_{95\%}$ = 10.8 -15.8%), 2.7% ($CI_{95\%}$ = 2 - 3.6%) were found in feedlots, beef and dairy farms, respectively. Higher sero-prevalence, varying between 9.5-30%, were estimated in cattle and buffalo in Ethiopia, Cambodia, Bhutan and Vietnam (Megersa et al., 2009; Dukpa et al., 2011; Ayelet et al., 2012; de Carvalho Ferreira et al., 2015; Tum et al., 2015). However, these were mostly based on sero-surveys conducted in small, often nomadic farms. This type of farming is markedly different from the intensive and semi-intensive management systems of the large cattle farms that exist in Israel, in which different control measures, including vaccination, are applied in order to prevent FMD outbreaks.

Beef cattle and feedlot calves

Most of the sero-positive samples of beef cattle were collected from farms in the Golan Heights region. Sero-prevalence was significantly higher in 2014 (vs. 2006) and was positively associated with previous FMD outbreak occurrence within the farm. Similar association was observed in Vietnam (de Carvalho Ferreira et al., 2015) and Bhutan (Dukpa et al., 2011). This is probably the result of the longevity of anti-NSP antibodies (subchapter 4a), which also resulted in the positive association between sero-positivity and older cows (i.e.

>2.5 years). Additional explanation of the association between age (i.e. >2.5 years) and sero-positivity can be that repeated vaccinations elicited antibodies against NSP. Yet, this is less probable as the commercial vaccine was purified from NSP (Doel, 2003) and only low percentage of sero-positive samples were previously found in cows repeatedly vaccinated with this vaccine (subchapter 1a, subchapter 4a).

In contrast to the high sero-prevalence among adult beef cattle, a negligible sero-prevalence was found in the feedlot calves. These calves originated, at least partially, from beef herds. The high sero-prevalence of infection in beef cattle dams, which was associated to age and previous FMD outbreaks and the absence of infection among young feedlot calves, indicates that there is probably no ongoing circulation of the virus in these livestock sectors. As this study estimated the sero-prevalence in high risk population, we can deduce that in the entire population the sero-prevalence is even lower and there is no continuous circulation of the virus.

Dairy cattle

The sero-prevalence in dairy cattle varied between the sampled years, yet the overall sero-prevalence was low in the high risk areas that were sampled. This indicates that the actual prevalence in the entire dairy cattle population in Israel is low and that ongoing circulation of the virus among dairy cattle is not likely. 'Location of up to 5km from the borders' and 'location of up to 3km from multiple outbreaks' were positively associated with sero-positivity.

Cattle are mainly infected by aerosol transmission from adjacent animals (Donaldson and Alexandersen, 2002; Kitching, 2002; Musser, 2004). Additional infection routes include mechanical transmission and for a lesser extent airborne infection (Donaldson and Alexandersen, 2002). Dairy cattle are bred in fenced farms and there is almost no introduction of animals into the farms. Additionally, during outbreaks quarantines are applied on all farms located up to 10km from the outbreak center. Therefore infection by aerosol transmission between animals in contact is less likely. Additionally, until recently all FMD outbreaks in Israel involved only cattle, SR and rarely mountain gazelles (based on the data published on the IVS yearly reports and the WAHID interface). It was previously estimated that 100 cattle or SR can generate a virus plume which is capable of infecting cattle only up to less than 1km away (Donaldson and Alexandersen, 2002). Therefore, airborne infection is also less likely. Accidental mechanical transmission, especially while multiple outbreaks occur can explain the positive association of infection with proximity (up to 3km) to multiple outbreaks.

Positive association between proximity to the borders and infection was previously described in Zambia (Hamoonga et al., 2014) and Bhutan (Dukpa et al., 2011). In these studies, cattle were grazing next to the borders and could have encountered affected herds or wildlife. In Israel, dairy cattle are not grazing and due to the confined management system infection from aerosol from adjacent animals is less likely. A rare event of airborne infection is also less plausible explanation for several exposures in separate locations and different years. Unfortunately, we could not find any biological explanation for this association.

4.3.2.2 Determination of the risk factors for infection and the sero-prevalence of FMD among different wildlife species (**subchapter 4d**)

High sero-prevalence of FMD infection (7.7% ($CI_{95\%}$ = 4.4% - 12.1%)) was detected in wildlife species. However, sero-positive samples were obtained mainly from wild boar. A significantly higher sero-prevalence was found in wild boar samples collected during 2007, with a significant infection cluster located in the north east region of Israel. The high sero-prevalence in wild boar during 2007 was in accordance to previous less detailed report on FMD sero-prevalence in wild boar in Israel (ProMED, 2007). An infection cluster was located adjacent to previously reported FMD outbreak affecting mountain gazelles during 2007 (OIE, 2007; Berkowitz et al., 2010). Similarly, positive association between sero-positivity in wildlife and proximity to outbreak centers was observed in Bulgaria (Alexandrov et al., 2013). Yet, a sero-prevalence lower than that found in wild boar during 2007 in Israel was observed in wild boar samples obtained from Bulgaria and an adjacent area in Turkey, following an FMD epidemic affecting wildlife and livestock in Bulgaria (EFSA, 2012; Alexandrov et al., 2013). This might indicate of differences in the virus transmission to wildlife during these outbreaks.

It was previously demonstrated in experimental studies that infected wild boar, with varying clinical presentations, can infect other wild boar and domestic pigs (Mohamed et al., 2011; Breithaupt et al., 2012). Additionally, wild species, including wild boar, were suspected as infecting livestock in the field (Hargreaves et al., 2004; Valdazo-Gonzalez et al., 2012b; Alexandrov et al., 2013). Therefore it is possible that during 2007 wild boar played a role in the disease transmission in Israel.

The absence of sero-positive samples in all study years, but 2007, as well as the rare reports of FMD outbreaks in wildlife in Israel (based on the data published in the IVS yearly reports and the WAHID interface) indicates that there is no ongoing circulation of the virus in wildlife in Israel. These results are in accordance with previous studies suggesting that except for the African buffalo (*Syncerus caffer*) no other wildlife species were able to carry the virus for a long period (Thomson et al., 2003; Weaver et al., 2013).

Consistent decrease in mountain gazelle population size (Kaplan, 2002) that results in population size too small for propagating FMD epidemics (Morgan et al., 2006) may partially explain the infrequent FMD outbreaks occurrence in this species in Israel, while nearby livestock are affected. Several additional explanations may be suggested, such as variability in the virulence of different FMD serotypes and subtypes and in the susceptibility of the host that can lead to lower infection and transmission rates of the wildlife species (Thomson et al., 2003; Weaver et al., 2013). Additionally, fluctuations in the wildlife population densities in certain locations throughout the year (e.g. as a result of changing abundance of food or water resources) may increase the risk for virus transmission within the population and between wildlife and livestock (Thomson et al., 2003).

5. Summary

In this study we conducted for the first time an integration of the data regarding differences in vaccination and infection of livestock sectors and wildlife. The sero-prevalence of infection, vaccine effectiveness and the longevity of the NA response following vaccination were evaluated. This study's findings set the stage for a better estimation of FMD risk factors and dynamics in Israel and of the current FMD control regime in Israel.

Our study suggests there is no ongoing circulation of FMD viruses in wildlife and in different livestock sectors, except SR. These findings strengthen the importance of repeated incursions of FMD viruses from surrounding countries into Israel (Stram et al., 1995; Alkhamis et al., 2009; Stram et al., 2011) in the dynamics of FMD in Israel.

Endemic infection of SR with low sero-prevalence, widely distributed all over Israel was demonstrated. The ease of illegal movements of SR between farms and the minor clinical presentation in these species probably contribute to the disease dissemination. However, due to the overall effectiveness of vaccination (routine and emergency), the sero-prevalence remained low and the risk for FMD transmission was low. It is possible, though, that occasional FMD virus transmission from SR do occur. Yet, as all livestock populations in Israel are vaccinated, these events probably lead to only small scale outbreaks.

The sero-prevalence study findings suggest that ongoing circulation of FMD viruses between beef and feedlot farms is not probable. Yet, during FMD outbreaks sub-clinically infected beef calves may play a significant role in FMD dissemination into the feedlot, as was described in this study. The high sero-prevalence of FMD infection in grazing beef herds, located in the Golan Heights region indicates their high exposure to FMD viruses. The presence of young calves (<6 months old) in these herds increases the risk for FMD outbreak occurrence within the herd. These findings suggest that it might be beneficial to increase the intensity of surveillance in addition to increasing the frequency of vaccination in these herds or alternatively to administer additional vaccinations to cows before calving in order to increase the protection provided by MDA in the newborn calves.

The sero-prevalence in dairy cattle was in accordance with the low incidence of the disease in these farms. This is probably the result of the management system in these farms and the control measures applied against FMD. It is suggested that despite the long term low effectiveness of the routine vaccination, its importance is by mitigating disease spread until the administration of emergency vaccination. Therefore, our additional findings, which demonstrate rather consistent NA titers in cows after the administration of the 5^{th} routine vaccination, may indicate that it is possible to reduce the frequency of routine vaccination at that stage.

The high sero-prevalence in wildlife, which was restricted to wild boars in 2007, illustrates the mostly limited but variable role of wildlife species in the dissemination of FMD in Israel. The sporadic occurrence of FMD in wildlife may be attributed to changes in wildlife population dynamics.

Based on the findings about the dynamics of FMD and vaccine effectiveness, an additional study should be conducted. This should aim at evaluating different control regimes and surveillance plans in different livestock sectors and in high risk areas. The possible influence of these on disease dynamics should be evaluated, while considering the cost and benefit of each strategy. This will enable decision makers to choose the best strategy for the control of FMD in Israel.

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