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Isolation of a cytopathogenic virus from a case of porcine reproductive and respiratory syndrome (PRRS) and its characterization as parainfluenza virus type 2

Brief Report

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Summary. From a lung of a fetus of a breeding sow showing PRRS-like symptoms a viral agent could be isolated. It was characterized as an enveloped, hemagglutinating RNA virus. Ultrastructural examination of purified virus revealed paramyxovirus-like pleomorphic virions of approx. 200 nm in diameter. The helical nucleocapsids were about 18 nm in diameter. The virus was found to be antigenically related to simian virus 5 (SV5) a prototype strain of parainfluenza virus type 2, but not to bovine respiratory syncytial virus, parainfluenza virus type 1, parainfluenza virus type 3, and Newcastle disease virus as determined by western blot analysis.

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The family *Paramyxoviridae* consists of the genera *Paramyxovirus, Morbillivirus, Rubulavirus* and *Pneumovirus* [12]. Based on serological properties the mammalian parainfluenza viruses (PI) are divided into 4 serotypes. Parainfluenza virus types 2 and 4 belong to the genus *Rubulavirus*, whereas PI virus types 1 and 3 are members of the *Paramyxovirus* genus. Other members of the *Rubulavirus* genus are mumps virus and Newcastle disease virus. PI virus of avian, bovine, canine, and human origin is associated with respiratory tract disease [3]. Experimental studies on the pathogenesis have identified PI viruses as primarily respiratory pathogens. Parainfluenza virus type 2 (PI-2) plays an important role in respiratory tract disease in dogs. To this group belongs simian virus 5 (SV5) [14].

Infections of swine with bovine parainfluenzavirus type 3 without development of clinical signs have been reported [6]. In Israel, paramyxoviruses

serologically related to avian paramyxoviruses have been isolated from normal slaughter pigs [10]. A novel paramyxovirus designated La Piedad Michoacan paramyxovirus (LPMV) was identified as the infectious agent of an endemic disease occurring in Mexico since 1986 with symptoms comparable to PRRS [11]. This so-called blue-eye disease leads to high mortality in suckling piglets suffering from non-suppurative encephalomyelitis, interstitial pneumonia and corneal opacity. In sows, symptoms of reproductive failure like abortion, still birth, mummification and repeated breeding were reported [16]. LPMV was classified as a member of the *Rubulavirus* genus [11, 18]. Comparison of the deduced amino acid sequence of the hemagglutinin-neuraminidase and the matrix protein gene revealed the highest degree of identity to the mumps virus and simian virus 5 [1, 17]. A different porcine paramyxovirus was isolated from the lung of a sow originating from Germany, showing PRRS-like symptoms [2]. The relationship to other paramyxoviruses is unclear. A part of the symptoms of PRRS could be induced after intravenous inoculation into piglets and sows [2]. In a serological survey 14.3% of finishing pigs (n=265) from farms with severe respiratory disorders had antibodies against this porcine paramyxovirus isolate [7]. The etiological relevance of other members of the family *Paramyxoviridae* for swine diseases has not yet been determined.

This report describes the isolation of a paramyxovirus from a stillborn piglet. First results of its comparison to other members of this genus are presented.

From a swine breeding herd, where a clinical outbreak of PRRS was diagnosed, one sow undergoing an abortion was euthanized and necropsied. For virus isolation the lung of a fetus was homogenized and incubated in a solution of 0.25% trypsine for 4 hours at room temperature. After filtration and subsequent low speed centrifugation (1000×**g**, 10 min), the cells were washed with phosphate buffered saline (PBS), suspended in MEM supplemented with 5% FCS and then grown in 80-cm^2 culture flasks. By appearance of a cytopathic effect (CPE) the culture supernatants were diluted 1:10 in MEM and passaged to confluent monolayers of ED (equine derma epithelium cells), EBK (embryonic bovine kidney cells), NBL-6 (equine derma fibroblasts), PK-15 (porcine kidney cells), and Vero cells (monkey kidney cells). The cultures were observed daily for CPE and harvested by freezing and thawing if 50% CPE was attained or after 7 days of incubation, respectively. Non-infected cell cultures served as controls. After three consecutive passages the supernatants of cultures showing CPE were tested for hemagglutination (HA) using guinea pig erythrocytes.

Nine parts of infective culture supernatant were mixed with one part of chloroform and incubated one hour at room temperature. After centrifugation at 1000×**g** for 10 minutes the supernatant was assayed for infectivity by inoculation onto confluent Vero cells. For measuring the effect of iododeoxyuridine (IUDR) on the virus replication, virus suspensions were adsorbed to confluent Vero cells in 24-well plates for one hour, then washed twice with PBS and further incubated in MEM supplemented without (control) or with IUDR in a concentration of 50 μg/ml. When CPE occurred the supernatants were harvested, clarified by low

Table 1. Antisera used for western blot analysis

Specification	Origin
Anti-porcine virus isolate	rabbit (this work)
Anti-BRSV	cattle (this work)
Anti-canine PI-2	rabbit [4]
Anti-bovine PI-3	cattle (this work)

speed centrifugation and tested for viral infectivity. In both experiments reovirus type 1 and pseudorabies virus served as controls.

Concentrated virus grown in Vero cells was centrifuged through a 30–60% sucrose gradient using a SW41 rotor (Beckmann, 100,000 $\times$ **g**, 18 h, 4 °C). The virus was harvested with the Fraction Recovery System (Beckmann) and detected at 280 nm in a flow photometer (Uvicord SD, Pharmacia). The sucrose concentration was determined with an Abbé refractometer (Zeiss).

Virus suspensions were adsorbed to formavar coated copper grids and negatively stained with a 1% solution of phosphotungstic acid in PBS and examined in a Zeiss EM 10.

Concentrates of the following viruses were employed for western blot analysis: Unknown porcine virus isolate (propagated on Vero cells), bovine respiratory syncytial virus[1] (strain 375, EBK cells), parainfluenza virus type 1[2] (avian strain, allantois fluid), parainfluenza virus type 2[2] (SV5, Vero cells), bovine parainfluenza virus type 3[1] (strain SB, EBK cells), parainfluenza virus type 3[2] (porcine isolate, PK-15 cells), and Newcastle disease virus[2] (strain Montana, Vero cells). Virus samples containing 3 μg of protein (BioRad protein assay) were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) under non-reducing conditions on slab gels (BioRad Mini Protean). The polyacrylamide concentrations were 7.5% for the separating gel and 4% for the stacking gel. As controls un-infected Vero and PK-15 cells were used. Electrophoresis was performed in the discontinuous buffer system of Laemmli (1970) [9]. The viral antigens separated by SDS-PAGE were transferred to Immobilon-P membranes (Millipore) by western blot using the semi-dry technique described by Towbin (1979) [19]. The membranes were stained by using the antisera listed in Table 1 as primary antibodies and corresponding IgG(H+L) alkaline phosphatase conjugates (KPL) as secondary antibodies. The membranes were developed in a NBT/BCIP solution.

After one week of cultivation, the primary lung tissue culture showed a slowly spreading CPE in form of shrinking cells. After passaging to PK-15 cells, CPE occurred 6 days after inoculation which was characterized by destruction of

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Table 2. Sensitivity of the porcine paramyxovirus isolate to chloroform and iododeoxyuridine (IUDR)

	Chloroform		IUDR	
	10%	0%	50 μg/ml	0 μg/ml
Porcine paramyxovirus isolate	<1.5[a]	>7.5	6.25	6.5
Reovirus type 1	6.0	6.5	6.5	6.75
Pseudorabies virus	<1.5	5.0	2.5	6.25

[a] Viral infectivity in $\log_{10}$ $TCID_{50}$/ml

approximately 10% of the monolayer cells. Further passages did not enhance the CPE. First signs of CPE on Vero cells could be detected 3 days after inoculation. The cells were shrinking, their boundaries became invisible and some cell fusion appeared. 24 h later the CPE proceeded with cell deaths. After 4 to 6 passages 40% to 100% of the cell monolayer was affected. However, neither CPE nor other signs of virus replication could be detected in the other cell cultures examined (EBK, ED, NBL-6). Supernatants of the third passage in PK-15 and Vero cultures hemagglutinated guinea pig red blood cells up to titres of 1:64.

Treatment with chloroform inhibited the virus infectivity of the porcine isolate completely. IUDR did not influence the virus replication (Table 2). Examination of the virus infectivity in fractions collected after sucrose gradient centrifugation revealed a peak at a density of 1.19 g/cm^3 (42.5 to 43.5% sucrose). Viral particles identical to paramyxoviruses were identified by electron microscopy. The virions were pleomorphic and contained nucleocapsids of helical symmetry. The size of the particles ranged from 175 to 250 nm, the filamentous nucleocapsids were 18 nm in diameter (Fig. 1).

The western blot analysis revealed a strong reactivity of the rabbit antiserum raised against the isolated paramyxovirus to its homologous virus and PI-2. No cross reactions to the other viruses were visible. The rabbit anti-canine PI-2 serum gave an identical activity pattern: cross reaction between the porcine virus isolate and PI-2, no reaction to the other viruses. The serum from BRSV vaccinated cattle reacted strongly with BRSV and to a less extent with both PI-3 strains. The latter, the porcine as well as the bovine PI-3-isolate were clearly detected by the homologous bovine antiserum attained from PI-3 vaccinated cattle. Some cross reactions of these antisera to PI-1 were observed.

Objective of this study was to characterize a viral agent isolated from fetal lung tissue of the unborn offspring of a sow showing PRRS-like symptoms. The agent was identified as an enveloped, hemagglutinating RNA virus, with the morphological characteristics of a paramyxovirus. Cells from monkeys (Vero) and pigs (PK-15) were permissive for the virus. By means of western blot analysis, a strong cross reactivity to SV 5, the prototype strain of parainfluenza virus type 2, was demonstrated. To our knowledge, the isolation of PI-2 from pigs has not

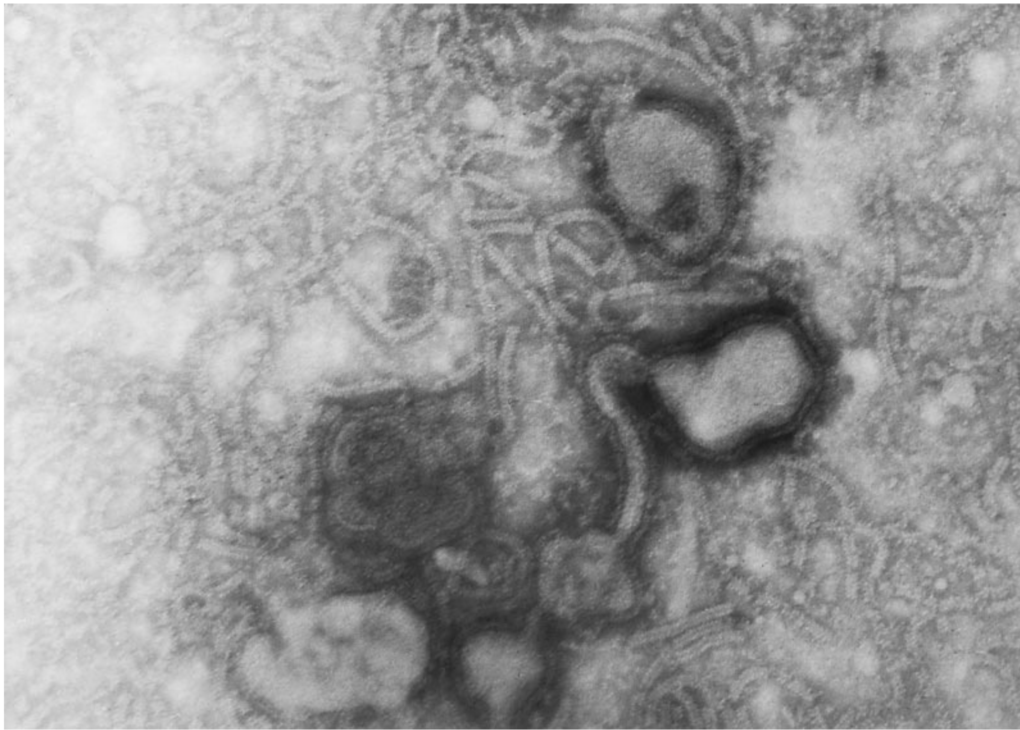

Fig. 1. Electron micrograph of partially disrupted virus particles of the porcine isolate

yet been reported. SV5 was first isolated from rhesus and cynomolgus monkey kidney cell cultures [8]. The natural host spectrum of SV5 and related viruses includes man, monkeys, and dogs [5]. Additionally, cats and laboratory rodents were shown to be susceptible to experimental infections [15]. Between human, simian and canine isolates no significant antigenic differences were observed [13]. In dogs, the natural infection of PI-2 seems to be restricted to the upper respiratory tract, although the isolation from cerebrospinal fluid has been reported [4]. The porcine paramyxovirus LPMV, now classified as a *Rubulavirus*, was isolated from cases of blue-eye disease in Mexico where clinical findings similar to PRRS were described. This isolate was serologically distinct from other members of the family *Paramyxoviridae* [11]. Highest homology of 41–46% of the deduced amino acid sequence of the hemagglutinin-neuraminidase and the matrix protein gene, however, was found in mumps virus and simian virus 5 [1, 17]. Furthermore, the occurrence of a paramyxovirus has been reported in connection with PRRS in 1992 [2]. It has not been characterized in detail, but it could be clearly distinguished from LPMV [7]. Even though this virus seems to be widely distributed in Germany, its significance for the induction of PRRS-like symptoms remains still unclear.

We are continuing our work on the immunological response of swine to mixed infections including PI-2-infections, to prevent infertility and abortions in sows.

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