

First Outbreak of Porcine Reproductive and Respiratory Virus (PRRSV) in Swine Farms in Israel

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ABSTRACT

This article summarizes the clinical findings and confirmation laboratory procedures occurring during the first outbreak the American strain of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), in swine farms in Israel. So far, (2017), Israel has been considered free from PRRS. At least 7 pig farms were involved in the PRRSV outbreak, in the North Region of Israel, involving about 10,000 breeders. Main clinical signs were premature farrowings, increase of stillbirth and pre-weaning mortality. Abortion waves, typical of PRRSV outbreaks, were not observed. PRRSV involvement in the outbreak was confirmed through ELISA-antibody test on blood samples from sows and piglets and RT-PCR on blood and organs. Sequencing and genetic analysis confirmed the involvement of the American PRRSV strain. The outbreak was contained through vaccination of breeders before insemination and at mid-pregnancy, and of piglets before weaning. The source of introduction of PRRSV into Israel remained unknown.

Keywords: premature farrowings, stillbirth, RT-PCR, sequencing.

INTRODUCTION

PRRS is the acronym for Porcine Reproductive and Respiratory Syndrome (PRRS), a viral disease characterized by clinical signs of the reproductive system (failures in breeding, abortions, stillbirths, early 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). Clinical signs of the syndrome, attributed to PRRSV, started appearing in the USA in the late 1980's, then in Europe in the early 90's (1990-1992); the etiology was confirmed in Holland in 1991 (1). Two genotypes are of PRRSV known: type 1, so-called Lelystad virus, due to its identification by the Dutch Veterinary Institute in Lelystad, The Netherlands, and type 2 or USA genotype. Today both types are largely diffused throughout the world (1) even though type 1 is largely

dominant in Europe and type 2 in North America and Asia. Nucleotide sequence of type 1 and 2 differ of around 44% (1), but intra-type nucleotides sequences may also vary up to 30% in type 1 and $\geq 20\%$ 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. Current ELISA-antibodies techniques are indeed able not only to confirm the presence of anti-PRRSV antibodies and roughly quantify them, but also to differentiate between the two different types (2).

So far Israel has been considered free from PRRS on the basis of previous random epidemiological investigations in single pigs farms or performed in the course of other diseases outbreaks (3, 4, 5, 6).

This paper summarizes the clinical findings and con-

firmation laboratory procedures occurring during the first outbreak of PRRSV in swine farms in Israel.

MATERIALS AND METHODS

Farms and animals

At least 10,000 breeders (sows and gilts) were involved in the outbreak and at least 7 different closed-cycle (far-row to finish) farms were known to have been affected, of which 6 farms were tested. Farms were located in Ibbilin Municipality area, Galilee, North Region of Israel, where almost all the swine farms of Israel are located. Concerning clinical and productive data, it was not possible to achieve reliable and complete data from all the 7 farms, however in this paper, data and the evolution of the outbreak in one closed-cycle farm of 1,400 breeders were summarized and analyzed.

Samples

From 7 farms, 33 piglets (stillborn and dead piglets) and one placenta; internal organs from one dead sow, were submitted to the Kimron Veterinary Institute, Beit Dagan, Israel, for pathology examinations. Seventy blood-sera samples, 34 organs homogenates from the 33 piglets and 1 placenta, were submitted to the Istituto Zooprofilattico Sperimentale Lombardia Emilia Romagna (IZSLER) Animal Health Institute, Brescia, Italy, for ELISA-PRRSV antibody test on blood-sera, Real Time Polymerase Chain Reaction (RT-PCR) on piglets' and placenta's homogenates and blood-sera and sequencing and genetic analysis of identified PRRSV.

RT-PCR

PRRSV RNA was extracted from 200 μ L of each the 34 organs homogenates (spleen, lung, tonsils, placenta) and the 52 blood samples, using a magnetic bead based commercial kit (NucleoMag[®] Vet kit, Macherey-Nagel, Düren, Germany), according to the manufacturer's instructions. An exogenous internal control RNA, was added to specimens prior to RNA extraction to verify the success of the procedure and the absence of inhibitors. The extraction was carried out on the Biosprint 96 instrument (Qiagen, Hilden, Germany), using the NucleoMag Vet 200 protocol. Nucleic acids were eluted into 100 μ L of elution buffer and immediately subjected to RT-PCR. PRRSV detection was carried out using the LSI VetMAX (TM) PRRSV EU/NA Real-Time PCR

kit (Applied Biosystem, Monza MB, Italy), as specified by the manufacturer.

Sequencing and genetic analysis

Partial ORF7 (282 bp) was amplified by means of one step RT PCR kit (Qiagen, Hilden, Germany) using primers and conditions previously described (7).

Amplified products were purified by Nucleospin Gel and PCR clean up Kit (Macherey-Nagel) and Sanger Sequencing was accomplished by BigDye Terminator Cycle Sequencing kit v1.1 on 3500xl genetic analyzer (Thermo Fisher Scientific, Waltham, USA.)

Sequences were analyzed using Lasergene software v 10.0 (DNASar, Madison, USA). Nucleotide BLAST (blastn) algorithm (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was employed to confirm the identification of PRRS virus. Sequence alignment was performed by ClustalW using MEGA6 (8). Phylogenetic analysis was conducted using Maximum Likelihood method (ML) and Kimura 2-parameters and discrete Gamma distribution (K2+G) model based upon best-fit model of nucleotide substitution selection based on corrected Akaike Information Criterion (AICc) and Bayesian Information Criterion (BIC) as implemented in MEGA6 (8). The robustness of the ML trees was statistically evaluated by bootstrap analysis with 1000 bootstrap samples.

Serology

Seventy blood samples from sows and piglets, with or without clinical signs, were subjected to a competitive in-house ELISA test (9) for the presence of PRRSV antibodies.

RESULTS

Clinical aspects

Clinical events in the analyzed farm, named "R", a closed cycle unit of about 1,400 sows and pregnant gilts, are hereby described. In the second week of June, the incidence of stillbirths sharply increased from 7.5-8.0% to 18.92%, and then up to 40.29% in July. In parallel, pre-weaning mortality increased from 15.8-23.5% to 47.5% in June and then up to 63% in July. Reproductive data (number of farrowings, piglets/sow/farrowing; stillbirths; mortality until weaning) are summarized in Table 1. The incidence of stillborn piglets appear in Figure 1. The inspection of sows' data relative to insemination dates and expected farrowing dates revealed

Table 1: Farrowings, productive and mortality data before and during the PRRSV outbreak in a selected closed-cycle farm of 1,400 breeders.

month	farrowings	total piglet/farrowing	stillbirth,%	preweaning mortality, %
Apr-17	231	10.94	7.95	16.85
May-17	264	9.80	7.50	23.53
Jun-17	216	10.55	18.92	47.48
Jul-17	249	9.64	40.29	62.96
Aug-17	114	11.80	25.00	33.40
Aug-17*	107	14.00	8.80	12.8
Sept-17*	233	10.41	14.19	26.24
Oct-17*	190	10.62	12.00	26.20
Nov-17*	183	10.95	10.03	43.70
Dec-17*	210	10.54	10.30	19.80
Total	1997	<i>* productive and mortality data after vaccination</i>		



Figure 1: Stillbirths: some piglets appear still wrapped in their amniotic sacs.

that the stillbirths were caused by premature farrowing, or late pregnancy abortion, which occurred at 109-111 days instead of 114-115 days.

Gross necropsy findings

A great number of dead piglets appeared still wrapped in their amniotic sacs, with typical soft and curled hoofs. On the R farm a few cases of sows' mortality (4 in total) also occurred; dead sows appeared intensely hyperemic, but not hemorrhagic, with intense conjunctivitis, as in Figure 2.

GROSS PATHOLOGY AND HISTOLOGY

The heart showed multifocal, mild, myocardial degeneration and hemorrhage and marked congestion. The liver revealed diffuse centrilobular, mild or acute hepatocellular degeneration. Examination of the placenta revealed diffuse hyperemia and multifocal micro-ulcerations (0.5 cm diameter).

The internal organs of the stillborn piglets appeared without any apparent pathological changes. The examination

of dead piglets revealed non-specific pathological findings which included consolidation of upper pulmonary lobes, fibrinous changes on abdominal serous tissues with hyperemia of omentum and intestines.

RT-PCR

Samples (blood and/or piglets' organs homogenates) from 6 farms were submitted to RT-PCR; all 6 tested farms and 19 out of 86 samples (22.1%) were found to be positive of which 10 out of 52 (19.2%) blood samples and 9 out of 34 (26.5%) homogenates (organs; placenta) were positive.

Sequencing and genetic analysis

Partial Opening Reading Frame 7 (ORF7) of Virus RNA encoding the nucleocapsid structural protein (N) resulted in an abundance of cells infected by PRRSV representing the immunodominant antigen in the pig immune response to PRRSV (10). The ORF7 sequence has been reported as highly stable during virus persistence in a groups of animals and therefore ORF7 was used for detection and diagnosis



Figure 2: intensely hyperemic dead sow with conjunctivitis.

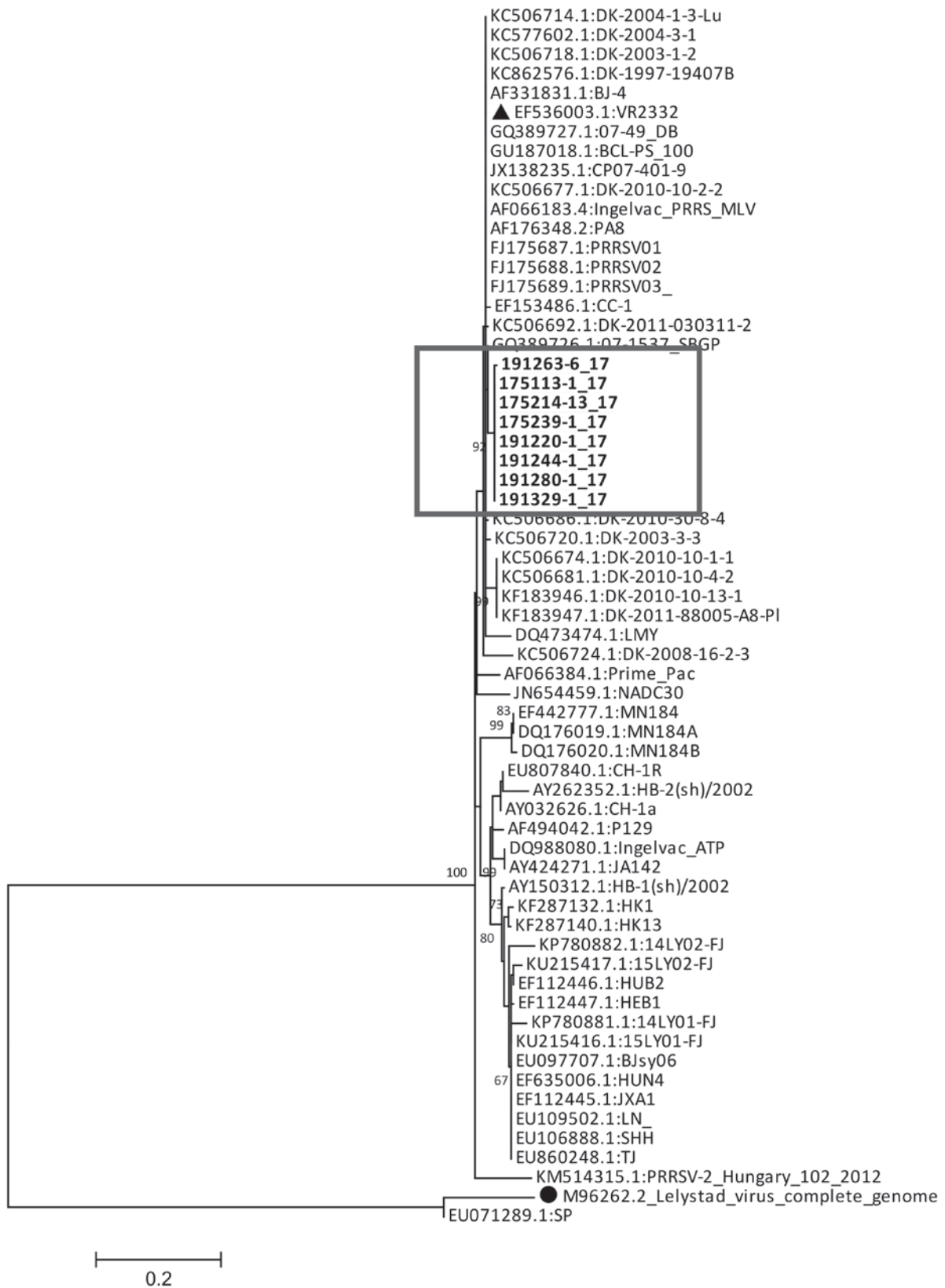


Figure 3: Phylogenetic tree of partial ORF7 Israeli sequences (strains isolated in Israel are represented in bold)

(11). A 282 base-pair long ORF7 fragment of each PRRSV isolate from the 6 examined farms was subjected to sequencing. Blast analysis of ORF7 obtained sequences revealed in all the farms a >98% of nucleotides identity with reference to the American strain PRRSV available in GenBank. Phylogenetic analysis of the obtained sequences compared to type 2 PRRSV sequences from GenBank and Lelystad PRRSV type 1 prototype sequence showed as an out-group confirming the designation of the Israeli sequences to Type 2 PRRSV group (Figure 3).

Phylogenetic tree of partial ORF7 Israeli sequences

Type 2 PRRSV strain retrieved from GenBank and Lelystad PRRSV type 1 prototype sequence as the outgroup was constructed by the Maximum Likelihood (ML) method using the best-fit model K2+G within MEGA6. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. Only bootstrap values higher than 60 are reported. PRRSV strains of the present study are represented in the frame, for quick identification, and in bold. Type 1 PRRSV prototype sequence (●) and Type 2 PRRSV prototype sequence (▲) are indicated respectively. Israeli strains' sequences have been submitted to Genbank by Animal Health Institute "IZS-LER", Brescia, Italy, which performed the described virus demonstration and sequencing tests. (<https://www.ncbi.nlm.nih.gov/genbank/>)

Serology

All the 7 tested farms and 46 out of 70 tested blood sera (65,7%) showed positive PRRSV-ELISA antibodies. Sampling for each farm, laboratory investigations and results are summarized in Table 2.

DISCUSSION

Contrary from the literature, this first PRRSV outbreak in Israel did not cause the typical waves of abortions at any stage of pregnancy but was limited to the last stage of pregnancy with late abortions or premature farrowings around 109 to 111 days of pregnancy and farrowing of already viremic piglets and stillbirths. Cases of reabsorption and mummification of fetuses were also observed.

Fetuses are exposed to PRRSV infection after the 72nd day of gestation; PRRSV multiplies at high titers, and piglets may die either as a consequence of infection or acute disease and fever in the sow, with abortion at any stage of pregnancy. Sows may also deliver viremic piglets, which contribute to diffusion of the infection and/or die in first days after birth.

It seems, from the examination of the reproductive data of the farms involved in the outbreak that the first clinical signs appeared by the end of May and/or during the first days of June in one farm and then in after 7-10 days of spread in the environment involved several other farms. The source of infection remains unknown, but considering that Israel is surrounded by countries without reared pig populations, suspicion was linked to uncontrolled import of boars' semen from abroad, similar to that in previous outbreaks in PRRSV-free countries in Europe (12).

In four affected farms, the first samples were collected by June 23rd and IZS-LER confirmed involvement of PRRSV by June 26th; with 50% seroconversions already and 14.2 to 30% piglets' homogenates RT-PCR positive. It is therefore the authors' opinion that there was a one month delay, at least, in diagnosing the outbreak or in understanding the significance of the increasing stillbirths and preweaning mortalities.

Table 2: Sampling from each farm, laboratory investigations and results.

Month	Farm	Serology			RT - PCR			% pos	PRRS ORF-7 Sequencing	
		material	number	positive	material	number	positive		reference strain	% nucleotidic identity
Jun-17	R	blood	9	3					VaccResp PRRSV-Amer	98,7%
	K	blood	9	6					""	98,7%
	A				placenta	1	1	100.00	""	98,7%
	N				piglets homogenates	10	3	30.00	""	98%
Totals	4		18	9		32	7		American strain	≥ 98%
Jul-17	K				piglets homogenates	2	2	100.00	VaccResp PRRSV-Amer	98,7%
	R	blood	11	8	blood	11	2	18.18	""	98,3%
	H	blood	16	12	blood	16	3	18.75	""	98,3%
	A	blood	12	10	blood	12	2	16.67	""	98,7%
	Y	blood	13	7	blood	13	3	23.08	""	98,7%
Totals	5		52	37		54	12		American strain	≥98%

Following laboratory confirmation of the American strain PRRSV, the Veterinary Services allowed the exceptional import of PRRSV vaccine, even though the vaccine was not registered. Both inactivated and modified live vaccine (MLV) – American strain were imported and vaccinations started during the first days of August, a two month delay, following observation of the first clinical signs.

The relatively low seroconversion rates, 50% in June and 71% in July, were considered indicative that a segment of the breeding population that was still naïve to PRRSV. In such a situation, taking into account potential adverse effects of MLVs in naïve pregnant sows (13), a cautious vaccination plan was implemented. Pregnant breeders were vaccinated twice with the inactivated American vaccine strain, then with the MLV in the first week after farrowing and again with the MLV at sixty days of pregnancy. Breeders which did not complete the four-course vaccination because of pregnancy progress (>90 days of pregnancy), received the fourth vaccination with the inactivated vaccine.

Piglets from vaccinated sows were RT-PCR tested, 2 from each sow, at different ages, from 1 to 7 weeks of age in order to investigate the probable beginning of PRRS viremia (data not presented). Results indicated that piglets converted to RT-PCR positive after weaning (at 28 to 35 days) in all but one farm. These results allowed the implementation of piglet vaccination, with MLV, before the expected transition to viremia.

By mid-October, stillbirths were reduced to an average 6% in the examined farms, with stillbirths of 12% in farm “R”, the most severely affected. Pre-weaning mortality was reduced to an average 15% in the examined farms but again with 26% dead piglets in farm “R”.

An increase yet again of pre-weaning mortality occurred in farm R starting in September, in contrast to the appreciable reduction of stillbirths, together with enteric clinical signs in piglets, suggesting the occurrence of other pathologies other than PRRS. Pigs farms in Israel are since 2004 occasionally affected by short outbreaks of Transmissible Gastro Enteritis (TGE), especially at autumn/winter (14). Indeed the Israel “Kimron Veterinary Institute” confirmed TGE as causative of the enteric clinical signs and the increase of the preweaning mortalities in farm R. This event severely affected the beneficial results obtained with the implementation of PRRS vaccination in terms of neonatal mortality. Lack of available vaccines suggested the implementation of

“feedback” of pregnant sows from ill piglets’ intestines (15), which contributed to yet another reduction of pre-weaning mortality in farm R by December.

This description constitutes to the best knowledge of the authors the first outbreak of PRRSV in swine farms in Israel. The outbreak was caused by a Type 2 – American strain of PRRSV which profoundly affected several farms in the North Region of Israel (Galilee) where all but one of the pig farms were located in Israel. The delay between the first clinical signs and starting of preventive measures (MLVs) was of 3 months at least, of which one month or more due to lack of diagnosis and one further month due to delay in vaccines supply. Mass vaccination of all the breeders, alternating inactivated and MLVs, reduced stillbirths and pre-weaning mortalities to acceptable levels. The cautious vaccination plan was able to reduce rapidly premature farrowings, stillbirths and early mortalities, the main clinical signs in this outbreak, without causing any side effect in the vaccinated breeding population.

The Source of introduction of the PRRSV strain involved remained unknown; while its sequencing indicated a high similarity to type 2 – American strains circulating in Northern Europe Countries, from which boars’ semen for insemination is often imported to Israel. This combination may suggest the uncontrolled import of boars’ semen as the causative event of PRRSV outbreak in Israel.

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