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Rapid Diagnosis of TGEV-Like Coronavirus in Fattened Pigs by Indirect Immunofluorescence Labelling in Nasal Cells

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With 2 figures and 2 tables

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Summary

Diagnosis of TGEV-like coronavirus was made by an immunofluorescence technique using monoclonal antibodies directed against TGEV. Six, 15 weeks-old pigs were inoculated intratracheally with the strain P 6008 ST 4. No clinical sign was observed during the course of the infection. Viral antigens were detected in the cytoplasm of nasal cells obtained from swab in five animals as early as one day p. i. and during several days. The sensitivity and specificity of this method appeared to be comparable to virus isolation of this cell-adapted TGEV-like strain.

Key words: Diagnosis, TGEV-like coronavirus, nasal cells, immunofluorescence

Introduction

In the last few years, a great number of fattening pig herds in France and in different countries showed seroconversion against the transmissible gastro-enteritis (TGE) coronavirus without clinical digestive signs. In the infected herds, about hundred per cent of the animals are contaminated in few weeks. The fast virus spreading is probably in relation with an infection by the respiratory way (Brown et al., 1986; Jestin et al., 1987; Pensaert et al., 1986). The causal agent has been isolated in cell culture in Belgium, in Denmark, in Great-Britain (see in Jestin et al., 1987), and in France (Duret et al., 1988). The virus was shown to be able to multiply in the respiratory tract, not in the digestive tract (Pensaert et al., 1986). These results confirmed the existence of a respiratory porcine coronavirus which is related to TGE virus. Antigenic studies showed that this coronavirus is strongly related to TGE virus. The three non-structural proteins are quite similar, nevertheless differences in the epitopic map have been identified in protein E1 and E2 (Laude et al., 1988). The diagnosis of this new agent by isolation in cell culture or seroconversion is time consuming. We have recently reported the rapid detection of influenza and Aujeszky's disease viruses in nasal cells by immunofluorescence (Onno et al., 1988; Jestin et al., 1988) here we present evidence showing that the rapid diagnosis of this TGEV-like coronavirus is possible in experimentally infected pigs.

Material and Methods

1. Virus

The strain P 6008 ST 4 of the TGE-like coronavirus was isolated in December 1986 in one herd in Brittany (DURET et al., 1988). The seroconversion was associated in the herd with respiratory symptoms, hyperthermia and anorexia, without gastro-enteritis signs. The infectious titre of the viral suspension was $10^{5,9}$ $TCID_{50}$/ml on swine testis cells (ST).

2. Experimental infection

Six 15 weeks-old SPF pigs received intratracheally 10^6 $TCID_{50}$ under 10 ml. Six control animals were inoculated with MEM medium under the same conditions. The rectal temperature was recorded daily before and after inoculation. Each animal has been weighed weekly from the second week before inoculation to the third week post-inoculation (p. i.).

3. Preparation of nasal cells

Cotton swabs were taken daily from the nasal cavities for nine days p. i. except the 8th day. The swabs were allowed to elute in 2 ml of MEM medium plus antibiotics (1,000 IU/ml Penicillin, 1 mg/ml Streptomycin). Immediately upon arrival in the laboratory the swabs were shaken and the half part of the medium containing the resuspended cells was transferred into a sterile centrifuge tube, used in virus isolation experiments. The remaining part of the suspension was centrifuged at 400 × g for 10 minutes at 4 °C to spin down the cells. The cell deposit were suspended in 2 ml of phosphate buffer saline (PBS) (pH : 7.4) by gentle pipetting. After a second centrifugation step, the resulting cell deposits were resuspended in 100 µl of PBS. 25 µl cell suspension containing about 20,000 cells were spread on an area of four slides for immunofluorescence[1]. The cell smears were then processed for IF either the same day or the following day after freezing at − 70 °C.

4. Antibodies and immunofluorescence staining

Swine polyclonal antibodies against TGE viruses produced by injection of PURDUE strain and seven monoclonal antibodies (Mabs) directed against TGE virus were used. The characteristics and polypeptid specificity of anti-TGEV Mabs have been described (LAUDE et al., 1986). The hybrids producing monoclonal antibodies have been obtained from the Institut National de la Recherche Agronomique and were multiplied in our Laboratory according to standard methods. Ascitic fluids prepared in 2 month old, pristane pretreated, Balb c mice were centrifuged for 5 min at 13,000 × g and stored in aliquots at − 20 °C.

5. Immunofluorescence staining

The Mabs giving good patterns of fluorescence were used for staining the nasal smears. Fluorescein isothiocyanate conjugated anti-mouse globulins were obtained from diagnostic Pasteur.

The smears were incubated in presence of 20 µl of the appropriate dilution in PBS pH 7.4 of the ascitic fluid. The slides were incubated in a humidified chamber at 37 °C for 30 min, washed in PBS for 10 min and dried. The smears were then detected with 20 µl of a 1 : 100 dilution of the conjugate with Evans blue as counter stain (0.04 %). The slides were reincubated, washed, dried and mounted with buffered glycerol.

The slides were examined under a UV microscope at magnification of × 400. Smears samples were recorded as positive when at least on 20,000 examined cells in each cupule, two definite specifically steppled fluorescent nasal cells were seen.

6. Virological and serological tests

For isolation of the TGE-like coronavirus. 0.1 ml of nasal secretion was inoculated in ST cells. Three passages were made before the specimens were considered as negative. Seroneutralization tests were carried out on paired sera collected before inoculation and two weeks later (TOMA and BENET, 1976).

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[1] Biomerieux.

Results

Seven Mabs against TGE coronavirus were tested for the detection of the TGE-like coronavirus by immunofluorescence in infected cells (Table 1). Four Mabs gave fluorescent cells but only two antibodies, IV 5.1 and VII 20.9, were revealed as good candidates for diagnosis. They could be used at dilution 1 : 250 and 1 : 500, respectively. Viral antigens were detected in the cytoplasm in fluorescent stipples form.

Table 1. Detection of TGE-like coronavirus by indirect IF assays with Mabs in cells collected from infected animals

Specificity	Mab		IF
NP (nucleoprotein)	IV	5.1	+(C)
	IX	22.6	+(C)
gp E_2 (Spicule)	VII	20.9	+(P)
	XV	51.13	+(P)
	XV	3b.5	–
gp E_1 (membran)	VII	25.22	–
	XV	49.22	–
Polyclonal serum			+(C)

C: fluorescence is located in the cytoplasm. P: fluorescence is located at the periphery of the nucleus.

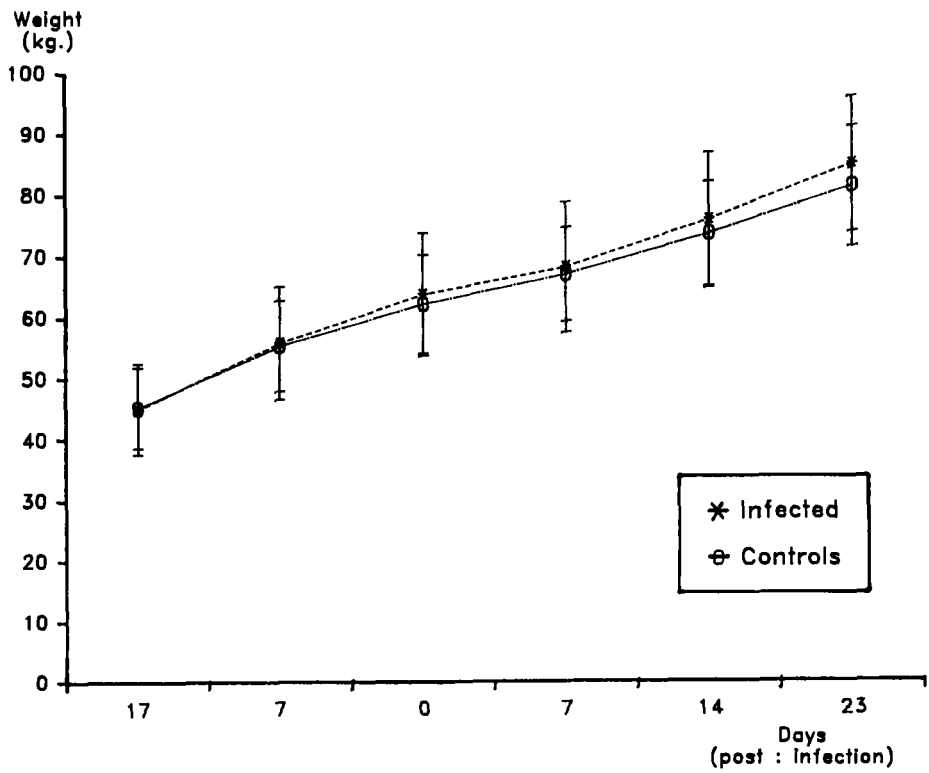

Fig. 1. Growth of the pigs from the control and TGEV-like inoculated groups

These two Mabs were used mixed for the detection of the TGE-like coronavirus antigens in nasal cells of experimentally infected animals. To improve the method as a diagnostic tool for the TGE-like virus respiratory infection, the aspect of fluorescence in the cells and the kinetic of their occurrence in the nasal cavities were described and studied. Granular fluorescence was observed in the cytoplasm of epithelial cells. The number of fluorescent cells was high and in some collected samples more than fifteen positive cells on 20,000 deposit cells were positive. The positive cells occurred in the nasal cavities from first post-inoculation day to the 7th p.i. days. No fluorescent cell was found in the swabs collected from the control pigs.

In five positive samples by VI test, no fluorescent cells were detected. In three positive samples by IF test no virus was isolated. TGE neutralizing antibodies appeared in the sera of 5 among the 6 pigs of the infected group as soon as the 7th day p.i. The neutralizing TGE antibodies titers were high and homogeneous in the sera collected the next 2 weeks (Fig. 2).

No clinical sign was observed after inoculation. Rectal temperature remained normal as well in the infected group as the control one (Table 2). The growth of the pigs decreased in the 2 groups. No significant statistical difference could be demonstrated between the growth of the pigs in the 2 groups (Student and Colin-white tests: $p = 0.01$) before and after inoculation. Anesthesia was not probably responsible for the observed slackening of the growth rate.

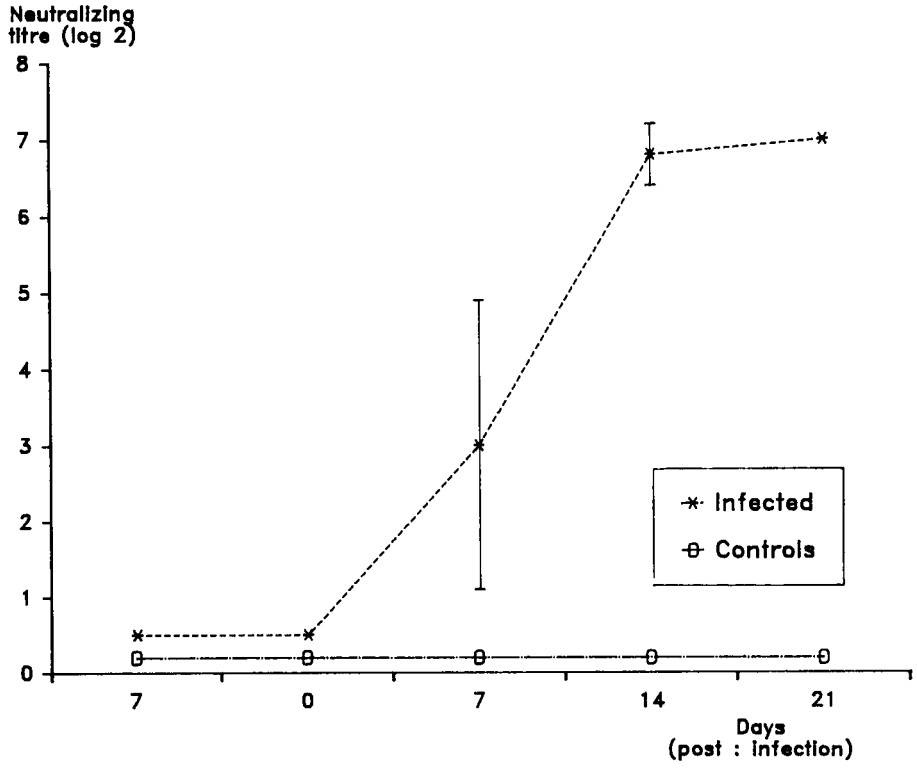

Fig. 2. Kinetic of TGEV neutralizing antibodies (mean titre = SEM) in the sera of TGEV-like virus and mock-infected pigs

Table 2. Detection of TGE-like coronavirus by immunofluorescence (IF) or viral isolation (ISO)

No Pig	no 1 IF	ISO	T	no 2 IF	ISO	T	no 3 IF	ISO	T	no 4 IF	ISO	T	no 5 IF	ISO	T	no 6 IF	ISO	T
J_0	–	–	39,7	–	–	39,2	–	–	39,2	–	–	39,2	–	–	39,2	–	–	39
J_1	+++	+	40,6	++++	+	40,2	–	–	39,3	+++	+	39,5	++++	+	39,5	++++	+	39,5
J_2	++++	+	39,5	+++	+	39,2	++++	+	39,3	+	+	39,6	++++	+	39,4	++++	+	39,8
J_3	–	+	38,9	–	+	39,2	++++	+	39,1	–	+	39,2	++	+	38,9	++	+	38,9
J_4	++	+	39,1	++	+	39,1	++	+	39,8	++++	+	39,5	++	+	39,1	+	–	39,5
J_5	++++	+	38,9	++++	+	39,3	+++	+	39,5	++	+	39	++++	+	39,3	+++	+	39,3
J_7	++	–	39,1	+	–	39,2	–	+	39	–	–	39	–	–	39,4	–	–	39,6
J_9	–	–	ND	–	–	ND	–	+	ND	–	–	ND	–	–	ND	–	–	ND

T = rectal temperature; ISO = virus isolation; IF = indirect immunofluorescence; ND = not determined; + = 1 < 5; ++ = 5 < 10; +++ = 10 < 15; ++++ = 15 .035.

Discussion

It is possible to detect the TGE-like coronavirus with Mabs directed against TGE coronavirus in infected cells by immunofluorescence. This technique has been used to detect TGE virus on frozen sections of small intestine using polyclonal antibodies, it was possible to make a specific diagnosis within two hours after collecting samples (PENSAERT et al., 1968). The positive cells are characterized by fluorescent stipples around the nucleus or in all the cytoplasm. For the detection of viral antigens in nasal cells, we have selected two Mabs specific of the peplomer protein E_2 and of the nucleoprotein. These results are in agreement with *in vitro* studies on TGEV-like virus (LAUDE et al., 1988).

Monoclonal antibodies are very promising for convenient use in rapid diagnosis because of the absence of non-specific immunofluorescence in control animals, and the possible ease of standardization for others respiratory viruses (JESTIN et al., 1988). In this experiment this technique appeared as sensitive as virus isolation or serological tests. It allowed to detect the virus few hours after the infection and several days after the inoculation. It should be stated, however, that the strain used is a tissue-culture adapted virus. Thus it might be necessary to get additional informations in the conditions of the fields concerning the most convenient time of sampling.

In our experimental conditions the infection of animals was not found to be associated with symptoms, anorexia and hyperthermia, such as recently described by other authors (DURET et al., 1988).

The diffusion of the new respiratory coronavirus among the fattening herds cause problems for the serological diagnosis of TGE coronavirus infection. The monoclonal antibodies that we have used are not specific of the TGEV-like coronavirus and do not permit a differential diagnosis between the respiratory and the enteropathogenic strains. Nevertheless by the use of Mabs in competition ELISA test, it is possible to differentiate TGEV from TGEV-like infected pigs (Per HAVE, personnal communication). Alternatively it might be interesting to prepare specific monoclonal antibodies against the TGEV-like coronavirus.

This technique used Mabs specific for all isolated strains of TGEV-like coronavirus (LAUDE et al., 1988). Thus, it would be possible to use these reagents in herds in different countries.

A specific and rapid diagnosis can be a great help for both management of the herds and control of TGEV-like coronavirus spreading. The above results demonstrate that rapid diagnosis of the TGE-like coronavirus by immunofluorescence staining of nasal cells is feasible. The preparation of cell smears is simple and practicable in most laboratories. A

diagnosis of TGEV-like coronavirus infection can be achieved on the day of collection. The sensitivity of this method has been found to be larger than the current techniques of virus isolation. For these reasons this method seems to be suitable for the epidemiological surveillance of coronaviruses.

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Zusammenfassung

Die schnelle Diagnose von TGEV-ähnlichem Coronavirus bei Mastschweinen mittels indirekter Immunofluoreszenz von Zellen aus Nasentupfern

Die Diagnose von TGEV-ähnlichem Coronavirus erfolgte mittels einer Immunofluoreszenztechnik, die monoklonale Antikörper gegen TGEV anwendet. Sechs Schweine im Alter von 15 Wochen wurden intratracheal mit dem Virusstamm P 6008 ST 4 geimpft. Während der Infektion wurden keine klinischen Erscheinungen beobachtet. Im Zytoplasma von Zellen, die aus Nasentupfern gewonnen wurden, konnte bei 5 Tieren schon ab dem ersten Tag nach der Infektion Virusantigen nachgewiesen werden. Die Empfindlichkeit sowie Spezifität dieser Methode waren vergleichbar mit der Isolierung von TGE-Virus.

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