

EFFECT OF STOMACH AND SMALL INTESTINE JUICES ON INFECTIVITY OF LOW AND HIGH PASSED STRAINS OF T.G.E. CORONAVIRUS.

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Genetic markers of T.G.E. virus connected with virulence are still unknown. Previous works on thermosensitivity of viral replication in tissue culture and *in vitro* stability of viral infectivity (thermolability, stability towards digestive enzymes and at low pH) did not permit separating the low passaged strains of T.G.E. coronavirus (MOSCARI 1980, LAUDE et al 1981). Stomach and gut contents have germicide activity because of low pH, proteolytic enzymes and bile. Intensity of the virulicidal activity is dependant of age and physiologic status (fasting or not) of the animal and also of duration of virus stay in stomach and gut fluids before penetration of virus particles into susceptible enterocytes. Knowledge of virus stability in digestive fluids is important for improvement of the methods for T.G.E. vaccine administration to pregnant sows by oral route. Post vaccinal immunity induction is dependant of the virus multiplication in gut epithelium which is dependant of maintenance of infectivity of the virus during transit between buccal cavity and intestine. We have investigated the conditions of stability of virus infectivity in stomach and gut juices harvested at 3 different periods after last meal in 22 three-six months old pigs. We have compared 4 different virulent strains of T.G.E. virus having low number of passages in tissue culture (L.P. strains : MILLER, D52, 6386 and SH.15) and 3 different attenuated strains having high number of passages (H.P. strains : PURDUE 115, SH.168, 137.SG).

Material and Methods.

Tissue culture, pig Kidney cell lines, and T.G.E. virus strains have been previously described (LAUDE et al, 1981). Infectivity titration was performed in tissue culture by means of a plaque assay under agarose overlay (LAUDE et al, 1981). Coming from a serologically negative herd, pigs were slaughtered 15, 24 and 48 hours after the last meal. Stomach and small intestine contents were harvested and immediately centrifuged at +4°C (15.000 R.P.M./60 mn). Supernatant was collected and stored at -70°C. Each virus strain was diluted 1 : 10 (v/v) in aliquot of stomach or gut juice. Mixture was placed in a 37°C water bath for designated period. Then mixture was diluted in ice-cold MEM + 10 % normal calf serum and residual activity was titrated immediately.

Results. A) Stability of T.G.E. virus in stomach juice : After some minutes, H.P. and L.P. strains of T.G.E. virus were strongly inactivated (2.6-4.5 log₁₀ titer reduction) in stomach juice insofar as the pH of which is lower than 2.5. There is no evidence of differences between H.P. and L.P. strains of T.G.E. virus. There is a correlation between infectivity titer reduction and value of pH, but no with the cell passage status of virus strain. 4 out of the 4 stomach juices collected 15 hours after last meal, have pH lower than 2.5 and thereby are strongly virulicidal for T.G.E. virus. Inversely 9 out the 13 stomach juices collected 24 or 48 hours after last meal have pH upper than 2.5 and thereby are poorly virulicidal for virus (Fig.A).

B) Stability of T.G.E. virus in small intestine juice.

After some minutes, H.P. and L.P. strains of T.G.E. virus were strongly inactivated when incubated in 9 out of the 22 samples of gut juice. The 5 samples which were free of virulicidal activity have been collected in pigs killed 24-48 hours after last meal. 7 samples have moderate inactivating activity. There is no relationship between titer reduction and the cell passage status of virus strain (Fig.B).

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Discussion : Enteric viruses which multiply primarily in the small intestine, such T.G.E. virus come into direct contact with gastric and intestinal fluids. Viral resistance to gastric and intestinal contents is clearly important if infection is to succeed especially in the case of oral immunization with live virus vaccine.

Our results bring good evidence of fragility of the T.G.E. coronavirus in stomach and intestine fluids of 3-6 months old pigs. But all samples of digestive fluids collected at different periods after last meal were not inactivating : 8 out the 18 samples of stomach juice and 5 out of the 22 samples of intestine juice are devoid of virulicidal activity. Factors influencing the intensity of virulicidal activity in digestive fluids remain to be investigated.

Our results bring new data on properties and behaviour of the T.G.E. coronavirus about which it will have to take into consideration for carrying on immunization of sow by oral route : On oral vaccination with live virus, from these data, we have to investigate conditions for maintenance of infectivity of viral suspension during its transit between buccal cavity to jejunum and ileum susceptible enterocytes. Then the right thing to do is : a) either to protect virus particles with special coating (capsule) or either to use a virus strain or mutant resistant to proteolytic enzymes and to low pH.

b) to modify physiologic status of the pig digestive tract (by fasting or pharmacological treatment) so as to insure a better survival of virus in stomach and intestine fluids.

Conclusion : stomach and intestine contents inactivated low and high passaged cell culture strains of T.G.E. coronavirus. The present findings suggest also that stability of T.G.E. virus infectivity in stomach and intestine juices could depend on physiologic status of digestive tract. We conclude that : 1) Behaviour of T.G.E. virus infectivity in digestive tract fluids is not involved in genetic markers of virus attenuation by cell culture passages. 2) Whatever the strain (H.P. or L.P.) T.G.E. virus infectivity is highly fragile in digestive tract contents of 3-6 months old swine.

Selected references : Furuuchi, S., Shimizu, Y. and Kumagai, T. : Natl. Inst. Anim. Health 1975, 15 : 159; Hess, R.G. and Bachmann, P.A. : Infect. Immun. 1977, 13 : 1624 ; Laude, H., Gelfi, J. and Aynaud, J.M. : Am. J. Vet. Res. 1981, 42 : 447 ; Mocsari, E.; Acta. Vet. Hung. 1980, 28 : 341.

