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PSEUDORABIES VIRUS STABILITY IN BOAR SPERM

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Pseudorabies virus (PRV) is a significant health as well as an economical problem in the swine industry. The virus can be isolated from the sperm of latently infected boars which, in the case of artificial insemination, accentuates the possibility of PRV spread. This fact is even more important in the field, where insemination is without control.

It has been shown that PRV is able to survive for 48 hours from the time of ejaculation in sperm taken and processed for artificial insemination (AI). After that period the sperm showed a toxic effect on cells in tissue culture which made further testing impossible.

Key words: pseudorabies, sperm, ejaculate

INTRODUCTION

Pseudorabies (PR) is an acute infective disease of warmblooded animal species. Man can be infected albeit seldom. The predominate clinical signs are due to the effect of pseudorabies virus (PRV) on the central nervous system. Localized pruritus is present except in swine where clinical signs vary depending on the age. Young piglets usually die suddenly, pigs up to 12 months of age have CNS symptoms but mature animals may have a latent infection, which is the major problem in diagnosis as well as in eradication of PRV from swine. Although other animal species seldom get PR, infection is usually fatal.

PRV belongs to the Herpesviridae family (alphaherpesviridae) and, although there is much Variability of virulence between isolates, there is just one serotype (Selivanov and Sedov, 1986). PRV is rather resistant to chemical and physical agents and to pH changes.

Swine become infected via the respiratory route (most often), per/os, during natural mating or AI. Virus is excreted through nasal and vaginal secretions, semen, milk and sometimes urine and feces (Van Oirschot and Gilkens, 1987). The duration of virus excretion depends on the age of the swine; e.g. mature swine for more than 180 days and piglets up to 6 months of age for more than 310 days (Selivanov, and Sedov, 1986).

The abovementioned as well as other data indicate (considering the specificities of AI in swine) that the viability of PRV in ejaculates is a very important factor in pseudorabies epidemiology. The aim of this paper was to investigate how long PRV can persist in the ejaculate which is used for AI.

Arteficial insemination technology followed the already described procedure (Miljković, 1979). Virus viability was checked after 15 and 30 minutes, 1, 2, 3, 4, 5, 6, 7, 8, 24 and 48 hours after obtaining ejaculates. Diluted as well as undiluted sperm was used.

MATERIALS AND METHODS

Virus: Field PRV isolate (from piglet brain was kept in liquid nitrogen. Virus was propagated and titrated in chicken embryo fibroblast tissue culture. A titer of 10_6 $TCID_{50}$ was used in the experiment. During the work the virus was kept at $+4^0C$.

Tissue culture: Primary cultures of chicken fibroblasts were grown in Eagle complete medium (Imunološki zavod Torlak) with 10% (v/v) calf serum.

Sperm: Serologically negative (serum neutralization test) boars were used as sperm donors (PKB farm "Vizelj"). According to a procedure already described (Miljković, 1979), the ejaculate was diluted (1:1) and virus suspension added (9 ml of diluted or undiluted sperm and 1 ml of virus suspension). Such a sperm/virus suspension was used in further work as the virus source for tissue culture inoculation.

Mice: Young mice (15 days old) were used for virus multiplication and virus titration using the serum neutralization test (SNT). The lethal effect of PRV was monitored.

SNT in tissue culture: In order to determine virus viability in sperm which has already been a procedure described was followed SNT in tissue culture: In order to determine virus viability in sperm an already descrabed procedure has been followed (Panjević, Đ. 1984). The cytopathological effect (CPE) of PRV in tissue culture was determined.

RESULTS AND DISCUSSION

Preliminary and control tests concerning the effect of native sperm, sperm diluent and diluted sperm on tissue culture are shown in Table 1. Table 2 shows the results for virus viability in undiluted sperm kept at room temperature (20^0 C) for up to 48 hours. Data on virus viability in diluted sperm ready to be used for AI during 48 hours are shown in Table 3.

The preliminary results indicated that undiluted sperm as well as sperm diluent did not have any pathological effect on the cells in tissue culture. Diluted sperm with PRV added, caused marked CPE for up to 24 hours from the moment of adding PRV to diluted sperm. The virus control and tissue culture control showed CPE and no effect on the cells, respectively (table 1). Twentyfour hours after obtaining the ejaculate, the semen changed in colour, smell and consistency acting toxically on the cells in tissue culture.

The results shown in Table 2 show PRV stability in undiluted sperm starting from 15 minutes up to 48 hours after obtaining the ejaculate. The PRV titer

after 15 and 30 minutes, 1, 2, 3, 4, 5, 6, 7 and 8 hours remained the same (10^6 $TCID_{50}$. After 24 hours (up to 48 hours) changes in semen (colour, smell and consistency) caused a toxic effect on the cells in tissue culture thus making further testing impossible. Such a toxic effect was present in undiluted as well as in diluted (with no virus) sperm.

Table 1. Preliminary testing of the effect of sperm, PRV and diluent on tissue culture

	TIME (hours)											
	15^x	30^x	1	2	3	4	5	6	7	8	24	48
Native sperm	—	—	—	—	—	—	—	—	—	—	—	—^{xx}
Sperm diluent	—	—	—	—	—	—	—	—	—	—	—	—
Diluted PRV infected sperm	+	+	+	+	+	+	+	+	+	+	+	+^{xx}
Virus control	+	+	+	+	+	+	+	+	+	+	+	+

(+) CPE in tissue culture
(—) no CPE in tissue culture
(x) minutes
(xx) toxic effect if sperm in tissue culture

Table 2. PRV titer in undiluted sperm.

Virus titer	TIME (hours)													
	15^x	30x	1	2	3	4	5	6	7	8	24	48	VC	CC
10 $TCID_{50}$	+	+	+	+	+	+	+	+	+	+	+	+^{xx}	+	
10_2 $TCID_{50}$	+	+	+	+	+	+	+	+	+	+	+	+^{xx}	+	10—2
10_3 $TCID_{50}$	+	+	+	+	+	+	+	+	+	+	+	+^{xx}	+	10—3
10_4 $TCID_{50}$	+	+	+	+	+	+	+	+	+	+	+	+^{xx}		10^{-4}
10_5 $TCID_{50}$	+	+	+	+	+	+	+	+	+	+	+	+^{xx}	+	10—5
10_6 $TCID_{50}$	+	+	+	+	+	+	+	+	+	+	+	+^{xx}	+	10—6

(+) CPE in tissue culture
(—) no CPE in tissue culture
(x) minutes
(xx) toxic effect if sperm in tissue culture
(VC) virus control
(CC) cell control

The PRV titer in diluted sperm kept at room temperature remained the same for the first 8 hours (10^6 $TCID_{50}$). After 24 hours the PRV titer dropped to 10^4 $TCID_{50}$. The virus titer was impossible to study after 48 hours because of the toxic effect of sperm on the cells in tissue culture. Those results as well as pserm (diluted and undiluted) and virus control results are shown in Table 3.

PRV was confirmed in infected sperm using the mouse test and SNT.

The stability of PRV in various media was so for investigated intensively reference. The virus was stable at 60°C for 30-60 minutes, 70°C for 3 minutes and 100°C for one minute. It stayed viable at +4°C for 20 weeks and for years at -40°. Such data indicate that, in pseudorabies control, virus stability in various media has to be taken in consideration. (Grandell, (1983), Valner, (1984), Kounw, (1984).

Table 3. PRV titer in diluted sperm.

Virus titer	TIME (hours)													
	15[x]	30x	1	2	3	4	5	6	7	8	24	48	VC	CC
10 $TCID_{50}$	+	+	+	+	+	+	+	+	+	+	+	+[xx]	+	
10_2 $TCID_{50}$	+	+	+	+	+	+	+	+	+	+	+	+[xx]	+	10—2
10_3 $TCID_{50}$	+	+	+	+	+	+	+	+	+	+	+	+[xx]	+	10—3
10_4 $TCID_{50}$	+	+	+	+	+	+	+	+	+	+	+	+[xx]		10^{-4}
10_5 $TCID_{50}$	+	+	+	+	+	+	+	+	+	+	+	+[xx]	+	10—5
10_6 $TCID_{50}$	+	+	+	+	+	+	+	+	+	+	+	+[xx]	+	10—6

(+) CPE in tissue culture
(—) no CPE in tissue culture
(x) minutes
(xx) toxic effect if sperm in tissue culture
(VC) virus control
(CC) cell control

The results presented in this paper show that PRV infective titer remained the same in the first 24 hours during sperm processing and preparation for AI. Proteins that are present in semen act as a protective factor for the virus titer as well. Moreover, the pH of semen (7,2) is in the range where PRV stays viable (5-8). Which means that PRV could stay infective for many years if kept under liquid nitrogen conditions. Swine ejaculate storage is still a problem. however, if boar sperm technology progresses. PRV could be another problem. The spread of the disease is so far restricted to a particular farm or small area. In the case of deep freezing of boar ejaculate such a route of disease spread could be accentuated. That is why it is of utmost importance to serocontrol sperm donors and to exclude positive reactors from the AI technology process.

On the basis of our results some conclusions could be drawn: During the first eight hours PRV infective titer does not change in diluted or in undiluted sperm prepared for AI. In the next 16 hours the infective titer decreased by log 2 of magnitude, whereas at 48 hours the sperm had changed colour, smell and consistency and acted toxically on the cells in tissue culture, thus making further testing impossible. Virus control in boar sperm is a prerequisite for keeping farms free from pseudorabies.

REFERENCES

1. *Andries, K., Pensaert, M. B. and Vandeputte, J.,* 1978. Effect of experimental infection with pseudorabies (Aujescky's disease) virus on pigs with maternal immunity from vaccinated sows. *Am. J. Vet. Res., 39.* 1282-1285
2. *Cvetnić, S.*, 1983, Virusne bolesti životinja.
3. *Donaldson, A. L., Wardley, R. C., Martin, S. and Harkness, J. W.,* 1984. Infuence of vaccination on Aujeszky's disease virus and disease transmission, *Vet. Rec., 11.* 121-124.
4. *Grupa autora,* (Đ.Panjević) 1984, Priručnik za laboratorijsku dijagnostiku-standardizacija dijagnostičkih metoda.
5. *Hagan and Bruner's* 1988 *Microbiology and Infectious Diseases of Domestic Animals, 8. edition.*
6. *McFerran, J. B. and Dow, C.,* 1975. Studies on immunisation of pigs with the Bartha strain of Aujeszky's disease virus. *Res. Vet. Sci., 19;* 17-22.
7. *McFerran, J. B., McCracken, R.M., and Dow, C.,* 1982. Comparative studies with inactivated and attenuated vaccines for protection of fastening pigs. *Curr. Top. Vet. Med. Anim. Sci., 17;* 163-170.
8. *Miljković, V.,* 1979., Veštačko osemenjavanje životinja. *Beograd, 1979.*
9. *Pensaert, M. B., DeSmet, K. and De Waele, K.,* 1990. Extent and duration of virulent virus excertion upon challenge of pigs vaccinated with different glycoprotein-deleted Aujeszky's disease vaccines, *Veterinary microbiology, 22,* 107-117.
10. *Selivanov, A. V. and Sedov V. A.* 1986, Aujeszky's disease, *Veterinaria, 5,* 71—73
11. *Vannier, P.,* 1986. Vaccination against Aujeszky's disease by different routes using live attenuated virus vaccines in pigs with or without passive immunity. *Zrntralbl. Vet. Med. B. 33,* 704-712
12. *Van Oirschot, J. T. and Gielkens, A. L. J.,* 1987. Vaccines against Aujeszky's disease: comparison of efficacy, DNA fingerprints and antibody response to glycoprotein *I. Vet. Q., 9.* 37S-49S

ODRŽIVOST VIRUSA MORBUS AUJESZKY U SPERMI NERASTA

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SADRŽAJ

Aujeckijeva bolest (MA) kod svinja predstavlja značajan problem kako zdravstveni tako i ekonomski. Poznata je činjenica da se virus MA iz inficiranog organizma izlučuje i spermom, da infekcija kod odraslih priplodnih svinja protiče kao letentna infekcija. Mogućnost širenja ove infekcije koitalnim putem, a naročito veštačkim osemenjavanjem, je velika. Ovo naročito dobija na značaju u uslovima terenske prakse kod nekontrolisanog priplodnog materijala.

Ispitivanje održivosti virusa MA u spermi nerasta uzetoj za veštačko osemenjavanje (VO) u uslovima u kojima se sperma nerasta obrađuje i priprema za inseminaciju pokazala su da virus MA u spermi ostaje aktivan i posle 48 časova od momenta uzimanja sperme. Sperma držana u ovim uslovima se menja organoleptički, deluje toksično na kulturu tkiva i onemogućava dalje ispitivanje delovanja virusa MA.