Clinical Description of an Outbreak of Foot and Mouth Disease in a Pig Close-Cycle Unit

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ABSTRACT

In November 2015, a Foot and Mouth Disease (FMD) outbreak occurred in a farrowing unit of a farrow to finish pig unit of 350 breeders, caused by FMD virus Type O. The outbreak caused mortality of 59% of piglets and of two sows. Sows totaled two deaths, two were euthanized and nine were sent to the slaughterhouse for urgent slaughtering, constituting a total 4% losses, as a consequence of the disease. Direct losses after weaning, growing/fattening units were negligible (2.5% in total) in terms of mortality, but mainly caused as a result of delays in slaughter of about 40 days, due to movements restrictions. The FMD outbreak was probably the result of the uncontrolled passage of infected small ruminants coming from Palestinian Authority Territories, in which the same Type O was identified about 10 days before.

Keywords: Foot and Mouth Disease; FMD; Pigs; Vesicles; Mortality; Vaccination.

INTRODUCTION

Foot and Mouth Disease (FMD) is a feverish, clinically acute, highly contagious disease of cloven hoofed both domesticated and wild animals (swine, ruminant) (1). FMD is listed in the Terrestrial Animal Health Code of the World Organization for Animal Health (OIE) (2) to which outbreaks are reported, and for which diseases the OIE establishes an official list of disease-free countries and procedures for which countries and/or zones can be recognized as free. Israel is endemic for FMD, with several outbreaks per year in ruminants, however there are no records relating to FMD outbreaks in pigs in Israel from the data on OIE (2).

FMD is caused by a virus belonging to Picornavirus family, Aphtovirus genus, of which seven immunological types are recognized: O, A, C, SAT1, SAT2, SAT3, and ASIA (1, 3), with no cross-protection between them. Several tens of sub-types are known with variable degrees of heterologous protection (4).

Clinical lesions in pigs are represented by vesicular lesions on the snout, tongue, teats, coronary band and ventral aspect of the hoofs. These lesions may be quite easily accessible. More difficult to identify are lesions on the prepuce of males. Vesicles and lesions are accompanied by high fever, lameness, "sitting-dog" posture (1), reluctance to move because of pain, and/or to eat, because of tongue lesions. Vesicles break in 4-5 days; the damaged epithelium tears away; then the damaged area is covered in few days by fibrinous exudate which heals in about two weeks. Mortality is low in fattening pigs and breeders (1 to 3%); but it is particularly high in suckling piglets and weaned piglets up to 8 weeks of age as a result of high fever and acute myocarditis. Myocarditis lesions are characteristic in piglets (white-greyish punctuations or necrotic myocardial tissue streaks), but not always present and/or noticeable at gross-pathology. High fever (up to 42°C) is probably the cause of abortion in pregnant breeders (3).

This article describes the clinical evolution of an FMD outbreak in a close-cycle pig unit in the Northern Region of Israel.

MATERIAL AND METHODS

Farm

A farrow to finish (close-cycle) pig farm of 300 sows and about 50 pregnant gilts, of which 66 were post-partum lactating sows and 12 were pre-partum (already introduced in the farrowing units, as usually practiced 5 to 7 days before expected farrowing date); about 620 weaned piglets and 820 growing-fattening pigs and 6 boars for artificial insemination (AI).

The FMD outbreak developed in the farrowing units of the farm. Farrowing units were divided into different rooms of 8 sows/farrowing pens each, rooms being adjacent to each other with shared corridors. The farm was located in the Northern Region of Israel, about 3.5 kms from the border with Lebanon. The farm was organized into different buildings, separated between them by a local-traffic route (Figure 1). The farm was characterized by a lack of any bio-security preventive measures. Different buildings were badly fenced with their gates almost always open. The local route served as a traffic route also for grazing sheep and goat flocks and beef in the surrounding areas. The area was populated by wild boars and contacts with them may have been possible, as demonstrated by an outbreak of Classical Swine Fever (CSF) which occurred in the past, during which wild boars found dead in the surrounding of the farms also resulting in positive cases to classical swine fever (CSF) (5, 6).

Clinical description

On the 19th November 2015, the Veterinary Services received a phone-report from the farm Veterinarian concerning clinical evidence in a farrowing unit: a sudden sharp increase of mortality in suckling piglets; fever in sows (>41°C); sows showing reluctance to stand up; the death of one sow; the presence of vesicles on the snout of one sow and lesions on hoofs of a dead sow. Nothing suspicious was reported from other units of the farm from any of the areas.

On the next day the Veterinary Services personnel from Central office and local Akko District visited the farm. Some sows in this unit presented with vesicular lesions at the snouts, teats, hoofs, possibly representing different developmental stages (Figure 2).

One sow in the farrowing unit presented with a uterine prolapse already in an advanced stage which was apparently irreducible. Considering animal welfare implications and due

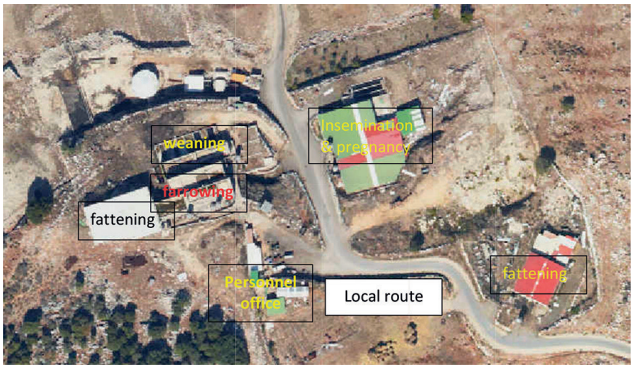


Figure 1: Farm organization and surroundings.
A local traffic route crosses through different buildings of the farm.

to the clinical situation, it was decided on euthanasia and at the same time to collect biological material to be sent to the diagnostic laboratory. The sow presented with vesicular lesions in an advanced stage on the snout, tongue and hoofs (Figure 2).

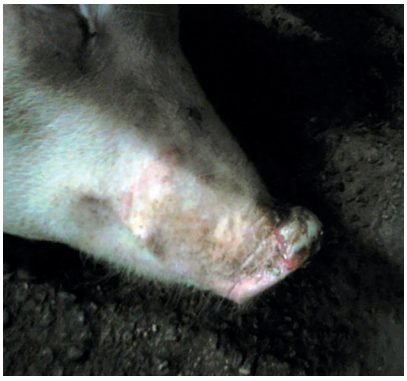
On the basis of the clinical signs in sows with fever and high mortality in piglets, without other specific clinical signs, the suspicion of FMD arose. The farm was immediately quarantined; an infected area declared (3 km radius); animal movement and transportation prohibited in the area of a 3 km radius, until confirmation from the laboratory.

Since the first visit to the farm the clinical situation deteriorated: on the same day in morning 73 piglets were found dead. In the following 9 weeks (64 days) the outbreak involved 66 farrowings with mortality of over 400 piglets in less than one month (Graph 1).

Sample Collection and Laboratory Investigations

After immobilization using a hog-snare, double blood samplings were carried out (with and without EDTA) from 3 sows with clinical symptoms, using vacutainer tubes and a fresh needle for each sow, from the right-side jugular vein. Snout vesicles from the above mentioned sows were swabbed and tongue epithelium samples from a dead sow was also collected. Two dead piglets and 4 hearts from other dead piglets, locally examined for gross-pathology, and samples were also collected for laboratory investigation.

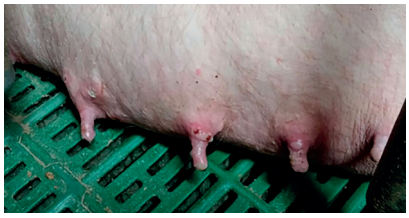
All the samples were submitted to Central FMD Laboratory of Kimron Veterinary Institute, Beit Dagan, Israel, for FMD virus identification, type identification, isolation and for the demonstration of antibodies.

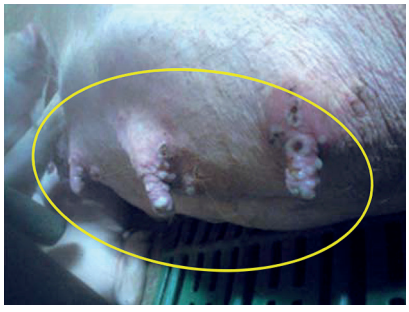


Snout: lesions estimated at day 1st-2nd

Snout: lesions estimated at day 3rd-4th

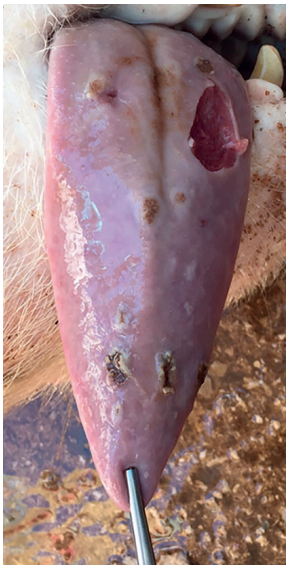
Snout: lesions estimated at day 5th-6th





Teat: lesions estimated at day 1st-2nd

Hoofs: lesions estimated at day 5th-6th (euthanized sow)



Tongue: lesions estimated at day 5th-6th (euthanized sow) (tissue from one vesicle has been removed for Laboratory test)

Figure 2: Vesicular lesions in sows with estimated stages of development

FMD Virus Identification

- FMD virus nucleic acid identification: FMD Type O identification was carried out using Polymerase Chain Reaction (PCR) and Reverse Transcription (RT)-PCR methods according to the OIE Manual for Terrestrial Animals 2017; chapter 2.1.8 B:1.3 and B:1.3.4) (7).
- FMD antigen identification was performed using a monoclonal Antigen Elisa test according to OIE Manual for Terrestrial Animals 2017; chapter 2.1.8 B:1.2.1) (7).
- FMD Type O identification was carried out with an ELISA monoclonal competitive antibody kit (IZS-LER kit;) (Istituto Zooprofilattico Sperimentale Lombardia ed Emila Romagna "IZS-LER", Via Bianchi 7, Brescia, Italy); (according to OIE Manual for Terrestrial Animals 2017; chapter 2.1.8 B: 2.2) (7).
- FMD antigen detection and serotyping was performed using an ELISA (according to "Partnership ELISA" kits with IZS-LER and The Pirbright Institute). (https://www.pirbright.ac.uk/diagnostic-development-team-products/foot-and-mouth-disease-reagents# panel-6445)

Differential antibody test

Non-Structural Protein (NSP) antibody titration (3,4) was executed with the Anigen Rapid FMD NSP Ab Test

Kit, BioNote (Kr) (according to the guidelines provided by manufacturer; BioNote Inc., Republic of Korea; www.bionote.co.kr).

FMD virus isolation

Identification of cytopathic effects were established using the cells-line IB-RS-2 from adult pig kidney cells tissue cultures (according to OIE Manual for Terrestrial Animals 2017; chapter 2.1.8 B: 1.1) (7).

All the above mentioned investigations were conducted at FMD Laboratory, Kimron Veterinary Institute, Beit Dagan, Israel, and according to OIE procedures (7). Isolates from the outbreak were then submitted for phylogenetic lineage determination to The Pirbright Institute, UK. (https://www.wrlfmd.org/western-and-central-asia/israel#panel-3542)

RESULTS

On the following day from samples collection, the Laboratory tests confirmed the presence of FMD virus Type O. Samples used, test methods, reference reagents and results are summarized in Table 1 below.

Phylogenetic sequence positioned the FMD isolates in the: Type O; topotype ME-SA; PanAsia viral lineage (Figure 2); it results correlated to Far East FMD isolates (7).

Following the FMD virus confirmation, a protection area of 10 km radius was immediately added with restrictions on animal movements.

Interventions in the farm

Considering the clinical signs and the type of lesions and furthermore taking into account that neither Vesicular Disease (Picornavirus; Enterovirus) nor Vesicular Stomatitis (Rhabdovirus; Vesiculovirus) were ever reported in Israel and that Israel is endemic for FMD, it was decided to start immediately a mass/blanket vaccination of the farm. This was carried out despite the lack of Laboratory results, which would anyway have arrived the following day.

A mineral double oil emulsion vaccine, trivalent (Types O, A, Asia 1), (Aftopor; Merial SAS, 29, av. T. Garnier F-69 007 Lyon, France) (containing the antigens O Manisa, O 3039, O Israel 85, O Pan Asia 2, A Saudi 95, A Iran 05, Asia 1) already available at Veterinary Services inventory and already in use in cattle was used for the pig farm vaccination. Pigs were vaccinated twice, 3 to 4 weeks apart, at dosage of 2 ml, intra-muscle application. The whole farm population was vaccinated: boars, gilts, sows; suckling piglets, weaned piglets, growers and fatteners.

At this time, the outbreak was apparently limited to the farrowing units, therefore indications were given for a division between personnel in charge of the farrowing unit and personnel in charge of rest of the farm. Both disinfection and protective clothing/dressing/undressing points were instituted at the entrance of the farm; both disinfection at the entrance of different units; a disinfection point for vehicles entering/leaving the farm (e.g. feed trucks) and a 24/7 surveilled road block was instituted. A cremation pit for dead animals was established within the farm perimeter, with prohibition to move dead animals outside the farm. Disposable protective clothes used for visits were also locally disposed of in the same pit.

Table 1: Samples, tests, reference reagents used and results

Samples	*blood; vesicles fluids*	*blood; vesicles fluids*	*virus isolate from hearts*	*epithelian suspension; vesicular fluids*	*blood*	*blood*
Tests and reference reagents	RT-PCR	PCR	Inoculation on swine kidney cells culture (IB-RS-2 line)	ELISA Antigen (Pirbright IZS-LER kit – UK)	ELISA Monoclonal Antibodies (IZS-LER-I)	NSP Differential Antibody (Anigen BioNote – Kr)
1st run	RT-PCR against all Types	PCR against Type O	evaluation of cytopathic effect	against all Types	against Type O	
Result	**Positive**	**Positive**	**Positive**	**Positive to Type O**	**Positive to Type O**	Negative*
2nd run	RT-PCR against Type O					
Result	**Positive**					

* antibody response towards NSP requires at least 4 to 5 days following infection.

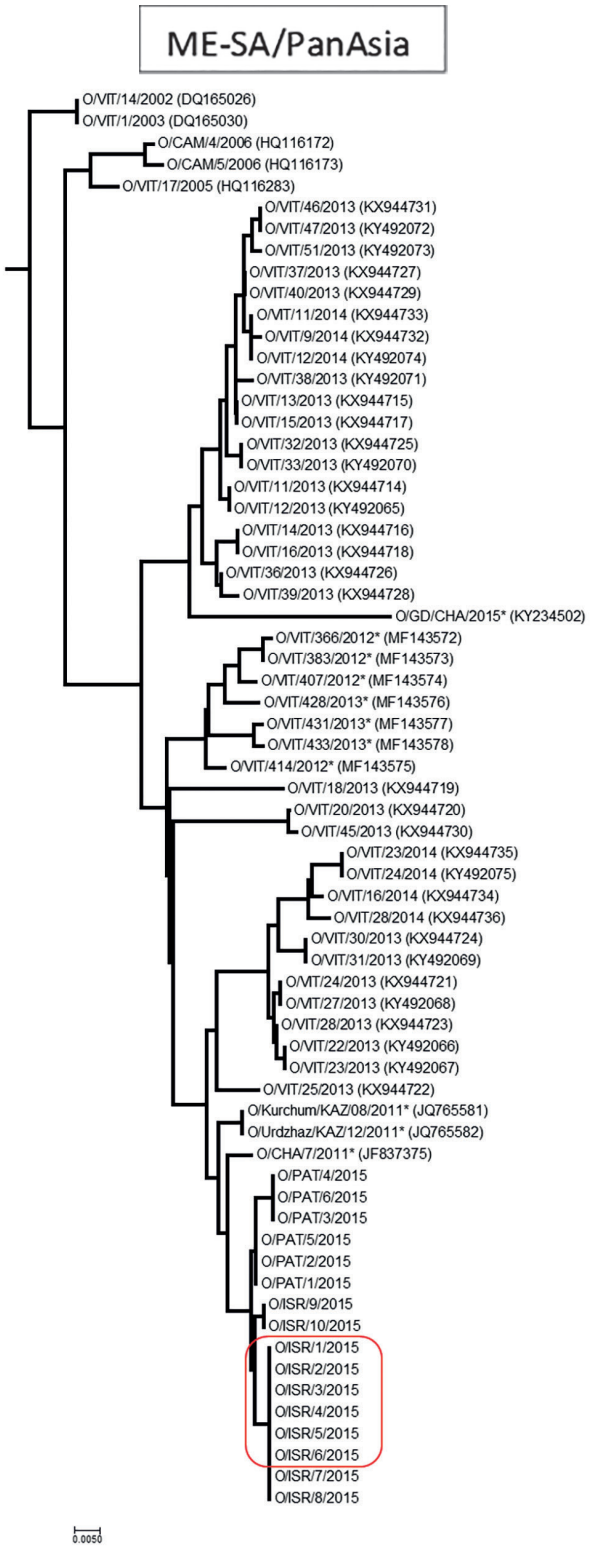
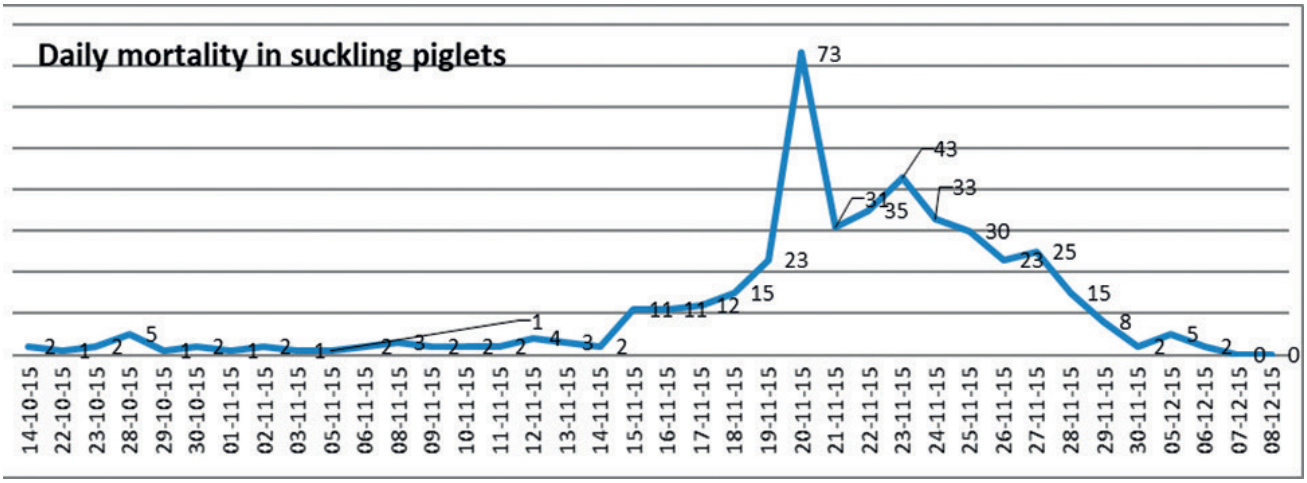


Figure 2: Lineage of FMD isolates.

Isolates from pig farm outbreak are framed in red and classified from O/ISR/1/2015 to O/ISR/6/2015. (FMD Virus isolated from piglets hearts).
O/ISR/9/2015 represents the isolate from cattle outbreak below mentioned;
Other isolates (7, 8) derived from FMD virus replications at Kimron Laboratory in course of the diagnostic process.



Graph 1: Suckling Piglets Mortality

Outbreak evolution:

Farrowing unit

The outbreak involved 66 farrowings in 49 days. In this period of time, suckling piglets mortality attributed to FMD virus reached 441 units (Graph 1).

The farm was organized as continuous flow, with farrowings almost every day (average 2 farrowings/day over a period of 7/week). This fact simplified describing mortality rate changes, and the sharp increase which started 16/11/2015. Peak mortality occurred on 20/11/2015. Evaluation of sows' lesions, estimated at their 5th-6th day of evolution on 20/11/2015, (Figure 2) allowed for speculation that outbreak started on 12 to 13/11/2015, at the earliest.

On 03/12/2015, the clinical situation appeared to stabilize (no dead piglets and no lesions on sows) in the farrowing units.

Growing and fattening units

On the 03/12/2015, a visit to the farm revealed that clinical symptoms appeared in one of the two fattening units: lameness in around 10% of fattening pigs; recumbence; reduction of daily feed intake and reluctance to get up and move. In spite of the lack of cleanliness, lesions at hoof coronary bands and of snouts of few pigs could be observed. It should be noted that at this time, the pigs had not yet received their booster vaccinations, which was given 3 to 4 weeks after the first vaccination, as above mentioned.

On 10/12/2015, no new clinical signs could be observed. Pigs returned to their normal activity and appetite despite a few pigs still suffering with lameness. In weaned piglets and growing/fattening units overall mortality remained limited: 33 pigs out of 621 (5.31%) and 3 out of 820 (0.36%), respectively. These mortalities were difficult to attribute specifically to FMD virus or to other causes.

Insemination and pregnancy unit

Eleven sows, previously exposed to FMD virus in the farrowing units, developed complications on their hoofs, which compromised a full recovery: One died and one was euthanized for animal welfare considerations; nine excluded from reproductive cycle (Figure 3) and sent to slaughter as soon as the farm was released from quarantine.

The total mortality and losses which were attributable to FMD virus are summarized in Table 2.

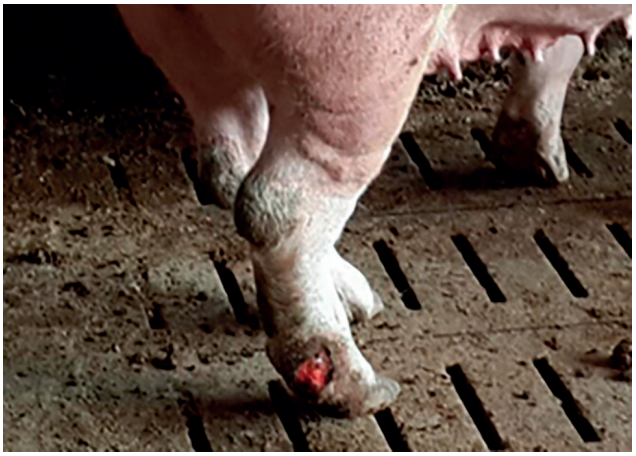
Contrarily to the literature, no abortions were recorded, which may indicate that the initial point of the outbreak was indeed in the farrowing units itself.

FMD outbreak was declared as resolved on 25/12/2015: 42 days after the estimated commencement; 35 days since the

Table 2: overall mortality and losses during FMD outbreak

Overall mortality and losses			
Category	**Dead/losses**	**Out of:**	**Percentage**
Stillbirth	93	835 total born	11,13%
Suckling piglets	441	742 livebirth	59,43%
Weaned	33	621	5,31%
Growers/fatteners	3	820	0,36%
Sows	13*	350	3,71%

* 2 dead sows; 2 euthanized; 9 wasting and sent to slaughterhouse



Lateral nail detachment; swelling

Cracks in the coronary band

Wasting, recumbent (euthanized)

Weight loss, wasting

Figure 3: compromised recovery in sows

beginning of vaccination plan and 15 days from the disappearance of fresh clinical signs. The first transportation of pigs to the slaughterhouse was permitted on 31/12/2015, 49 days after the estimated onset of the disease.

DISCUSSION

The FMD outbreak developed mainly in the farrowing unit; other units in the farm (pregnancy unit; weaners and growing/fattening pigs units) were only slightly affected. The early start of mass/blanket vaccination in the farm may have contributed to limit the spread of the outbreak, together with personnel movement limitations between different units. Economic damages in growing-fattening units were limited in terms of direct damages due to mortality (2.5% overall). The quarantine prolonged the permanence in the farm of fattening pigs of around 40 days (+22% with respect to regular growing time); this delay contributed to the economic costs suffered by the farm in addition to the direct mortality.

Israel undergoes FMD outbreaks almost every year in ruminants (8, 9). There is no eradication policy in Israel. Endemic nature of FMD in Israel, the proximity between farms with susceptible animals, movements of sheep, goats and beef cattle on account of grazing practices, necessitate regular vaccination campaigns organized by the Veterinary Services. Swine breeders are also included in these vaccination campaigns. Similar to ruminants, in swine breeders a mass/blanket vaccination is carried out once a year only, generally in autumn/early winter, just before the season which

is considered a risk for outbreaks (10, 11). The farm under consideration was vaccinated in November 2014. It appears that FMD vaccination strategies for swine in Israel may be deficient: either according to the time table (seasonality of vaccination), modality (mass/carpet vaccination), frequency (single yearly intervention) or category of vaccinated animals (breeders only).

It is therefore proposed to modify the vaccination plan against FMD in the swine populations and suggested to adopt the following:

- Basic vaccination (priming and booster) in young breeders (gilts; young boars);
- Biannual vaccination in boars;
- Biannual vaccination in pregnant breeders, around 30-35 days before farrowing (80 days pregnancy).

This vaccination plan would guarantee:

- More uniform coverage in vaccinated animals (all the breeders) (1, 3, 12)
- Passive protection of piglets, due to maternal derived antibody (MDA) titers (1, 2, 4, 12) which would contributes to population immunity (12) till 8 to 12 weeks of age (according to vaccines of choice).
- An acceptable level of protection at any given time of the year against FMD virus (4, 12).

In the case of a FMD outbreak, growing/fattening population of pigs (10 to 26 weeks of age) would remain at risk, but with less direct economic implications due to lower levels of mortality/losses (1,3). This population should be promptly vaccinated either with a mono-valent vaccine relative to the type involved or, again, with a ready-to-use vaccine in the inventory.

Origin of the outbreak

On 12/11/2015, Israel veterinary Services were informed about a mortality outbreak in a goat flock of 450 goats and 150 kids in the Nablus area (Palestinian Authority territories) (PA), with symptoms attributable to FMD. Biological samples were forwarded to the FMD Laboratory of the Kimron Veterinary Institute. On 16/11/2015, a FMD virus Type O was identified and isolated. Lineage of the isolate from the goat farm, performed at Pirbright Institute (UK), was shown to be close to that of the pig farm isolate (Figure 2: O/PAT/ isolates). On 22/11/2015, in a grazing beef cattle farm of 43 cows, 4.5 km far from the described pig farm, two bulls and 20 calves were suspected with FMD of which two clinical cases of FMD were confirmed in calves. The isolated FMD virus, indicated as O/ISR/9/2015 in the Lineage (Figure 2) was again, close to same FMD Type O isolated from goats and pig farms outbreaks. Therefore it appears that the FMD virus isolate from the goat farm was the first in the outbreak chain.

It was not possible to identify the entry source of FMD virus into the pig farm, but the presence of grazing livestock in the area surrounding the farm, the concurrency of an FMD outbreak in goats in the PA territories, reinforces the suspicion of uncontrolled movements of animals through the border between Israel and the PA territories.

CONCLUSIONS

To the best knowledge of the authors this is the first clinical description of a FMD outbreak in pigs in Israel. The outbreak developed mainly in farrowing units, and it was responsible of heavy losses caused by high mortality in suckling piglets. High mortality in piglets was attributable to lack of immunological protection in piglets, which is strongly dependent from maternal derived antibodies (MDA) and the sows' immunity (1, 3, 4, 12). Damages and losses in other units (weaned piglets; growers; fatteners) were limited and mainly as a result of delays in slaughter caused by the quarantine on the farm, slaughter of few sows (2, 57%) and mortality and/ or euthanasia of others (1, 43%).

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REFERENCES

1. Alexandersen, S., Knowles, N., Dekker, A., Belsham, G., Zhang, Z. and Koenen, F.: "Picornaviruses", in Diseases of Swine", 10ma Ed., Zimmerman J., Karriker L., Ramirez A., Schwartz K., Stevenson G. Wiley & Sons, (Editors), Ames, Iowa, USA, pp. 587-620, 2012
2. OIE, Animal Disease Information, 2015 http://www.oie.int/fileadmin/Home/eng/Media_Center/docs/pdf/Disease_cards/Q_A-FMD-EN.pdf
3. Cavirani, S.: "Malattie Vescicolari" in "Le Patologie del Maiale", 1a Ed. Point Veterinarie Italie Ed., Milano, Italia, p. 298-3131, 2013.
4. Ferrari, G., Paton, D., Duffy, S., Bartels, C. and Knight-Jones, T.: "Foot and mouth disease vaccination and post-vaccination monitoring – Guidelines", The Food and Agriculture Organization of the United Nations and the World Organization for Animal

Health: Metwally, S. and Münstermann, S. (Editors) Ch.3: 23,37; 2016.
5. David, D., Pozzi, P.S., Ozeri, R., Hadani, Y., Yadin, H., Schmeiser, S., Bashara, R., King, R. and Perl, S.: "An Outbreak of Classical Swine Fever in Israel", Isr. J. Vet. Med., 67: 225-231, 2012.
6. David D., Edri, N., Yakobson, B., Bombarov, V., King, R., Davidson, I., Pozzi P., Hadani, Y., Bellaiche, M., Schmeiser, S. and Perl, S.: Emergence of classical swine fever virus in Israel in 2009, The Vet. J. 190:146-149, 2011.
7. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2018; Ch.2.1.8 http://www.oie.int/standard-setting/terrestrial-manual/access-online/
8. OIE and FAO World Reference Laboratory for Foot-and-Mouth Disease The Pirbright Institute; OIE and FAO World Reference Laboratory for Foot-and-Mouth Disease https://www.wrlfmd.org/western-and-central-asia/israel#panel-3542
9. OIE: World Organization for Animal Health https://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail , update 31/12/2018
10. Israel Veterinary Services, Ministry of Agriculture, Yearly Report 2014-2015; https://www.moag.gov.il/DocLib/shnaton_vet_2014-2015.pdf
11. Galon, N., "FMD in Israel", at 40th General Session of the EuFMD , 22-24 April 2013, Rome, Italy, 2013; http://www.fao.org/fileadmin/user_upload/eufmd/40thGeneral_session_documents/40General_Session/App22_IsraelNGalon.pdf
12. Ferrari, G., Paton, D., Duffy, S., Bartels, C. and Knight-Jones, T.: "Foot and mouth disease vaccination and post-vaccination monitoring – Guidelines", The Food and Agriculture Organization of the United Nations and the World Organization for Animal Health; Metwally, S. and Münstermann, S. (Editors), Ch. 2:15,21; 2016.