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DEMONSTRATION OF NEUTRALIZING ANTIBODIES AGAINST THE AUJESZKY'S DISEASE VIRUS IN BLOOD SERUM AND OVARIAN FOLLICULAR FLUIDS OF SOWS

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Abstract

J e ř á b e k J., L. D e d e k, M. V y s k o č i l: *Demonstration of Neutralizing Antibodies Against the Aujeszky's Disease Virus in Blood Serum and Ovarian Follicular Fluids of Sows*. Acta vet. Brno,56,1987:123-128.

Specific neutralizing antibodies were detected simultaneously in 100 % of blood serum samples and ovarian follicular fluids from 38 slaughtered sows from a site of previous Aujeszky's disease (AD) outbreak. Virus neutralizing antibodies were also detected in 97.5 % of blood serum samples and ovarian follicular fluids from 40 slaughtered sows from an AD focus where vaccinations and revaccinations had been carried out with inactivated AD vaccine. Samples of blood serum and ovarian follicular fluids collected from 42 slaughtered control sows from a herd free of AD yielded negative results.

Aujeszky's disease, virus neutralizing antibodies, blood serum, follicular fluid.

Demonstration of IgG, IgM and IgA and their quantitative distribution in the blood serum, follicular fluid, uterine and vaginal secretions of cows in the course of bovine viral diarrhea (BVD) and infectious bovine rhinotracheitis (IBR) were successfully done by W h i t m o r e and A r c h b a l d (1977). The authors also studied the relations between these immunoglobulins and virus neutralizing antibodies against BVD and IBR. They found high IgG concentrations in blood sera and follicular fluids and a positive correlation between titres of virus neutralizing (VN) antibodies against BVD and IBR in blood sera and follicular fluids of the examined animals. They imply that these antibodies provide a certain protection to ova immediately after ovulation. T h a c k e r et al. (1981) compared haemagglutination-inhibiting antibodies against porcine parvovirus in blood sera and ovarian follicular fluids of 17 sows and 12 gilts. In 26 cases positive results were obtained both in blood sera and follicular fluids. Blood serum and follicular fluid titres were in all animals, with one exception, within one 2-fold dilution of each other. Antibody was not detected in either

serum or follicular fluid of other 3 animals. Their results indicated that either blood serum or ovarian follicular fluid can be used for serological examination for porcine parvovirus.

The aim of the present study was to assess the suitability of ovarian follicular fluid for serological AD detection apart from the blood serum.

Materials and Methods

Blood sera and ovarian follicular fluids were obtained from fattened sows of 3 herds. The herd NT was free of AD. For our comparative study, blood serum and follicular fluid samples from 42 animals were collected.

The herd B was a so-called quiescent AD focus (a site of a former AD outbreak) where no vaccination against AD had been carried out. From this herd, blood sera and follicular fluids of 38 fattened sows were collected.

The herd VN had been systematically vaccinated and revaccinated against AD with an inactivated vaccine. The veterinary service records indicated that the animals had been revaccinated 6 weeks after vaccination an slaughtered 14 weeks after revaccination. Here blood sera and follicular fluids of 40 sows were collected.

Blood was taken during the slaughter procedure at a slaughterhouse, the animals were then identified and after exenteration, follicular fluids were collected from several ovarian follicles of the respective animals, using a syringe. The amount of the mixed follicular fluids collected from several follicles varied from 0.5 to 2.0 ml in the individual sows. For the comparative study, a total of 120 blood and 120 follicular fluid samples were collected. All samples were inactivated in a water bath (30 minutes at 56°C) prior to neutralization test (NT).

Neutralization test (NT)

Cell line

For NT, a rabbit kidney cell line RK 13 was employed. For cell line culture and dilution of blood sera and follicular fluids the MEM with 10 % inactivated bovine serum for tissue culture was used in the following composition:

MEM (Eagle) ÚSOL	10 ml
H_2O bidest. ad	100 ml
7.5% $NaHCO_3$	1.2 ml
inactivated bovine serum for TC	10 ml
antibiotics	1 ml

(the final antibiotic concentration in 1 ml of the medium was as follows: penicillin 100 I.U., streptomycin 100 µg, kanamycin 100 µg, polymyxin B 50 µg, fungicidin 50 µg).

For NT, the RK 13 cell line was grown in Roux bottles (volume 1200 ml) and after trypsination it was suspended in 60 ml of medium (cell density ± 600 000 to 800 000.ml^{-1}).

AD virus

employed for NT was the S+V strain (Bioveta, Ivanovice na Hané), cultured on cell line RK 13. The dose employed was 100 $TCID_{50}$/0.05 ml.

Neutralization test

was carried out in 96-well plates, using microdiluters and capillary transfer pipettes (0.05 ml capacity) by Dynatech, Switzerland. Seven wells were coated with a drop of medium each, the 8th well serving for control of cell culture growth was coated with 2 drops (0.1 ml) of the medium. The first well was then coated with 0.05 ml examined sample. The sample was diluted,

using the microdiluter, 2-fold, 4-fold, 8-fold, 16-fold, 32-fold, 64-fold and 128-fold. One drop (0.05 ml) containing 100 $TCID_{50}$ of the virus was added to the diluted samples. This viral dilution was further diluted 10^{-1}, 10^{-2}, 10^{-3} and added in wells for $TCID_{50}$ control in the test. At NT examination 1 positive serum serving as laboratory standard was used as control. After adding the virus the suspensions were shaken and incubated with 5 % CO_2 at 37°C for 1 h. Thereafter, 1 drop of cell suspension was added to each well and after shaking the samples were icubated with 5 % CO_2 at 37°C.

NT was evaluated on days 3 and 5 (the final evaluation). Reaction in wells with CPE + was still assessed as neutralization, CPE ++ and more was assessed as no virus neutralization.

Each sample was examined twice in one row. With different results (a difference in 1 dilution) always the higher dilution was recorded. The results were assessed statistically (C y h e l s k ý 1985).

Results

Examinations of blood sera and follicular fluids of 42 slaughtered control sows from the herd NT free of AD revealed no virus neutralizing antibodies.

Examinations of blood sera and follicular fluids of 38 slaughtered sows from the herd B (a quiescent AD focus) are summarized in Table 1, indicating virus neutralizing antibodies detected in 100 % of samples under study.

Table 1
Neutralizing antibodies against the AD virus in blood sera and follicular fluids of sows from the herd B

No. of sows	Blood serum + titre	Follicular fluid + titre
1	4	4
5	4	8
2	4	16
1	8	2
1	8	4
10	8	8
8	8	16
1	8	32
1	16	2
1	16	4
4	16	8
3	16	16
n = 38	$\bar{x}$ = 9.05	$\bar{x}$ = 10.74

+ reciprocal values are given

Statistical assessment of the herd B is presented in Table 1a. With regard to great variability of values of both blood sera and follicular fluids in the individual sows the paired t-test was used to test the differences between blood sera and follicular fluid antibody titres.

$$t = \frac{|\bar{d}| \cdot \sqrt{n-1}}{s_d}$$

($\bar{d}$ = the mean difference between the values of blood sera and follicular fluids, n = number of pairs, s_d = standard deviation).

Table 1a
Results of the statistical assessment of the herd B

	Blood serum	Follicular fluid
n	38	38
$\bar{x}$	9.05	10.74
s	4.17	5.72
v	46.1 %	53.3 %

n = number of cases, $\bar{x}$ = sample mean value, s = sample standard deviation, v = variation coefficient.
n = 38, $|\bar{d}| = 1.68$, $s_d = 7.25$.

No statistically significant differences were found between the blood sera and follicular fluid titres. Similarly, no correlation was found between antibody titres in blood sera and follicular fluids when using the t-test ($t = |r| \cdot \sqrt{\frac{n-2}{1-r^2}}$, where r = correlation coefficient, n = number of cases).

Results of blood sera and follicular fluid examinations of 40 sloughtered vaccinated and revaccinated sows from the herd VN are given in Table 2. Virus neutralizing antibodies were detected in 97.5 % of examined samples.

The statistical assessment of the VN herd is given in Table 2a. In this case, paired t-test indicated that the follicular fluids contained significantly higher titres of virus neutralizing antibodies ($\alpha < 0.01$).

Table 2
Neutralizing antibodies against the AD virus in blood sera and follicular fluids of sows from the herd VN

No. of sows	Blood serum titre +	Follicular fluid titre +
1	< 2	< 2
5	2	4
7	2	8
4	4	4
8	4	8
1	8	4
9	8	8
2	8	16
2	16	8
1	16	16
n = 40	$\bar{x}$ = 5.4	$\bar{x}$ = 7.4

+ reciprocal values of titres are given

Table 2a
Results od the statistical assessment of the herd VN

	Blood serum	Follicular fluid
n	40	40
$\bar{x}$	5.4	7.4
s	3.9	3.17
v	72.2 %	42.8 %

n = number of cases, $\bar{x}$ = sample mean value, s = sample standard deviation, v = variation coefficient
$n = 40$, $|\bar{d}| = 2$, $s_d = 3.58$

Similarly, a highly positive correlation was found between the antibody titres in blood sera and follicular fluids ($\alpha < 0.01$).

Discussion

Blood serum collected both from living and slaughtered animals has been traditionally used for serological diagnosis of infectious diseases. The aim of the present study was to verify the possibility to employ the ovarian follicular fluid instead of blood serum for serological diagnosis of AD. In agreement with other authors (W h i t m o r e and A r c h b a l d 1977) who investigated this question in BVD and IBR in cattle, and in porcine parvovirosis (T h a c k e r et al. 1981) we found that the NT for AD yields similar results in blood sera and follicular fluids. Despite small differences (in the range of 1 - 3 dilutions between the blood sera and follicular fluid titres) it can be stated that in pilot screening examinations follicular fluids can be employed instead of blood serum for virus neutralization test. In no case a positive result with blood serum and simultaneously collected follicular fluid yielding a negative result occurred, and vice versa. The only disadvantage so far of this procedure using the follicular fluid is a relatively frequently occurring cytotoxicity of these fluids (J e ř á b e k and D e d e k, unpublished data).

Examination of blood sera and follicular fluids in sows from the AD-free herd the NT yielded negative results. In sows from a herd considered as a quiescent focus (i.e. site of previous AD outbreak) with no clinical symptoms of the disease, where no vaccination against AD had been carried out, we failed to show a statistically significant difference between blood sera and follicular fluid antibody titres. However, the antibody titres in follicular fluids were significantly higher than those in blood sera of vaccinated and revaccinated sows, as indicated by statistical assessment of our results. In this case also a positive correlation was found between the antibody titres in blood sera and follicular fluids. We cannot explain this finding satisfactorily as yet, however, the possible effects of vaccination and revaccination cannot be ruled out.

Průkaz neutralizačních protilátek proti viru Aujeszkého choroby v krevním séru a v tekutině z vaječníkových váčků prasnic

Neutralizačním testem byly specifické virusneutralizační protilátky prokázány shodně ve 100 % vzorků krevních sér a tekutin z vaječníkových váčků od 38 poražených prasnic z klidového ohniska Aujeszkého choroby (ACH). Virusneutralizační protilátky byly rovněž prokázány shodně v 97,5 % vzorků krevních sér a tekutin z vaječníkových váčků od 40 poražených prasnic z ohniska ACH, kde se prováděla vakcinace a revakcinace inaktivovanou vakcínou proti ACH. Vzorky od 42 kontrolních poražených prasnic z chovu prostého ACH byly vyšetřeny s negativním výsledkem.

Определение нейтрализационных антител против вируса болезни Ауески в кровяной сыворотке и в жидкости яичниковых пузырьков свиноматок

Нейтрализационным тестом были специфические вирусонейтрализационные антитела выявлены тождественно в 100 % образцов кровяной сыворотки и жидкости яичниковых пузырьков 38 битых свиноматок из очала болезни Ауески (ACH). Вирусонейтрализационные антитела были также установлены тождественно в 97,5 % образцов кровяной сыворотки и жидкостей яичниковых пузырьков 40 битых свиноматок из очала болезни Ауески, где проводили вакцинацию и ревакцинацию инактивированной вакциной против болезни Ауески. Образцы 42 контрольных битых свиноматок без наличия болезни Ауески были исследованы с негативным результатом.

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