Porcine Reproductive and Respiratory Virus European Type 1 (PRRSV1) in Swine Farms in Israel

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ABSTRACT

This communication summarizes the clinical findings and confirmatory laboratory results occurring during an outbreak of Porcine Reproductive and Respiratory Syndrome Virus, European type (PRRSV1), in swine farms in Israel. In 2017, an outbreak of PRRS Virus, American type (PRRSV2), had already occurred in Israel. In 2021, several pig farms in the Northern District of Israel (Galilee) were affected by the PRRSV1 type. Main clinical signs were typical of PRRS virus: premature farrowing, increase of stillbirth, high pre-weaning and post-weaning mortality. PRRSV1 was confirmed at the Animal Health Institute "IZSLER" of Brescia (Italy) through ELISA-antibody test and RT-PCR on blood samples from sows and piglets. Open Reading Fraction-7 (ORF-7) sequencing and genetic analysis of field isolate confirmed the presence of the European type of PRRSV. PRRSV control is based on use of type-specific vaccine as there is no cross protection between the European and American types, therefore, a PRRSV1 type modified live vaccine (MLV) was introduced. Identity sequencing of PRRSV1 isolates pointed to suspicion of an uncontrolled introduction of PRRSV1 infected material.

Keywords: PRRSV1; Premature Farrowing; Stillbirth; RT-PCR; Sequencing.

INTRODUCTION

Porcine Reproductive and Respiratory Syndrome (PRRS) is a viral disease characterized by clinical signs of the reproductive system (failures in breeding, abortions, stillbirths, premature farrowing) in gilts and sows, and respiratory disease in pigs of any age. PRRS virus (PRRSV) is an enveloped single stranded RNA-genome virus, genus Arterivirus, family Arteriviridae (1). Two genotypes are of PRRSV:

PRRSV1, European type; and PRRSV2, American type; both are largely spread throughout the world (1). Nucleotide sequence of type 1 and 2 differ around 44% (1), but intra-type nucleotides sequences may also vary up to 30% in type 1 and 20% in type 2, because of inherent errors in PRRSV-RNA transcription (2). Both inter-type and intra-type nucleotide variability affects the immunological response of pigs vaccinated against type 1 or 2 of PRRSV.

So far Israel has been considered affected only by PRRSV2 type, as in previous epidemiological investigations in the Northern District (Galilee) pigs farms in 2017 and described (3). This communication summarizes the clinical findings and the laboratory results during the first outbreak of PRRSV1 in swine farms in Israel.

MATERIALS AND METHODS

Farms and animals

Six different closed-cycle (farrow to finish) farms (A, B, C, D, E, F) were clinically affected and investigated. Farms were located in Ibblin Municipality area, Galilee, North Region of Israel, where almost all the swine farms of Israel were located. Three farms (B, E, F) reported both reproductive problems in breeders and wasting disease, high mortality in piglets. Three other farms (A,C,D) reported mainly clinical problems in piglets with wasting, respiratory disease, and lameness. The six farms belonged to a unique property, with some personnel moving farm to farm. Four of them (A,B,C,D) were localized in a single complex of farms, with shared service roads, sewer, feed-supplier, animals transportation trucks, etc. All these farms constituted therefore a unique epidemiological unit. As a consequence of PRSSV2 type outbreak in 2017, all the farms, breeders and piglets, were vaccinated with a PRRSV2 American type Modified Live Virus MLV (3).

Samples

From 6 farms, 34 sows' and 170 piglets' blood-sera samples, were collected and submitted for:

- ELISA-PRRSV antibody test (3,4).
- Porcine CircoVirus type 2 (PCV2) ELISA antibody test (4).
- *Mycoplasma hyopneumoniae* ELISA antibody test (4).
- *Actinobacillus pleuropneumoniae* ELISA Apx IV antibody test (4).
- Swine Influenza Virus (SIV) Hemagglutinin Inhibition (HI) antibody test; subtypes H1N1, H1N2, H3N2 (4)
- Real Time Polymerase Chain Reaction (RT-PCR), in pools; then sequencing and genetic analysis for PRRSV of Open Reading Frame 7 (ORF7) of Viral RNA according to methods described (3,4).
- Quantitative (qn)RT-PCR for PCV2, (from pools of blood) (4).

Farms were sampled as described in Table 1.

Four joint aspirates were collected from growing pigs with severe joint swellings and submitted for PCR for *Mycoplasma* spp and *Haemophilus parasuis* investigation (4, 5). Sera from these piglets were also submitted to serology and RT-PCR investigations as described above.

All the samples were submitted to the Istituto Zooprofilattico Sperimentale Lombardia Emilia Romagna (IZSLER) Animal Health Institute, Brescia, Italy, for the above mentioned investigations. It is not the purpose of this communication to describe the laboratory methods, for which we refer to in references 3, 4 and 5.

RESULTS

Clinical aspects and gross necropsy findings

Reproductive problems were reported in farms B, E and F, mainly consisting of premature farrowing (around one week before expected farrowing time), still-births and early mortality. As typical in premature farrowing in PRRS epidemics, a great number of dead piglets appeared still wrapped in their amniotic sacs, with typical soft and curled hoofs (3), as illustrated in Figure 1.

As in the PRRS of 2017, only few abortions were reported: 13 cases in farm E; premature farrowings were not separately registered, rather the stillbirth (direct consequence of premature farrowing) was enumerated. In farm E, 79 sows' mortality cases in 14 months out of 1,166 (average presence), amounted to 5.8% yearly, also transpired. All the examined farms reported wasting and respiratory problems in surviving weaned piglets and growers; lameness in weaned piglets and growers and serous arthritis with swollen joints, often ulcerated, are illustrated in Figure 2.

Gross necropsy of dead growers revealed interstitial broncho-pneumonia, and subacute fibrinous pleuritis (5), as illustrated in Figure 3. Typical cranio-ventral lesions induced by *Mycoplasma hyopneumoniae* were scarcely observed (data not quantified).

Table 1: Farms, sows and piglets sampling

Sampling		
Farm	**Piglets**	**Sows**
A	9	
B	7	3
C	12	
D	11	
E	22	7
F	16	4
	60	20
	33	
Total	170	34

Figure 1: Premature farrowing:
Dead piglets appear still wrapped in their amniotic sacs with typical soft and curled hoofs.

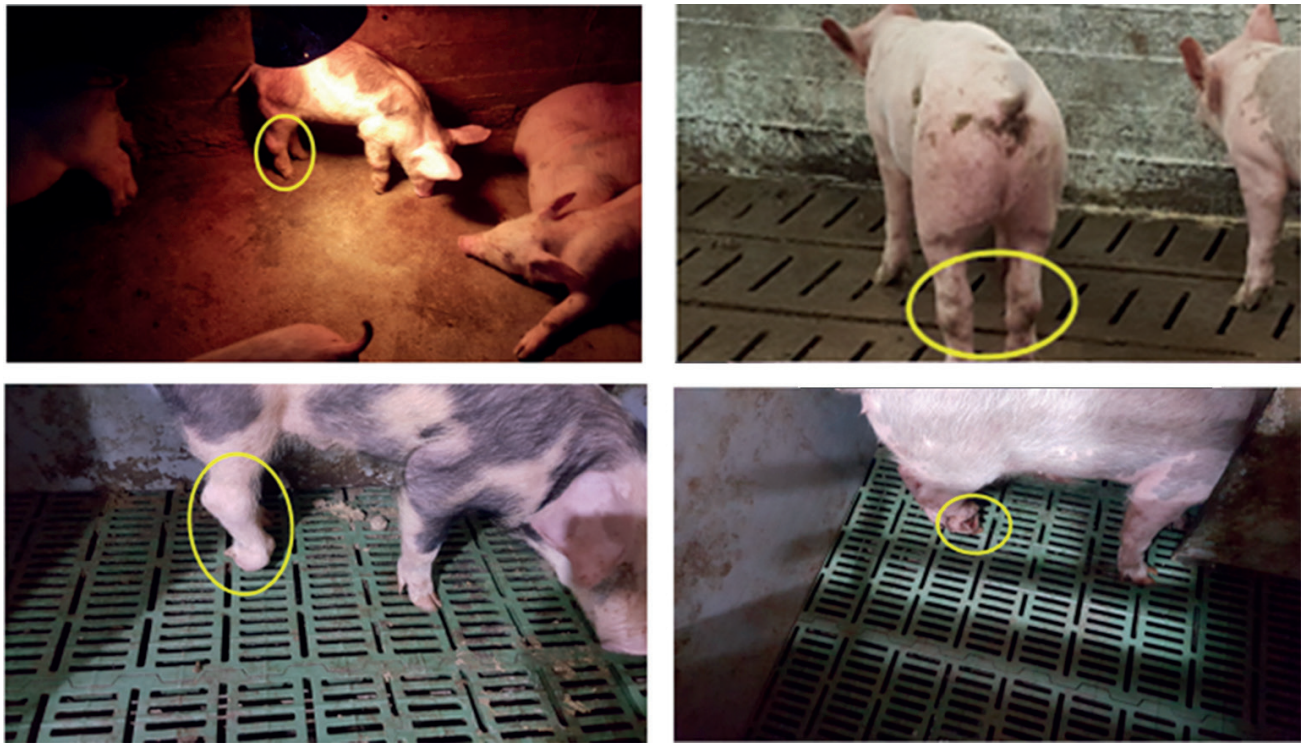


Figure 2: Swollen joints in weaned and growing pigs caused by *Mycoplasma hyorhinis* and *Haemophilus parasuis* arthritis.

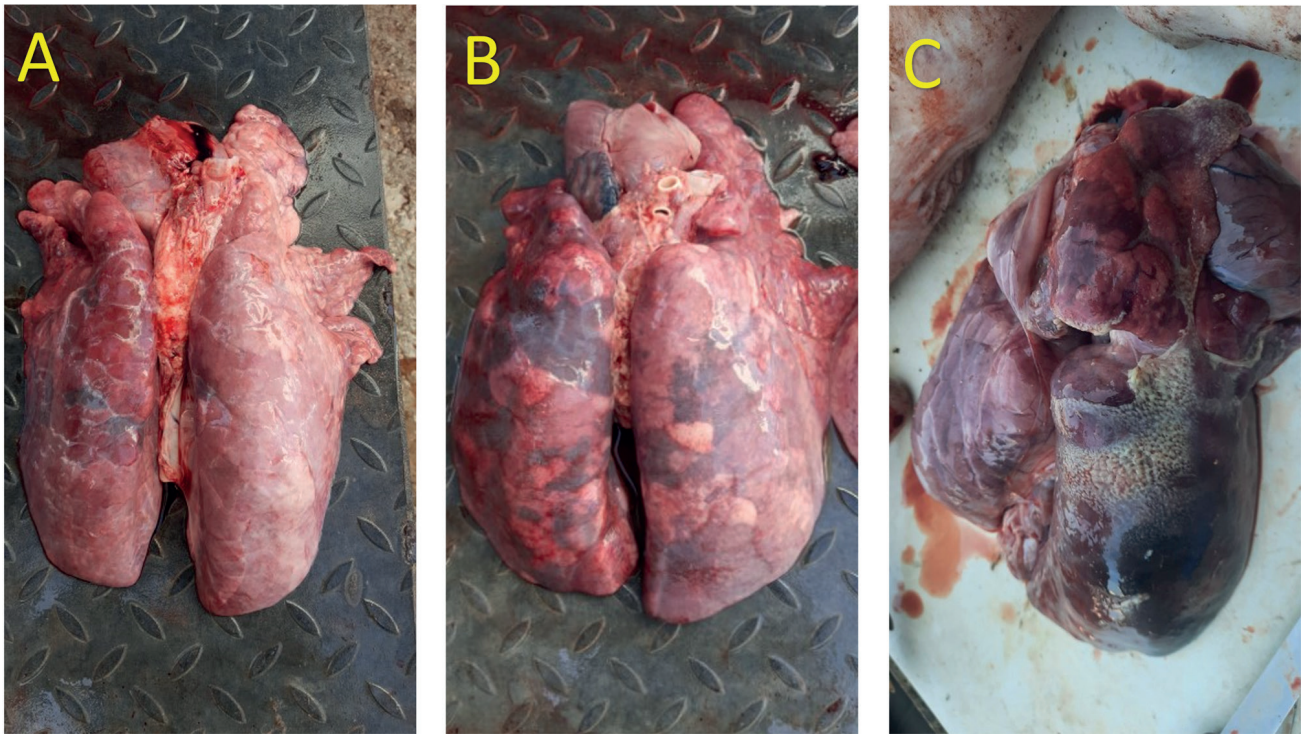


Figure 3: A, B: Interstitial bronco-pneumonia in growing pigs in course of the epidemic of PRRSV1 type.
C: fibrinous pleuritis and pneumonia, compatible/plausible with *Mycoplasma hyorhinis* and *Haemophilus parasuis* coinfection with PRRSV1 (5).

RT-PCR

Blood samples from sows on 3 farms, and piglets in 6 farms, were submitted for RT-PCR PRRSV. Thirty four sows' blood samples in 7 pools (3 to 7 blood sera each), and 170 piglets' blood sera in 23 pools (4 to 10 blood sera each) were examined. All 6 tested farms were found RT-PCR positive to PRRSV1: 2 pools out of 7 from sows in 2 farms; 14 pools, out of 23 (60.9%) from piglets in all the 6 examined farms (Table 2). Because PCV2 may be associated in wasting disease syndrome, respiratory problems with interstitial pneumonia in piglets (7), the 23 pools from 170 piglets' blood sera were also submitted for quantitative (qn) RT-PCR for PCV2. Only 2 pools from two farms resulted qnRT-PCR positive for PCV 2, with the farm B having a 2,000 viral load/ml and farm D a 128,000 viral load/ml (Table 3).

Table 2: Sows and piglets sampling in the examined farms; PRRSV RT-PCR results on pooled samples for each category of animals and each farm

Farm	sows	PRRSV RT-PCR pools		piglets	PRRSV RT-PCR pools	
		tested	positive		tested	positive
A				9	2	2
B	3	1	1	7	1	1
C				12	2	2
D				11	2	2
E	7	1	1	22	4	3
F	4	1	0	16	3	1
	20	4	0	60*	6	3
				33	3	0
Total	**34**	**7**	**2**	**170**	**23**	**14**

* young gilts

Sequencing and genetic analysis

Blast search of the sequences at GenBank for ORF7 revealed nucleotide identity of > 98% to strains belonging to PRRSV1 type in all the farms, both in sows and piglets.

Phylogenetic analysis of the obtained sequences compared to Netherlands/Lelystad NC 043487 PRRSV1 and USA/VR2332 PRRSV2 prototypes sequences as outgroup, retrieved from GenBank, confirmed the assignment of the Israeli sequences to PRRSV1 type group (Figure 4).

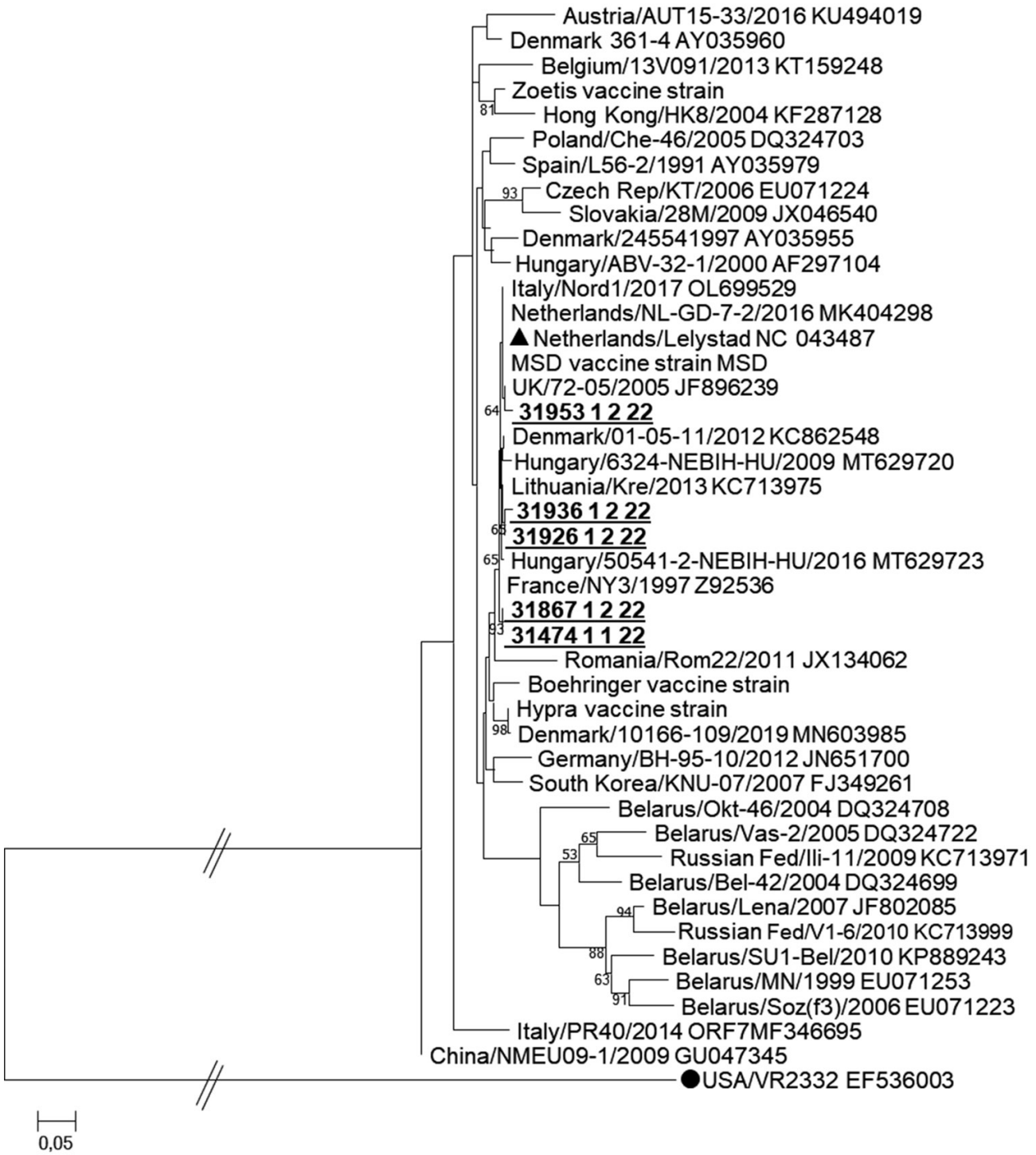


Figure 4: Phylogenetic tree ORF7 Israeli sequences (**in bold**) was constructed by Maximum Likelihood (ML) method using the best-fit model K2+G within MEGA X. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. Only bootstrap values higher than 50% are reported.
PRRSV strains of the present study are **underlined** and in **bold**.
PRRSV1 prototype sequence and PRRSV2 prototype sequences are used as outgroup.

Table 3: Serology and qnRT-PCR for PCV 2 results

Farm	Piglets;	Serology; positive results			RT-PCR	
	Total samples	App	M.hyo Elisa-Ab	SIV (3 subtypes)	PCV2	
		Elisa ApxIV-Ab		HI-Ab	tested pools	positive
A	9	3	2	0	2	0
B	7	4	3	0	1	1a
C	12	0	1	0	2	0
D	11	1	8	0	2	1b
E	22	20	11	0	4	0
F	16	14	0	0	3	0
	60	47	nt	0	6	0
	33	nr	nt	0	3	0
Total	**170**	**89**	**25**	**0**	**23**	**2**
Notes	nr	piglets < 10 weeks of age; passive antibodies; not relevant				
	nt	not tested				
	1a	2,000 RNA copies/ml; no clinical significance				
	1b	128,000 RNA copies/ml; no clinical significance				

Table 4: RT-PCR on joints aspirates and on blood sera

samples	RT-PCR on blood	RT-PCR on joint aspirates	
	PRRSV1	*M. hyorhinis*	*H. parasuis*
1	pos	pos	
2	pos	pos	
3	pos	pos	
4	pos	pos	pos
Total	**4**	**4**	**1**

Serology

All the 6 tested farms showed positive PRRSV-ELISA antibodies at various degrees: the PRRSV-ELISA antibody test used in this test does not differentiate between European and American types of PRRSV, therefore these data are of less relevance, and not presented in this communication (9).

All the 6 tested farms resulted negative to HI for SIV subtypes H1N1, H3N2, H1N2.

Five out 6 tested farms were scarcely positive to *Mycoplasma hyopneumoniae as* all the farms practice vaccination against *Mycoplasma hyopneumoniae*.

Two farms (E, F) resulted positive at high percentage, 90.9% and 80.3% respectively, to antibodies anti-ApxIV toxin of *Actinobacillus pleuropneumoniae* (App), the toxoid of which is not included in vaccines and only expressed, as toxin, in the course of infection. In farms which pigs were vaccinated against App, anti-ApxIV positivity reflected infection or, in young piglets until 10 weeks of age, passive antibodies from (previously infected) dams. App was not demonstrated in 11 lungs' samples from two farms submitted for bacteriological analysis.

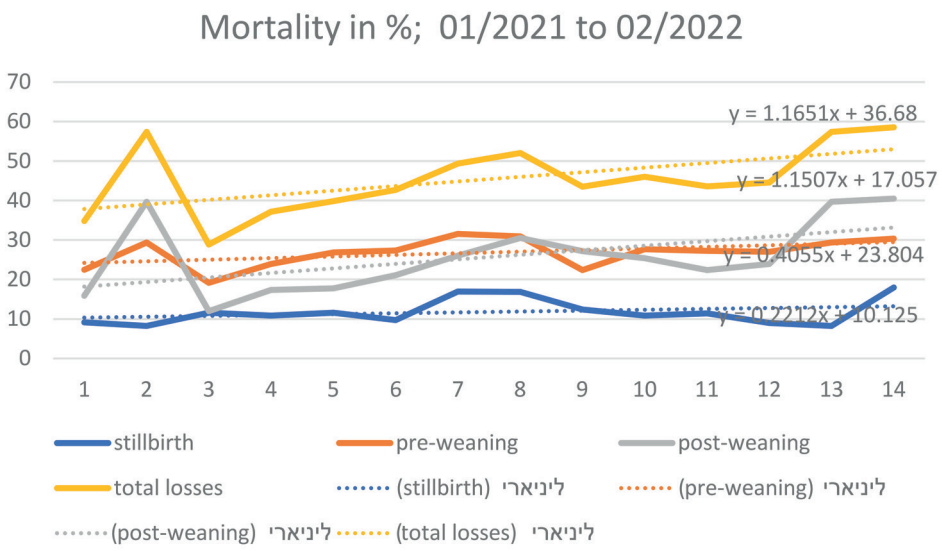
Serology and qnRT-PCR for PCV 2 are summarized in Table 3.

RT-PCR on joints aspirates

The four aspirates from swollen joints were all positive to *M. hyorhinis.* One of the aspirates was demonstrated coinfected with *H. parasuis*. The four piglets blood sera resulted in RT-PCR positive for PRRSV1 type, as summarized in Table 4.

DISCUSSION

As above mentioned, at gross necropsy performed at farm level, lung lesions induced by *Mycoplasma hyopneumoniae* were scarcely observed and the limited antibody titers, which resulted from the serological tests, should be attributed to vaccination, which induces low, short-lasting, serological responses (6). The authors' opinion is that the pathogen did not play a role in the respiratory disease complex observed.



Graph 1: Mortality in percentages, during the period January 2021 to February 2022.

As in the first PRRSV2 type outbreak in Israel, also the PRRSV1 type caused premature farrowings, stillbirths, farrowing of previously viremic, ill, piglets. Moreover RT-PCR demonstrated PRRSV1 in weaned and growing piglets affected by respiratory disease, wasting syndrome and noticeable arthritis caused by secondary bacterial infections.

Relative to PCV2, two pools proved positive to qnRT-PCR for PCV2, with low viral loads (2,000 and 128,000 genomic copies/ml, respectively). Severe signs of Post Weaning Wasting Syndrome (PMWS) in piglets are associated with loads ≥10^9 of viral DNA copies in 1 ml blood and/or in 500 ηg tissue (7); therefore the low viral loads resulted in the exclusion of PCV2 as responsible or for being a significant co-factor in piglets wasting, mortality and respiratory problems.

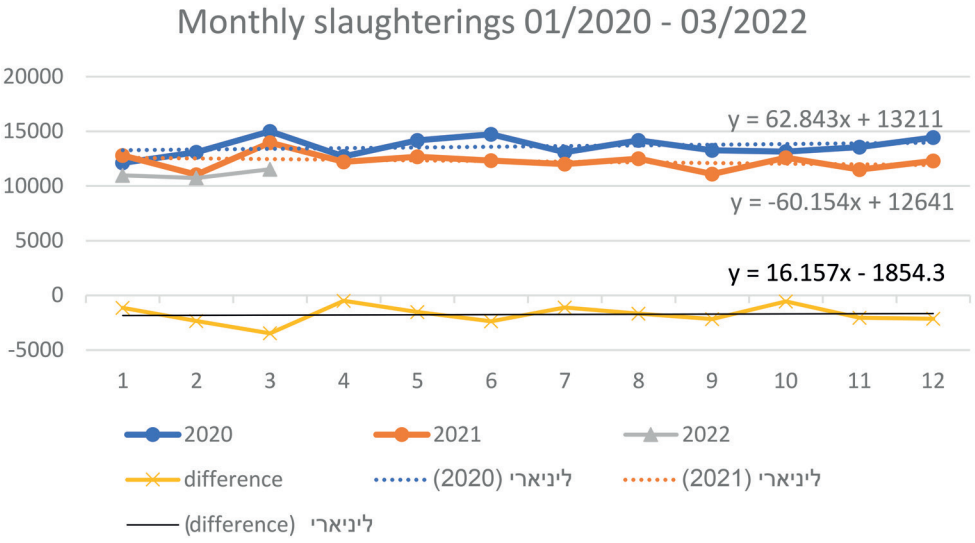
According to the "Procedure for Implementing the "Pig Keeping Regulations for Agricultural Purposes, 2015" (8), farmers are requested by the Israeli Veterinary Services to register the daily/monthly performances of pig farms (Appendix 3: Farm Data Log Diary Form) (8), the therapeutic treatments, and keep these data available for at least two years. Therefore, it was retrospectively possible to examine the productive and mortality data starting from January 2021.

From these data it appeared that first clinical signs started at January-February 2021, with an increase of mortality in pre-weaning and weaned piglets. A 14 months, distribution of productive performances (January 2021 to February 2022) showed a "wave" pattern, with a first wave from January 2021 to February 2022; a second wave during July to August 2021, and then a third from January 2022 to February 2022. Wave patterns of clinical signs were considered typical for course of PRRSV outbreaks (9) Graph 1.

Table 5: Losses/mortality in 2020 and 2021 in examined farms. Dead sows in Farm E.

Year	2020	2021
phase	mortality in %	
stillbirth	6.81	12.98
pre-weaning	18.79	28.32
post-weaning	16.83	29.47
total losses	35.49	49.38
Sows (data for E farm only)	5.8	6.8

Intensive pigs farming typically suffers losses/mortality through stillbirth, pre-weaning mortality, growing/fattening or post-weaning mortality, sow mortality, all of which can vary widely in different countries, breeding conditions, seasons, etc. (10,11,12). PRRSV is recognized to boost losses in all of these breeding phases, including sows mortality (1, 3, 13). Examined farms data (8) allowed an evaluation of breeding performances and losses in 2020 (PRRSV2 type vaccinated farms) and 2021, in which the PRRSV1 type broke out. Table 5 below summarizes the losses/mortality, in examined farms in 2020 and 2021.



Graph 2: monthly pigs slaughtering data at Northern District pigs' slaughterhouse (15).

The authors'opinion was that high values in stillbirth reflected the premature farrowing, which is typical in PRRSV.

In farm E, sow mortality totalized 79 dead sows in the period 01/2021 – 02/2022, that is, 5.8% yearly. Peaks of mortality occurred during 07/2021, during 08/2021 and during 02/2022, in correspondence with the second and third "waves" of the PRRSV1 outbreaks. Sow mortality peaked during the hottest months of the year (July-August) (12) amounting to 24 sows in 2021, and 17 sows in 2020, that is, 2.06% and 1.56% of the annual sow population, respectively.

There is only one pig slaughterhouse in the Northern District (Galilee). Results of inspections at slaughterhouses are important indicators of the health of food animals at both the abattoir and at the farms level (14). Indeed slaughtering data of Northern District pigs' slaughterhouse show dramatic changes in slaughtered animals in the first months of 2022, along with all of 2021, with respect to 2020. The average monthly slaughtering was 12,727 heads in 2020, then 11,463 in 2021, which was reduced to 11,077/month in first three months of 2022. Overall, the number of slaughtered pigs decreased more than 16,000 heads, with respect to about 163,000 heads slaughtered in 2020 (15). Graph 2 above illustrates monthly slaughtering in 2020, 2021, first months of 2022, and average monthly slaughtering difference. While the trends in slaughtering in 2020 were slightly increased, in 2021 and 2022 they appeared to decline.

The analysis of the slaughterhouse data may contribute to the knowledge and identification of the level of health problems in livestock productions (14). Declines in slaughtering data appeared to resemble the overall losses which increased at farms level. The authors' opinion was that there were several months delay in diagnosing the outbreak, or in understanding the significance of the increasing stillbirths, pre-weaning and post weaning mortality, and appreciating the reduction of slaughtering, with respect to previous years.

The source of infection remains unknown, but considering that Israel is surrounded by countries without reared pig populations, PRRSV1 introduction through movement of animals should be excluded. Suspicion was linked to introduction of infected material from abroad, similar to that in the previous outbreak in 2017 (boars' semen) and as in other outbreaks in PRRSV-free countries (3,16). While the study of the phylogenetic tree and genetic homology of PRRS1 Israeli isolates revealed similarities with old/dated European strains, the most recent is represented by the Hungarian isolate from 2016: Hungary/50541-2-NEBIH-HU/2016.

Sequencing showed the presence of strains of PRRSV1 circulating in the farms, with a limited genetic difference (from 2.17% to 0.36%) in weaning and finisher pigs. Only in sows there was evidence of coinfection of two strains (mixed sequence, data not show). It is not possible to infer about if the origin of the strains (mutation from one original strain or introduction of two different strains). Anyway, this data, considering also the distribution of RT-PCR positivity and the clinical signs, supporting a long-term circulation of the virus.

Following laboratory confirmation of the PRRSV1 type, the Veterinary Services allowed the exceptional import of a PRRSV1 vaccine, even though the vaccine was not registered. Modified live vaccine (MLV) – European strain was imported and vaccinations started during March 2022, two months following confirmation of PRRSV1 type.

This description constitutes, to the best knowledge of the authors, the first outbreak of PRRSV1 type in swine farms in Israel. The outbreak profoundly affected several farms in the North Region of Israel (Galilee) where all but one of the pig farms are located in Israel. The delay between the beginning of clinical signs and starting of preventive measures (MLVs) was of 15 months at least, of which 12 months or more due to lack of diagnosis and 2 further months due to a delay in vaccines supply.

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