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Isolation of a porcine respiratory, non-enteric coronavirus related to transmissible gastroenteritis

M. Pensaert, P. Callebaut, and J. Vergote¹

SUMMARY A porcine respiratory, non-enteric virus which is related to the coronavirus transmissible gastroenteritus virus (TGEV) has been isolated in pigs and in cell culture. The isolate was designated TLM 83.

It has become very widespread and enzootic among the swine population in Belgium and in other swine raising countries. It causes an infection of the lungs and appears to spread by aerogenic route. It does not replicate in the enteric tract. The experimental infection in conventional and gnotobiotic pigs in isolation remains subclinical. The infection, either experimental or in the field, results in the formation of antibodies which neutralise the classical enteric TGEV. Based on this relationship, this virus is assumed to be a new TGEV-related porcine respiratory coronavirus or TGEV itself which has totally lost its tropism for the enteric tract.

INTRODUCTION

Transmissible gastroenteritis (TGE) in swine is caused by a coronavirus, called transmissible gastroenteritis virus (TGEV), which was first isolated in 1946 (1). The virus induces diarrhoea in swine of all ages, and pigs infected during the first week of age usually die from dehydration. TGEV has a worldwide prevalence. The pathogenesis of TGE has been studied in detail and the virus replicates primarily in absorptive epithelial cells covering the small intestinal villi. Virus replication in these cells results in degeneration, villous shortening, and diarrhoea. TGEV is, therefore, a specific enteropathogenic virus (4). It has, however, also been reported to be able to replicate in the tonsils and in the respiratory tract (3, 6). This respiratory infection with field strains of TGEV appears to be secondary to the enteric infection and does not result in respiratory disorders. Field strains of TGEV which replicate in the respiratory tract always infect the enteric tract in the same host.

In the authors' laboratory, serological surveys have been carried out at regular time intervals between 1968 and 1984 on sow sera, collected in slaughterhouses, for the purpose of determining the prevalence of the TGEV in Belgium. The percentage of sow sera with neutralising antibodies varied between 12 and 24%, and a certain degree of correlation was present with the number of clinical TGE outbreaks reported and diagnosed during the winter preceeding the survey.

In May 1984, however, such correlation was not seen in another survey in which 68% of total of 265 sow sera were found to have seroneutralising (SN) antibodies against TGEV, even though the number of outbreaks in the previous winter had not been higher than usual. Vaccination against TGE is not performed in Belgium. This high incidence of antibodies among the Belgian swine population in the absence of clinical TGE outbreaks suggested to us that a change in the virus or in its epizootiologic behaviour had taken place.

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Similar observations of a high incidence of TGE antibodies in the swine population without disease have been made in other countries such as the Netherlands (8) and Denmark (2) but no explantation was available.

Research was conducted to elucinate this problem and the results will be reported here chronologically.

SEROLOGICAL EXAMINATIONS ON CLOSED BREEDING FARMS

Sera were collected in October 1984 from primiparous and multiparous sows on 11 closed breeding farms which had not experienced a clinical TGE outbreak for at least 4 years. All the sows on 10 to 11 farms had SN antibodies against TGEV (Purdue strain) in the microtitre neutralisation test (7). This finding was surprising, considering the absence of a history of TGE. Enzootic TGE was not likely to be present, since feeder pigs and fattening swine on several of these farms were devoid of TGE antibodies at the time of the examination. It could, however, not entirely be excluded, even though evidence existed that TGEV or a TGEV-related virus had caused infection without disease on these farms but had disappeared again, leaving the younger swine with no antibodies. Moreover the 11th farm, which was negative for TGE antibodies in October, had become positive in January and no diarrhoea had been observed.

A thorough serological examination and follow-up was subsequently carried out on 5 of these farms in an attempt to determine the nature of this TGEV-like infection. The number of sows on these 5 farms varied from 45 to 200. The examination was started in February 1985 and was continued until February 1986. A total of 49 groups of pigs (5-6 pigs per group) were serologically followed monthly, starting at the time of weaning (age 4 weeks) until the end of the fattening period.

Seroconversion against TGEV occurred on all 5 monitored farms in the pigs weaned during the months of February to April 1985. Pigs weaned between May and November, however, dit not seroconvert on 4 of the 5 farms (nrs 1, 2, 3, 5). These 4 farms had temporarily become free of the TGElike agent but they were reinfected again in December 1985. No abnormal diarrhoeal problems were observed throughout the year on these farms.

On one farm (nr 4), the infection remained present throughout the year 1985. This farm was followed more closely to determine the age of the pigs at which the infection took place.

The age until which the maternal antibodies persisted in pigs from TGE-sero positive mothers was first determined in the feeder pigs on the four farms on which the infection had disappeared. The results of this serological study showed that the antibodies of maternal origin were detectable in 100% of the pigs at the age of 3 to 6 weeks (geometric mean SN titre-GMT of 35,5), in 84% at 7 to 10 weeks (GMT of 7,9), in 29% at 11 to 14 weeks (GMT of 4,4) and in 1,5% at 15 to 18 weeks. It was thus concluded that maternal antibodies remained detectable at the most until 16 weeks of age and that the mean half-life was 12,04 days. Any deviation from this rate of decline was considered a result of infection. To determine at what age the infection took place in pigs on farm 4, the antibody titres were followed in 10 groups of pigs. A deviation of the normal maternal antibody decline and formation of active antibody titres occurred in all the groups. Maternally derived antibodies in the blood gradually changed into actively formed antibodies, proving that an infection with the TGE-like virus had taken place. From the slopes of the curves, it was concluded that 8 of the 10 groups had been infected prior to the age of 3 monts, 4 of the 8 had been infected within one month after weaning. All the animals had antibodies at the end of the fattening period (5 months) and the SN titres varied from 2 to 192. It was concluded from these results that the TGE-like virus was enzootic in farm 4.

A preliminary identification attempt of this TGE-like agent was made by the examining of 26 sow sera from the farms mentioned above. In these sera, the SN titre was determined not only against TGEV but also against 2 other coronaviruses: feline infectious peritonitis virus (FIPV) and canine enteric coronavirus (CCV), which are known to be related to TGEV (5). It was found that the SN titres against FIPV and CCV were respectively 5 and 3 times lower than those against TGEV. These results indicated that the agent was most closely related to TGEV and that it was neither a FIPV nor a CCV which had possibly adapted to swine.

ATTEMPTS TO DEMONSTRATE THE TGEV OR THE TGE-LIKE VIRUS IN FAECES

In order to exclude an enzootic enteric TGE viral infection, pooled faecal samples were collected from each of the 49 groups of pigs which were followed serologically on the 5 farms mentioned above. The samples were collected monthly and stored at -25° C. Only those samples which had been collected from groups of pigs that afterwards proved to have seroconverted against TGEV, and particularly those collected 1 and 2 months prior to seroconversion, were examined for the presence of TGE viral antigens. These amounted to a total of 173 pooled samples. Additionally, 275 faecal samples were collected from individual animals which had loose stools or diarrhoea during the period of experimentation on the farms.

All faeces samples were subjected to an ELISA test which is routinely applied for demonstrating TGEV in faecal material. The technique of this ELISA will be reported later but it is essentially a blocking test which has been proved to be specific, susceptible, and very reliable for the detection of the classical enteric TGEV in faeces of experimentally and field infected swine of all ages. None of the faecal samples examined from the groups or from the individual animals on the 5 farms were positive for TGE viral antigens.

Pooled ELISA-negative faecal samples from several groups of pigs of farm 4, which had been collected one and two months prior to seroconversion, were orally inoculated in 4 pigs which had no antibodies against TGEV and which were kept in individual isolation. None of the pigs became seropositive for TGEV, confirming that TGEV or a TGE-like virus was not present in the faeces.

These results excluded the presence of an enzootic infection with the enteric TGEV

and pointed towards the existence of a TGEV-related virus or a TGE-like virus without tropism for the enteric tract.

DEMONSTRATION OF THE TGE-LIKE VIRUS IN SENTINEL PIGS ON FARM 4

Eleven conventional 6 week-old sentinel pigs, without SN antibodies against TGEV, were introduced on farm 4, on which it had been shown that the infection with the TGE-like virus persisted throughout the year. They were intermingled with 30 freshly weaned pigs that had been born on the farm. Seven sentinel pigs were killed, one each at respectively 3, 5, 7, 9, 11, 14, and 17 days after inoculation and tonsils, trachea, lungs, and small intestine with its contents were collected for further examination. The 5 remaining pigs were used for serological follow-up. Also, faecal samples were collected every two days from the sentinel pigs.

The surviving pigs had built up SN antibodies against TGEV at 14 days after the introduction (titres of 3 to 24), proving that infection with the TGE-like virus had occurred in the sentinel pigs soon after their introduction. No clinical disease was observed. All 45 faecal samples collected were negative in respect of TGE viral antigens in ELISA, and no fluorescence was observed, with a TGEV conjugate, in the intestines of the sacrificed pigs. These results allowed the conclusion that infection with the TGElike virus had occurred in the sentinel pigs and confirmed that it was non-enteric.

VIRUS ISOLATION IN PIGS AND IN CELL CULTURES

A pool was made of suspensions (25%) of the respiratory tract tissues from sentinel pigs which had been sacrified at 3 and 5 days and at 7 and 9 days after introduction respectively. These suspensions were oronasally inoculated into two 10-day-old pigs which were devoid of antibodies against TGEV and which were kept in individual isolation. The pigs remained healthy and built up SN antibodies against TGEV (titres 192 and 96) within 14 days after the inoculation. Another pig which was orally inoculated with pooled intestinal contents of the sentinel pigs killed at 5 and 7 days remained seronegative for TGEV.

A second virus isolation experiment was performed in a colostrum-deprived hysterectomy-derived piglet (nr 1490) inoculated at the age of 17 days with respiratory tract suspension of the sentinel pig which had been sacrified 5 days after the introduction on the farm. The pig 1490 remained healthy but seroconverted against TGEV and had a SN titre of 256 at 21 days after inoculation. From these isolation experiments in pigs, it was clear that the TGElike virus was present in the respiratory tract of the sentinel pigs killed between 3 and 7 days after the introduction on the farm. Tonsillar swabs were collected daily from pig 1490 and were used for virus isolation in cell cultures.

Virus isolation attempts were made in primary pig kidney cells from the respiratory tract tissue suspensions of the same sentinel pig (killed 5 days) and from the tonsillar swabs of pig 1490. The attempts yielded a cytopathic (CP)agent. The CP agent was designated TLM 83. With this CP agent, preliminary identification studies have been performed both in cell cultures and in colostrum-deprived piglets.

Infected cell cultures show fluorescence with a conjugate prepared against TGEV but not against the other 2 porcine coronaviruses, namely hemagglutinating encephalitis virus and porcine epizootic diarrhoea virus. The isolate TLM 83 was neutralised by sera collected from experimental pigs which were inoculated with respiratory tissue suspensions and which seroconverted against TGEV. It was also isolated from the tonsillar swabs collected from pig 1490 between 3 and 7 days after inoculation.

The cytopathic effect in primary pig kidney cells is characterised by clustering of rounded up cells and becomes visible at 2 days after inoculation. Even though it progresses, it does not involve the entire monolayer and remains limited to a number of foci of degenerated cells. The CP virus does not have any haemagglutinating or haemadsorbing capacity. Up to 6 serial passages have been made successfully and a titre of $10^{3.5}$ TCID₅₀ per 0.5 ml of tissue culture fluid can be obtained. The CP virus, TLM 83, induces a subclinical infection upon oronasal inoculation of colostrum-deprived piglets. These pigs build up antibodies which neutralise TGEV. Pigs inoculated with the CP agent TLM 83 and sacrificed 3 or 4 days later show immunofluorescence with a TGEV conjugate in the lungs but not in the small intestine. With the intestinal contents of these latter sacrificed pigs, the infection can be reproduced neither in other pigs nor in cell cultures. It is, therefore, clear that the isolate TLM 83 is the respiratory TGElike virus.

GENERAL CONCLUSIONS

Based on the experiments described, it can be concluded that the isolate, TLM 83, is a respiratory but non-enteric virus which is related to the classical enteric coronavirus, TGEV. It is not pathogenic for conventional or gnotobiotic pigs in isolation. It causes a cytopathic effect in primary pig kidney cells. The respiratory infection can be reproduced with the CP isolate.

TLM 83 may be a new porcine respiratory coronavirus which is related to TGEV or it may be the classical TGEV which has totally lost its tropism for the enteric tract. It is very widespread among the swine population and has become enzootic.

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Theileria taurotragi in Zambia

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SUMMARY Theileria sp. (Bwengwa) of low virulence was isolated by feeding R. appendiculatus ticks collected from the field on a susceptible calf and subsequently transmitted between cattle by R. appendiculatus ticks- Theileria sp. (Bwengwa) was shown to be T. taurotragi on parasitological, clinical and serological grounds. T. taurotragi is the fourth Theileria sp. shown to be present in Zambia.

INTRODUCTION

Three species of *Theileria* in cattle have been reported from Zambia. The most important one, *Theileria parva*, transmitted by the tick *Rhipicephalus appendiculatus*, was first recorded in 1922 in the Northern Province (1).

T. parva parva-type parasites have been isolated from the field, whereas the presence of bovis- and/or lawrencei-types of T. parva remains to be confirmed (F. L. Musisi, unpublished). Theileria velifera was the second species reported from Zambia and was found throughout the country (8). A third species, Theileria mutans, was recently isolated and transmitted between cattle by the tick Amblyomma variegatum (7).

During studies on theileriosis in cattle in Zambia occasional mild strains of theileria were encountered. Mild theileria strains which are transmissible by *R. appendicula*- tus were identified as *Theileria taurotragi* in East Africa (5) and southern Africa (9). In this paper, we report the isolation of a mildly pathogenic *Theileria* sp. from cattle in Zambia and its subsequent identification as *Theileria taurotragi*.

MATERIALS AND METHODS

Isolation

Brown ear ticks were collected from cattle at Bwengwa, Monze District, Southern Province of Zambia and subsequently identified in the laboratory. Isolation of theilerial parasites was attempted by feeding unengorged adult *R. appendiculatus* ticks from this collection in earbags on experimental calf no. 132. Laboratory strain of *R. appendiculatus*.

A strain of *R. appendiculatus* was used which originated from a colony established at the former East African Veterinary Research Organisation, Muguga, Kenya. It was maintained by feeding on the ears of rabbits and cattle. Oviposition, hatching and moulting were performed at 28°C and 85 per cent relative humidity (rh), quiescent stages being kept at 20°C and

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