PORCINE CIRCOVIRUS TYPE 2 (PCV2) INFECTION OF PIGS IN ISRAEL: CLINICAL PRESENTATION, DIAGNOSIS AND VIRUS IDENTIFICATION.

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ABSTRACT

During the years 2006-2007, an increase of respiratory and enteric problems in piglets after weaning was reported from pig herds in western Galilee. These problems were accompanied by increased mortality and a lower growth rate of the affected piglets. A reduced growth rate was also noted in older pigs at these farms.
Sick piglets were bled on three affected farms, and on necropsy, lungs, inguinal and mesenteric lymph nodes were collected from carcasses, and submitted to laboratory examination. Evidence for the presence of porcine circovirus type 2 (PCV2), was based on positive ELISA serology; virus isolation; polymerase chain reaction (PCR) and immunohisto chemistry (IHC) on the lymphoid tissues.
To our knowledge this is the first case on PCV2 identification in Israel pigs.

INTRODUCTION

Porcine circovirus type 2 (PCV2) is a single-stranded DNA virus, belonging to genus *Circovirus,* family *Circoviridae*. It is considered ubiquitous and both domestic and wild pigs are the natural hosts (1). Oral-nasal infection is the most frequent and natural route of transmission (1). Alternatively, infected sows may shed virus into the colostrum and the milk, thereby infecting piglets by the oral route (2).
PCV2 is resistant at acid pH (pH<3) and stable for 15' at 70°C (3). Chlorexidine, formaldehyde, iodine and alcohol–based disinfectants can reduce the virus titer at room temperature within 10 minutes (1).
Despite the ubiquity of PCV2 in almost all pig herds worldwide, only in some cases does clinical disease develop presenting 5-30% morbidity and 50-60% mortality among the affected pigs. In such cases, there is a dramatic worsening of the health of weaned piglets, from between 8 to 16 weeks of age, represented by a **p**ost-weaning **m**ulti-systemic **w**asting **s**yndrome (PMWS) with emaciation, jaundice, reduced growth (Fig. 1,3), hind legs and dermatitis (**p**orcine **d**ermatitis associated with a **n**ephropathy **s**yndrome (PDNS) (Fig. 4,5), (1,4,5,6). Both respiratory and enteric disease appear in fattening animals, while in adult herds, ,reproductive problems affect breeders. All these syndromes are clinically indistinguishable from other diseases. Thus, PCV2 involvement in PCVAD, PMWS, PDNS must be confirmed by the virus isolation (1). The virus may be isolated on a pig kidney cell line, and a polymerase chain reaction (PCR) is available. Immuno histochemistry (IHC); immunofluorescence (IF) and serology (ELISA competition) can be used to detect PCV2 infection. Histological evaluation of lymphoid cells depletion in PCV2-target lymphoid organs such as the inguinal or mesenteric lymph nodes has been reported.
The purpose of this article is to describe PCV2-associated clinical signs in 3 swine farms of north Israel.

MATERIALS AND METHODS

Herd history

In 3 far-to-finish swine farms located in western Galilee, an increased incidence of respiratory and enteric problems was encountered from weaning at 5 weeks old and 12-14 weeks. There was high mortality (12-20%), and a decreased growth rate in piglets. An increased proportion of fattening pigs suffered from dermatitis that was unresponsive to antibiotic treatment. A tentative diagnosis of PCV2 infection was made

Samples:

Six blood samples from affected chronic piglets aged 8 to 12 weeks were collected from each farm.. In addition, 10 dead piglets were necropsied; and inguinal and mesenteric lymph nodes and lung tissue were collected from each piglet .The samples were processed at the Virology Department of the Kimron Veterinary Institute, Bet Dagan, and then submitted for further laboratory investigation at the IZSLER Animal Health Institute , Brescia, Italy.

Laboratory investigations:

All sera were submitted to serological testing for Porcine Respiratory and Reproductive Syndrome virus (PRRSV), Aujeszky Disease virus (ADV), Swine Influenza virus (SIV), PCV2 and erysipelas. The pooled blood samples were also tested for PRRSV-antigen.

Virology:
PCV2 was performed using an in-house sandwich-ELISA antigen test using a monoclonal antibody at the second passage on a pig kidney cell line.

Polymerase Chain Reaction:
The PCR test was performed on pooled tissue homogenates from each piglet (9). The following primers were designed to amplify 263 nucleotides sequence from opening reading frame 2 (ORF 2) of PCV2 associated with PMWS (10):

- CF8 5'-TAGGTTAGGGCTGTGGCCTT-3' (nucleotides 1323 to 1342)

- CR8 5'-CCGCACCTTCGGATATACTG-3' (nucleotides 1567 to 1586).

Immunohistochemistry:
The IHC test were performed with a PCV2 monoclonal antibody on 4 μ tissue slices, then compared with PCV2-PCR and *in situ* hybridization (ISH) positive and negative reference samples (1,7).

Serology:
Sera were tested using an in-house PCV2 ELISA-competition test,. The results are given as Log_{10} of reciprocal of last dilution, starting 1:10 = 1 Log_{10}. The results are summarized as mean titer; standard deviation (sd) and percentage of positive samples.

RESULTS

The PRRSV, ADV, SIV, and Erysipelas examinations were negative.

Clinical signs:
The weaned piglets appeared apathic and emaciated (fig.1). They were coughing; and had enlarged inguinal lymph nodes at palpation (fig 2). Runts, poor doers and anemic pigs were seen in the fattening areas (fig 3). Examination of the herds showed a few cases of fattening pigs affected by irregular, purple,necrotizing dermal lesions, primarily over the hind legs and perineum (fig. 4 and 5).
Post mortem of dead piglets showed poor bodily condition, enlargement of inguinal and the mesenteric lymph-nodes (fig 6, 7), interstitial pneumonia (fig 8), and in some cases, enteritis.

Laboratory results:

Virus isolation:
The test could be performed only on tissues from 5 out of 10 piglets and from only one farm, due to poor preservation of the material. All the 5 piglets yielded PCV2 virus..

PCR:
Four out of the 10 pooled lymphoid tissue homogenates originating from the 3 farms were PCR positive;

IHC
Two of the 10 piglets were PCV2 positive; representing 2 out of the three farms.

Serology: All sera were PCV2-Elisa positive, average titer = $log_{10}3.72$ sd = 0.57.
All the 3 farms tested positive.

DISCUSSION

The wasting disease of the pigs in the weaning area of the farms, together with the poor performance of the fattening pigs, the presence of dermal lesions and necropsy findings indicated the involvement of PCV2 From an epidemiological point of view, it can be considered that more than 50% of the sow population and the related pig production are concentrated in a small and densely populated area (11). Moreover, the farms are located fence to fence and are so interconnected that they represent a single epidemiological entity (12). This explains how PCV2 could spread among them so rapidly.
In connection with the clinical signs induced by PCV2, PDNS must be differentiated from:
- Classical and African Swine Fever (1) both are notifiable diseases, also considering interstitial nephritic lesions induced by PCV2 in PDNS;
- Erysipelas, by *E. rhusiopathiae*, a notifiable, zoonotic disease
- Pityriasis rosea (pustular psoriaform dermatitis), an apparently inherited disease, self-limiting, but not resembling human pityriasis.

Inactivated vaccines against the PCV2 for the use in breeders and piglets are registered and used in several European countries, as well as the USA and Canada. They are generally characterized by their cost-effective results in terms of mortality and in reduction of the incidence in clinical signs. The sow vaccines may be indicated for the passive protection

Table 1: A summary of PCV2 laboratory tests performed on piglet tissues.

	Piglets									
Test performed	1	2	3	4	5	6	7	8	9	10
Virological *	not performed					pos	pos	pos	pos	pos
ImmunoFluorescence	neg	neg	neg	pos	neg	neg	neg	pos	pos	pos
Immuno Histo Chimic	neg	neg	pos	pos	neg	neg	neg	neg	neg	neg
Polimerase Chain Reaction	neg	pos	pos	pos	neg	pos	neg	neg	neg	neg
* two passages on pig kidney cells line										

of the piglets in the presence of early clinical signs of PCV2 in the weaning period, and up to the age of 6-8 weeks. The vaccination of piglets at the third week of age is strongly indicated when there are PCV2 problems at the growing-fattening stages.
As an alternative to vaccination, the application of good management practice (13), rescheduling of other vaccination times (1) and dietary changes to include certain supplements (14), could give some benefit in affected farms.

To the authors' knowledge, this is the first report of PCV2-associated disease of Israel pigs.

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Fig 1: Growth retardation and wasting post-weaning (approx 2 months of age). Note prominent spinal vertebrae.

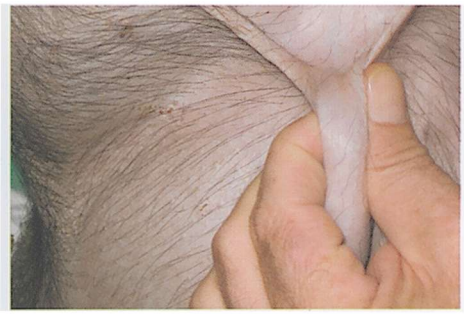

Fig 2: Enlarged inguinal lymph nodes.

Fig 3: Growth retardation at fattening (approx 4 months of age) all the pigs in the picture are the same age.

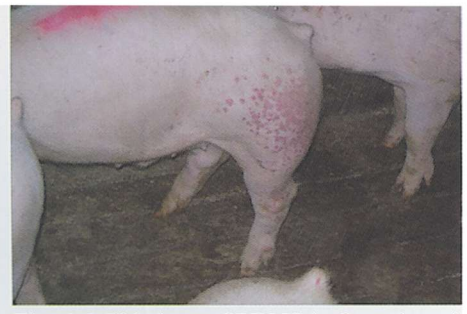

Fig. 4: Mild signs of PDNS in fattening pigs

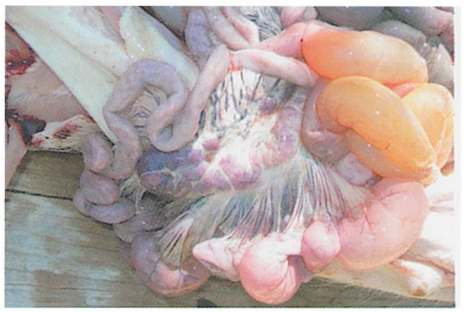

Fig 6: Enlarged mesenteric lymph nodes

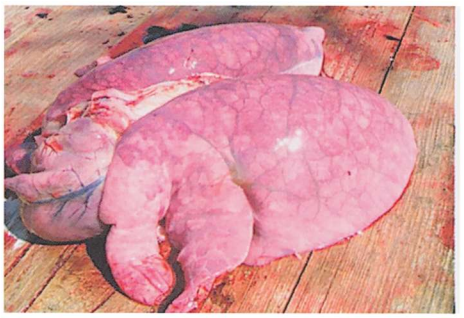

Fig 8: Interstitial pneumonia

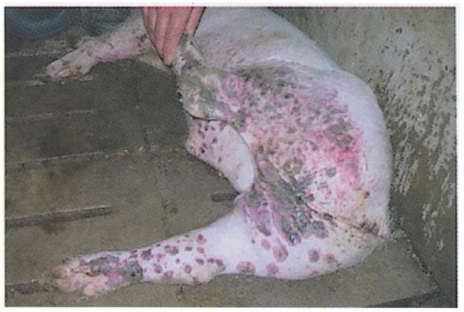

Fig 5: Severe signs of PDNS in fattening pigs

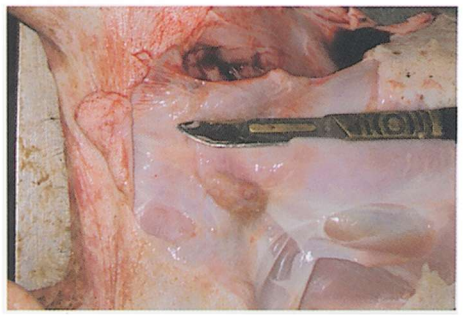

Fig 7: Enlarged inguinal lymph nodes