Investigation on Results from Vaccination of Sows Against Foot and Mouth Disease (FMD) using Different Vaccination Protocols in Israel

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ABSTRACT

Israel is endemic for Foot and Mouth Disease (FMD), with several outbreaks occurring every year; almost all of them occur in ruminants, however in November 2015 a pig farm was affected and FMD virus Type O was confirmed as the etiological agent. A compulsory mass/blanket vaccination of all pigs (breeders, growers) in Israel was thereafter implemented. During 2016, FMD vaccination continued to be implemented in Israeli pig farms, but it was limited to breeders and with different vaccination protocols. Six farms with different vaccination protocols in breeders were examined from May 2016 until December 2016 for levels of Virus Neutralizing Antibody Titers (VNT) in breeders and suckling piglets induced by repeated vaccinations. The highest VNT was found in sows vaccinated during pregnancy examined around 60 days after the last vaccination. Correspondingly, the VNT-Maternally Derived Antibodies (MDA) levels in their piglets was directly related to the antibody titer in the sow. Repeated vaccination with an inactivated vaccine of breeders during pregnancy, achieved results recommended by literature and the World Animal Health Organization (OIE): population coverage of >80% and VNT ≥1.5Log_{10} in both in sows and their piglets.

Keywords: FMD; Vaccination; Sows; Piglets; Protection.

INTRODUCTION

Israel is Foot and Mouth Disease (FMD) endemic, with 160 outbreaks between 2005 and 2018 (1); almost all the outbreaks occurring in ruminants (1, 2), however in 2015 also a pig farm was involved (2). Israel is counted by the World Organization for Animal Health (OIE) among the "Countries and zones without an official status for FMD" (3). FMD vaccination is mandatory in Israel against Types O, A, Asia (inactivated trivalent vaccine), with a vaccination protocol in ruminants consisting of 2-3 vaccinations in the first year of age, followed by a yearly single booster, with possibility of a further booster in the course of an outbreak (4). At that time (until 2015) the vaccination protocol in pigs was less structured, with a single mass/blanket vaccination of breeders only, once a year, in autumn (2). There is no eradication policy in Israel during FMD outbreaks, rather a compulsory vaccination policy in which animals are allowed to complete their normal life-cycle (5, 6) even in occurrence of an outbreak. Symptomatic therapy and/or euthanasia are implemented for ill animals, taking into account welfare implications (6). This policy has been defined as "*vaccine to live*" (5) and it may be considered as the most beneficial for animal survival and domestic continuity of business (5) in endemic situations, when no commercial implications for export exist.

In November 2015, an FMD outbreak occurred in a farrow to finish unit of 350 breeders, located in the Northern Region of Israel, induced by an FMD virus of Type O (2, 6). The outbreak clinically developed mainly in the farrowing unit, with high mortality in suckling piglets, but low or

negligible losses among breeders and fattening pigs (6), as described in literature (5, 7). As first clinical signs appeared, considering that vaccination against FMD may prevent animals from developing clinical lesions even when infected (8), mass/blanket vaccination against FMD was implemented on all the animals of the affected farm: breeders, piglets and growing/fattening pigs. Taking into account the endemic situation relative to FMD in Israel, the proximity of other pig farms in the Northern Region, and the pig population size in the Region (13,000 breeders and some 90,000 growing pigs at any given time) (9), within a few days compulsory mass vaccination of all pigs in the country was implemented. An inactivated trivalent vaccine (O, A, Asia 1) (Aftopor, Merial SAS, 29, av. T. Garnier F-69 007 Lyon, France; antigens: O-Manisa; O-3039; O-Israel85; O-PanAsia2; A-Saudi95; A-Iran05; Asia1), water/oil, double emulsion, intra-muscle (IM) application was used, as already in use for cattle. The vaccination schedule consisted of priming and booster vaccinations 4 to 5 weeks apart. Following the confirmation of Type O in the outbreak (6), subsequent vaccinations courses were implemented with water/oil, double emulsion-inactivated vaccine, containing Types O and A only, already available in the Country (Aftopor, Merial SAS, 29, av. T. Garnier F-69 007 Lyon, France; antigens: O-Manisa; O-3039; O-4625; A-Iran2005; A-4165).

The FMD outbreak was confined to the affected farm, and no spread to other pig farms was observed; nevertheless, discussions arose about the effectiveness of the current FMD prophylactic vaccination schedule on the swine population, which was based on single mass/blanket vaccination once a year in breeders only.

Literature (7) and OIE (3) strictly recommend that vaccination strategies should be designed to achieve "mass coverage" and that "coverage should be at least 80%" in the vaccinated population. Therefore, following the first vaccine and booster courses during the outbreak, different vaccination plans were implemented in Israel, aiming to achieve the recommended coverage targets. The purpose of this communication was to present and discuss the results obtained.

MATERIALS AND METODS

Following the mass/blanket vaccination of November 2015 on the whole population (outbreak situation), starting May 2016, FMD vaccination continued to be implemented on breeders only according to different protocols below described:

- Mass/blanket protocol: vaccination of all the sows and boars on the same day, every six months, irrespective from reproduction phase of sows (only, sows in the last two weeks of pregnancy were excluded from vaccination, in order to avoid side effects at last stage of pregnancy; then vaccinated immediately after farrowing).
- Pregnancy protocol: vaccination of sows around 80th day of each pregnancy; about 35 days before expected farrowing.
- Post-partum protocol: vaccination of sows after each farrowing, about 3rd week of lactation

Different vaccination protocols and vaccination times are summarized in Table 1.

Times of vaccination plans and serological tests performed

Times of vaccinations and serological tests, vaccination plans executed, and category of vaccinated animals are summarized in Table 1.

Farms and animals

Six farms, 62 sows and 100 piglets were included in this study; in 5 farms 41 sows and 100 of their piglets (2 to 3 piglets/sow) were tested; in one farm only 21 sows were tested. Serological tests were started in September 2016.

Details are summarized in Table 2 below.

Table 1: Timetable of vaccination plans and serological tests; category of vaccinated animals; vaccination plans executed

Date	Event	Vaccinated animals; vaccination protocols executed
11-2015	FMD outbreak; mass/blanket vaccination	Breeders: priming + booster Piglets, growers: priming + booster
From 05-2016	Prophylactic vaccination	Breeders only; 3 different protocols: a. mass/blanket vaccination b. pregnancy vaccination c. post-partum vaccination
09-2016	Serological test of sows and piglets	Breeders only; different protocols as above
12-2016	Serological test of sows and piglets	Breeders only; different protocols as above
After 01-2017		A standardized vaccination protocol

Table 2: Farms; vaccination plans; vaccinations dates; samplings dates; category and number of sampled animals.

Farm	Vaccination plan in breeders	Last vaccination	Samplings date	Sampled animals	Number of pigs
L	Mass/blanket	05/2016	09/2016	sows	8
				piglets	*21*
N	Mass/blanket	09/2016	12/2016	sows	21
				piglets	*0*
F	Post-partum	05/2016	09/2016	sows	5
				piglets	*9*
F	Pregnancy (~80 days)	09/2016	11/2016*	sows	6
				piglets	*12*
R	Post-partum	05/2016	09/2016	sows	5
				piglets	*19*
R	Post-partum	09/2016	12/2016	sows	11
				piglets	*27*
Y	Pregnancy (~80 days)	09/2016	11/2016*	sows	6
				piglets	*12*
			Total	**sows**	**62**
				piglets	***100***

*sows were sampled around 60 days post last vaccination.

All the sows were blood-sampled in their 3rd lactation week; they were immobilized with a hog-snare and sampled by using a new 40-mm length needle for each sow from the right jugular vein by using a blind-stick approach to the vein. Two to three piglets from each sow, from 41 sows, were also blood-sampled on the same day; manually up-side down immobilized; a new 20 mm length needle was used for each piglet, on the right jugular vein or the vena cava, using the blind stick approach. Red-cap vacutainers (without anti-coagulant) were used for both animal categories.

Considering that following vaccination (or infection), serum antibodies are induced against the outer capsid structural proteins of the FMD virus, the blood-samples were examined in order to detect FMD Virus Neutralizing Antibodies titers (VNT). The examinations were carried out at the FMD Laboratory of the "Kimron" Veterinary Institute, Beit Dagan, Israel, according to literature and OIE recommendations (10, 11). The VNT measures the ability of a serum to neutralize a fixed dose of FMD virus, preventing its cytopathic effect (CPE) in susceptible cell cultures.

Final VNT titers were calculated as the reciprocal of the last serum dilution neutralizing the CPE of 100 TCID50 of the reference FMD virus strain O1-Gheshur. VNT were classified as protective/non protective (12), according to Table 3 below.

Table 3: Classification of VNT titers (from 12; modified)

Dilution	Log_{10}	Classification
<1:32	<1.5	Negative/non protective
=1:32	=1.5	Weakly positive
≥1:45	≥1.65	Strongly positive
Protection probability	**1.1 – 1.7 Log_{10} = 50%**	**2.1 Log_{10} = 95%**

Data were analyzed using Excel Data Analysis ToolPak, 2013. Student's t-Test has been used to compare VNT, between pigs groups, resulting from different vaccination plans; and to compare between VNT in sows and MDA-VNT in their pigles. A probability value (P) of ≤0.05 was considered statistically significant.

RESULTS

Cumulative serological results in sows and in piglets

Cumulative average VNT in all examined sows was 1:31 (Standard Deviation (SD) ±67); 1.49Log_{10}; only 16 sows (25.8%) developed a ≥1.5Log_{10} titer. Cumulative VNT-MDA in all examined piglets was 1:23 (SD±32); 1.36Log_{10}; only 33 piglets (33%) developed a ≥1.5 Log_{10} titer. These data define the overall sow population, examined 2 to 6 months from last vaccination, as poorly protected (12) and as a consequence, suckling piglets population were equally inadequately protected (12), according to their VNT-MDA. When we excluded from the comparison the sows of farm "N" (see Table 2 above) from which no piglets were tested, there was no significant difference between VNT titer in sows and their piglets (t-TEST, P=0,072).

Cumulative results of serological test in sows and piglets are summarized in Table 4 below.

Serological results according to the different vaccination plans

VNT titers in sows and their piglets varied according to the vaccination plan executed in the sows; results and variations are summarized in Table 5.

Table 4: Cumulative results of serological tests in all sows and piglets.

Category	Number	VNT, average	VNT, Log_{10}	Number & (%) with ≥ 1.5Log_{10}	TTest
All sows	62	1:31	1.49	16 (25,8%)	
Sows excl. N	41	1:46	**1.67**	11 (26,8%)	**NS**
All piglets	100	1:23	**1.36**	33 (33,0%)	**P=0.072**

Table 5: Serological results in sows and in their piglets, according to vaccination plans executed in sows. For sows (N) no piglets were tested.

Category & (farm)	Animals, number	VNT, average	VNT, Log_{10}	Number & (%) with ≥ 1.5Log_{10}	T-Test
Pregnancy vaccination					
Sows (Y,F)	12	1:119	**2.08**	9 (75%)	**NS P=0.43**
Piglets (Y,F)	24	1:112	**2.05**	18 (75%)	
Mass/blanket Vaccination					
Sows (N)	21	1:1.3	0.11	0 (0%)	not relevant
Sows (L)	8	1:35	**1.54**	5 (62.5%)	**NS P=0.47**
Piglets (L)	21	1:34	**1.53**	11 (52.38%)	
Post-partum vaccination					
Sows (F,R)	21	1:10	**0.99**	2 (9.52%)	**NS P=0.26**
Piglets (F,R)	55	1:19	**0.97**	4 (7.27%)	

Pregnancy vaccination

The highest VNT was obtained in sows vaccinated during pregnancy and examined around 60 days after last vaccination: VNT = 1:119; 2,08Log_{10}; 75% of sows ≥1.5Log_{10}; and in their piglets: VNT-MDA = 1:112; 2,05Log_{10}; 75% of piglets ≥1.5Log_{10}. There was no significant (NS) difference between VNT in sows and in their piglets (t-TEST, P=0.43).

Mass/blanket vaccination

When sows were vaccinated at a fixed date every six months, irrespectively of reproduction phase, and they were tested after six months and immediately before next vaccination (like farms "L" and "N" in Table 1), VNT resulted 1:10 in average; 1.02 Log_{10}; 17% of sows ≥1.5Log_{10}.

Excluding the sows from farm "N" (piglets not tested), fixed date biannual vaccination in sows of farm L resulted in an average VNT of 1:35; 1.54 Log_{10}; 62.5% of sows ≥1.5 Log_{10}; and in their piglets: average VNT-MDA of 1:34; 1.53Log_{10}; 52.38% of piglets ≥1.5Log_{10}. There was no significant difference between VNT in sows and in their piglets (t-TEST, P=0.47) in farm L.

Post-partum vaccination

Post-partum biannual vaccination of sows, executed around 3rd week of lactation, resulted in an average VNT of 1:10; 0.99 Log_{10} (with slight differences between the two farms "F" and "R"); 9.52% of sows ≥1.5 Log_{10}; and in their piglets: average VNT-MDA of 1:9; 0.97 Log_{10}; 7.27% of piglets ≥1.5 Log_{10}. Again, there was no significant difference between VNT in sows and in their piglets (t-TEST, P=0.26).

Following the vaccination carried out on May 2016 and as a consequence of the results obtained on September 2016, on farm "F" it was decided to change the vaccination plan from "post-partum" (Table 1) to "pregnancy" (Table 5). At the next serological test, performed around 60 days after vaccination, average VNT increased significantly to 1:93; 1.97 Log_{10} in sows; 66.7% of sows ≥1.5 Log_{10}; and in piglets: an average VNT of 1:50; 1.69 Log_{10}; 66,7% of piglets ≥1.5 Log_{10} (Table 6). There were no statistical differences between sows VNT and their piglets VNT-MDA in farm Y (t-TEST, P=0.7) and F (t-TEST, P=0.26), both farms adopting the pregnancy vaccination scheme; neither statistical differences were observed between sows VNT of the two farms (Y,F), (t-TEST, P=0.22). A statistical difference (P=0.003) was found between the VNT-MDA of the two groups of piglets (Y,F) with higher VNT-MDA in piglets from farm Y. (Table 6 below).

We speculate that this difference may have been generated by the fact that farm Y, after the mass vaccination plan of September 2015 (FMD outbreak), immediately adopted the pregnancy vaccination plan, as opposed to farm F (see Table 2). Consistency and continuity of vaccinations during pregnancy in farm Y, after priming and booster, achieved the 95% protection-probability VNT titers in sows and piglets (≥2.1Log_{10}) in ≥80% population as recommended by FAO-OIE (12).

DISCUSSION

In September 2016, following the outbreak of FMD, 3 situations were assumed:

I. All the breeding sows already received at least one full vaccination course (priming + booster) in November 2015 and then a third vaccination starting May 2016

Table 6: Results among farms Y and F adopting the pregnancy vaccination plan

Category & farm	Number, animals	VNT, average	VNT, StDev	VNT, Log_{10}	Number & (%) with ≥ 1.5Log_{10}	TTest
Sows Y	6	1:145	±131	**2.16**	5 (83.3%)	**P=0.22**
Sows F	6	1:93	±74	**1.97**	4 (66.7%)	
Piglets Y	12	1:175	±156	**2.24**	10 (83.3%)	**P=0.003**
Piglets F	12	1:50	±43	**1.69**	8 (66.7%)	

according to one of the vaccination protocols as in Table 1.

Therefore, we did not consider a "time 0" blood sampling for serological tests

II. Rather, we considered that a serological test, at a fixed time, would reflect the immunological situation achieved in breeders populations at any time, and then could be compared with the literature and OIE recommendations (3, 7, 12, 13, 14, 15, 17): That are:
   - target population coverage of at least 80%
   - target protective Virus Neutralizing antibody dilution Titer (VNT) no less than 1:32; 1.5 Log_{10}

III. Dissimilar vaccination protocols in sows would affect the VNT-MDA in piglets in a different manner, thereby affecting their immunological status towards FMD vaccinations (3,7,13)

Overall results of the different vaccination protocols in sows and piglets would suggest the best vaccination plan to be adopted (12, 14). It should be emphasized that reduction in virus shedding in pigs, after infection, correlates to VNT (16).

As mentioned above, the purpose of this work was not to organize a field sero-conversion trial in pigs relative to FMD vaccination, but rather to verify the status of population coverage following an emergency mass vaccination (priming and booster) followed by at least, two more vaccinations, 6 and 12 months apart.

Confirming results from earlier studies (7, 13), we found that VNT-MDA levels in piglets depended very strongly on the antibody titer in the sow. No significant differences were found between VNT in sows and VNT-MDA in their piglets within each vaccination group/protocol.

Concerning sampling times in sows, we have to point out that sows in the "pregnancy vaccination" groups had been sampled earlier (around 60 days after last vaccination) than other sows vaccinated 4 to 6 months before. Earlier sampling might have affected serological results and this was expected. However, we must stress that VNT-MDA in piglets is associated with VNT in sows at farrowing (assuming an adequate colostrum intake by piglets), and that the aim of FMD vaccination in a pig population is to obtain the highest possible immunity level in the widest possible population, including piglets (VNT-MDA) as a result of sow vaccination.

The "mass/blanket vaccination" plan, performed every 6 months, in farms L and N, achieved lower VNT and VNT-MDA than in the "pregnancy vaccination" plan, with an estimate protection-probability = 50% (12) only, evaluated around 6 months since the last vaccination in sows and in their suckling piglets. In a field situation, it may be more acceptable and easy to vaccinate sows one or twice per year with a "mass/blanket vaccination", but it will probably result in a higher variation of VNT-MDA titers in the piglets (13) compared to a "pregnancy vaccination" protocol. Again, we stress the concept of piglets protection, via VNT-MDA, as part of population protection target.

In farm N, "mass/blanket" vaccination resulted in extremely low VNT: 0,11Log_{10} measured 6 months after the last vaccination and after previous vaccination courses (Table 2). In such a situation, mistakes in vaccination can be assumed (e.g. vaccination needle-length; or site of injection) and, if necessary, corrections in vaccination techniques should be introduced immediately.

The "post-partum vaccination" plan carried out in sows does not represent any advantage for piglet protection: VNT-MDA of 0.97 Log_{10} in piglets; protection probability less than 50%; with 7.27% of piglets ≥1.5 Log_{10}. This VNT is the result of vaccination completed in sows 6 months earlier, immediately after previous farrowing. It may be the most comfortable vaccination schedule for farmers and/or veterinarians, due to the relative immobilization of sows in the farrowing crates, but it gave the worst results in terms of expected protection of piglets, as well as being apparently without any advantage to sow immunity.

CONCLUSIONS

When adopting FMD prophylactic vaccination plans in pigs, based on periodic vaccination of "breeders only", it should be taken into account how passive immunity/MDA in piglets contributes to the immunity of the overall population (12); therefore, vaccination plans must also aim to increase VNT-MDA toward piglet protection. Young pigs develop poor immunity to FMD vaccines; their protection in endemic areas depends on proper vaccination of the sow (5).

The outbreak of November 2015 confirmed the fragility of the "breeders only – once a year only" vaccination scheme and how direct losses induced by FMD in unvaccinated/unprotected pig farms were mainly concentrated in piglets (5, 6, 7) (apart from economic damages induced by temporary quarantine). Even in the absence of information on direct correlation between serology results and protection, the in-

Table 7: Decision process

Vaccination policies	Objectives	Means	Plan
vaccine to live; breeders only	widest population coverage; containment of losses	protective immunity in breeders and piglets	compulsory vaccination of pregnant breeders*

* in boars: vaccination twice a year.

terpretation of the serological response should be consistent with expected immunity targets relative to the disease and the animal population involved (12): for example achieving the highest immunological response (12, 15) in the majority of pigs, with a target of 80% of the population (7, 17). Furthermore, the presence of individuals, or entire farms, with low levels of antibodies, facilitate the introduction and maintenance of FMDV in the population.

Specific farming systems, like production flow in pigs, require different vaccination plans to be adopted (18) with respect to other susceptible livestock. Unlike cows, in conventional pig herds, farrowings occur all year round; therefore, the purpose is to minimize losses by ensuring highest VNT also in piglets (17, 19). In this case, vaccination must be carried out on breeders (5, 18), when pregnant (19), and is more likely to be delegated to the farmer (18) an/or to the practitioner of the pig unit on continuous flow.

Israel adopted in pigs a "*no eradication*", "*vaccine to live*" (5) *and* "*breeders only vaccination*" (4) policy. In such a situation, a logical decision process should be strictly implemented: highest population immunity/protection *and* containment of losses; achieved by ensuring that breeders *and* piglets together acquire the highest VNT achievable (Table 7).

If FMD has seasonal patterns in certain farming systems or species (3, 4), vaccination should be scheduled accordingly (18), but specificities of all susceptible farming systems must be taken into account, especially in pigs (5, 17, 18). Therefore, according to this survey, the "pregnancy vaccination" plan in sows should be the one to be implemented (18, 19) in Israel, unless different data are provided.

VNT-MDA in piglets are assumed to remain at protective level until 60 to 90 days of age (12, 13); in case of an FMD outbreak this will contribute in containing the high losses expected in unprotected piglets (6). In case of an FMD outbreak, an emergency/mass vaccination, should immediately start in piglets, from 8 (13) to 10-12 weeks (5, 17) of age; then repeated in 2 weeks (5) and/or according to the vaccine of choice. Sows, lactating sows and suckling piglets may be considered as protected because of the "pregnancy vaccination" schedule, if regularly performed twice a year in line with reproductive cycle. It should also be considered that depending on the formulation used, conventional adjuvant oil vaccines in pigs may require a single injection (5) to promote a protective immunity, starting approximately after 8 days and lasting for about 6 months.

At the best of the knowledge of the authors, this is the first report dealing with immunological results following FMD vaccination in pigs in Israel. Results obtained were in line with experimental literature data (13, 18, 19), previous Pirbright Reference Laboratory data (20), and with expected targets recommended by FAO-OIE (12, 18, 19).

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