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FOUR LAYER ENZYME-IMMUNOASSAY (EIA) DEMONSTRATION OF A SPECIFIC IgG AGAINST THE AUJESZKY'S DISEASE VIRUS IN BLOOD SERA, FOLLICULAR FLUIDS AND OVA OF VACCINATED SOWS

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Abstract

J e ř á b e k J., L. R o d á k, A. L u b i n a, R. D o l e ž e l, M i l o s l a v a L o p a t á ř o v á: *Four Layer Enzyme-Immunoassay (EIA) Demonstration of a Specific IgG Against the Aujeszky's Disease Virus in Blood Sera, Follicular Fluids and Ova of Vaccinated Sows*. Acta vet.Brno,56,1987: 129-140.

In five sows vaccinated and revaccinated against Aujeszky's disease (AD) with an inactivated AD vaccine, specific IgG to the AD virus was detected by means of four-layer enzyme-immunoassay (EIA) in blood sera and disintegrated ova, and in three of these animals also in ovarian follicular fluids. In all sows under study, titres of antiviral antibody of the IgG class occurred and increased after vaccination and revaccination. The specific IgG was also detected in disintegrated ova from 4 of these sows. Its calculated amounts surpassed considerably those found in simultaneously collected blood sera of these animals.

Aujeszky's disease, IgG, blood serum, follicular fluid, ova, enzyme-immunoassay.

Interaction of viruses with unfertilized and fertilized ova in different periods of development has been studied by a number of authors. Porcine parvovirus was shown to adhere to the zona pellucida of porcine ovum but dose not penetrate it (W r a t h a l l and M e n g e l i n g 1979). Interaction of the porcine ova and embryos with Aujeszky's disease virus has been studied by B o l i n et al. (1981). The ova and embryos originated from sows free of neutralizing antibodies against the AD virus and the semen used for insemination was from serologically negative boars. After cocultivation with the AD virus on a cell line from porcine kidney no AD virus was demonstrated either in embryonic cells or in zona pellucida. Further electronmicroscopic study (B o l i n et al. 1983) revealed no productive infection with AD virus, porcine parvovirus or porcine enteroviruses. B o l i n and B o l i n

(1984) demonstrated the AD virus in porcine embryos harvested 6 and 10 d after intrauterine inoculation of bred gilts with AD virus. However, from available literature sources no information was obtained that could be employed as a basis for immunoglobulin demonstration in porcine ova.

In context with our previous work (J e ř á b e k et al. 1987) the aim of this study was to find whether specific antibodies against AD virus (porcine herpesvirus 1 - S z á n t ó et al. 1979; R o c k and R e e d 1980; S a b ó 1981) can be detected not only in the blood serum and in follicular fluids but also in porcine ova.

Materials and Methods

Experimental animals

Five sows after their first farrowing were used, three of them Duroc (No. 3 , 4 and 7), 2 crosses of the Large White and Landrace (No. 25 and 26). The sows were from AD-free farms.

The ova were recovered during natural (No.3) or hormonally stimulated heat. The hormonal stimulation was performed in the second half of expected cycle by administration of 1500 I.U. PMSG followed by 1000 I.U. HCG (Praedyn, Léčiva) 56 h later. Twenty four hours after Praedyn administration laparotomies were carried out estimating that 30 - 50 h after HCG application ovulation occurred (K o z u m p l í k and K u d l á č 1980). In this way sow No.25 was prepared for the first and second ova collection and sows No. 4, 7 and 26 for the second collection (in these animals the first collection was carried out during a natural ovulation).

Each sow was given antibiotics (Penstrepten, Biotika, 1 ml.10 kg)$^{-1}$ after the first laparotomy. The sutures were removed 8 - 9 d later. The second laparotomy was carried out on d 22 - 27 after revaccination (after slaughter) of the sows.

Vaccination

The sows were vaccinated and revaccinated with 5 ml in inactivated vaccine against AD i.m. (according to the manufacturer's instructions - Bioveta, Ivanovice na Hané). They were vaccinated after the first sample collection (d 0) and revaccinated on d 16 (No. 7 and 25) or 21 (No.3, 4 and 26) after the first vaccination.

Sample collection

Samples of blood, follicular fluids and ova were collected before vaccination (d 0), blood samples before revaccinatination, and blood, follicular fluids and ova on d 22 - 27 after revaccination. Blood sera and follicular fluids were stored at -20°C pending analysis.

The ova were collected from ovarian follicles, by flushing the oviducts or uterine horns. From sows No. 4 and 26 the ova were collected twice from the ovarian follicles, from sow No. 3 at the first collection by flushing, at the second collection from the follicles, from sow No. 7 at both samplings by flushing, from sow No. 25 at the first collection by flushing and at the second by both flushing and from the follicles. A fivefold washing of all ova in PBS after Dulbecco (B o n i n 1973) followed. The ova were then individually placed in test tubes containing 0.1 ml PBS. They were stored at -20°C and three times thawn and frozen prior to examination.

All samples collected from the individual sows were always examined simultaneously. For the four-layer EIA, they were diluted using a diluting and conserving solution (R o d á k et al. 1985): PBS supplemented with 0.5 % bovine serum albumin (BSA), 0.3 % Tween 20, 0.1 % EDTA-Na, 0.02 %

Table 1

EIA examination results in sow No. 3

Day 0 of experiment		Day 21 of experiment	Day 22 after revaccination	
Blood and ova collection vaccination		blood collection revaccination	blood and ova collection	
blood serum: negative		blood serum: titre 100	blood serum: titre 24 300	
ova	follicular fluids		ovum	follicular fluid
1 negative 2 negative 3 negative 4 negative 5 negative 6 negative	N		titre detected x 42 554	
			1 45 1 914 930	N

N = no sample collected

Table 2

EIA examination results in sow No. 4

Day 0 of experiment				Day 21 of experiment	Day 22 after revaccination			
Collection of blood, follicular fluids and ova, vaccination				blood collection revaccination	collection of blood, follicular fluids and ova			
blood serum: negative				blood serum: titre 2 700	blood serum: titre 24 300			
ova		follicular fluids			ovum		follicular fluid	
					titre detected	x 42 554	titre	
1	N	1	negative		1	5 212 770	1	6 561
2	N	2	negative					
3	N	3	negative					
4	N	4	negative					
5	N	5	negative					
6	negative	6	negative					
7	N	7	negative					
8	negative	8	negative					
9	N	9	negative					
10	N	10	negative					

N = no sample collected

sodium azide. Series of three-fold dilutions were prepared from the basic dilution of blood sera (1:100), follicular fluids (1:9) and PBS containing one disintegrated ovum (1:5).

EIA reagents

Viral and control antigen

The preparation of both antigens was described in detail earlier (R o d á k et al. 1985). Virus antigen (V-Ag) was obtained by growing AD virus in the RK 13 cell line. In the same way the control antigen (C-Ag) was prepared from non-infected cell cultures. After concentration, both antigens were stored at -70°C. For use in EIA, the antigens were diluted 1:200 in 0.05 M carbonate-bicarbonate buffer, pH 9.6.

Class specific antiserum to porcine immunoglobuline

Class specific antiserum to porcine immunoglobuline was prepared by repeated immunization of rabbits with heavy chains of porcine IgG. The method of preparation and specifity checks were described earlier (R o d á k 1984). For the four-layer EIA the rabbit antiserum was diluted 1:20 000 using PBST (PBS supplemented with 0.1 % Tween 20) with 1 % lactalbumin hydrolysate (LAH, Difco).

POD-SwARIgG conjugate

A pig weighing 50 kg was injected i.m. with 3 consecutive 10 mg doses of purified rabbit IgG (RIgG) in Al-Span Oil adjuvant (SEVAC, Czechoslovakia) administered at 3-week intervals. Specific antibodies to rabbit IgG (SwARIgG) were separated from hyperimmune porcine serum collected 10 d after the last injection by affinity chromatography (IgG covalently bound to CNBr Sepharose 4B, Pharmacia). These antibodies were conjugated with horse-radish peroxidase (POD; RZ = 3.0, Boehringer, Mannheim) using the periodate method (W i l s o n and N a k a n e 1978). The stock solution of the POD-SwARIgG conjugate contained 2 mg of specific antibodies with addition of 1% LAH in 1 ml. For the four-layer EIA the stock solution was diluted 1:1000 with PBST containing 1 % LAH.

Substrate solution

A solution of 8 mg of 5-aminosalicylic acid (FLUKA AG, Switzerland) in 10 ml 0.05 M citrate-phosphate buffer, pH 5.8, was supplemented with 1 ml 0.05% H_2O_2 immediately before use.

EIA method

All samples were examined by the four-layer EIA for specific IgG to AD virus. Microtitre plates with flat-bottom wells were used (Koh-I-Noor, Czechoslovakia). Each well contained 100 µl of all reagents. The wells were always washed three times using PBS.

Four-layer EIA

Vertical rows of wells in microtitre plates were alternatively filled with viral - V (rows 1, 3, 5 etc) and control - C (rows 2, 4, 6 etc.) antigens. After adsorption of antigens overnight at 20°C and washing the wells with bound V and C antigens were filled with threefold diluted samples. The blood sera were examined in dilution range 1:100 to 1:218 700, follicular fluids in the range of 1:9 to 1:19 683 and PBS with ovum (presumably disintegrated due to alternate freezing and thawing) in the range of 1:5 to 1:10 035. Examined samples were always diluted in the same way in wells coated with viral and control antigen and left in a moist chamber at 20°C overnight. The next day the wells were washed and filled with a solution of class-specific rabbit antiserum against the porcine immunoglobulin (RASwIgG). After incubation for 90 minutes at 37°C and washing another incubation with

Table 3

EIA examination results in sow No. 7

Day 0 of experiment		Day 16 of experiment	Day 26 after revaccination	
blood and ova collection vaccination		blood collection revaccination	blood and ova collection	
blood serum: negative		blood serum: titre 8 100	blood serum: titre 72 900	
ovum	follicular fluid		ova	follicular fluid
1 negative	N		1 negative 2 negative 3 negative 4 negative	N

N = no sample collected

the conjugate peroxidase-antibody to rabbit immunoglobulin (POD-SwARIgG) followed under the same conditions. After this incubation and washing the wells were filled with the substrate solution and the reaction was evaluated 60 min later spectrofotometrically at 492 nm.

Evaluation of EIA results

The results were evaluated by comparing the absorbance of the substrate solution in the wells containing V-Ag with that found for C-Ag incubated with the same sample dilution. A sample was considered positive if the V:C ratio was $\geq$ 2.0. Besides it was required that after incubation without serum or with a negative serum the ratio V:C be 1.0 $\pm$ 0.2.

The antibody titres are given in reciprocal values.

Calculation of the IgG levels in porcine ova

Calculation of the IgG amount in the porcine ovum was based on its diameter 165 µm (K o z u m p l í k and K u d l á č 1980), its volume then being 0.00235 mm^3 giving thus a 42 554fold dilution of one disintegrated ovum in 0.1 ml PBS. The EIA result (i.e. the IgG titre of a disintegrated ovum in PBS) was multiplied by this value (e.g. EIA titre of IgG 405 x 42 554 = 17 234 370 = the IgG titre in one ovum).

Results

The EIA examination results in the individual sows are given in Tables 1 - 5 and schematically in Fig. 1. They indicate that the specific IgG was found in the blood serum of all examined sows after their vaccination and revaccination. Comparison of the IgG concentrations in follicular fluids in two sows (No. 4, see Table 2, and No. 26, see Table 5) prior to and after vaccination and revaccination shows that these treatments resulted also in occurrence of the specific IgG in follicular fluids. IgG was also found in fluids from two follicles of sow No. 25 (Table 4), however, without sample collection before her vaccination. In 17 ova disintegrated in PBS collected from 5 experimental sows prior to vaccination, no specific IgG was demonstrated using the EIA test. In one animal (No. 7, Table 3) no IgG was detected by this method in 4 ova collected after revaccination. From the remaining 4 sows 17 ova were collected after revaccination. Among them 4 (1 from sow No. 25 and 3 from sow No. 26) yielded negative results whereas in 13 disintegrated ova (Tables 1, 2, 4, 5 and Fig. 1) the IgG titres were substantially higher than in simultaneously collected blood sera.

Discussion

The observation of virus neutralizing antibodies in follicular fluids of sows (J e ř á b e k et al. 1987) was an impulse for our further study with the aim to find whether and in what amounts specific antibodies are present in ova of sows vaccinated and revaccinated against AD. Virus neutralizing antibodies to the virus of mucosal disease and to the virus of infectious rhinotracheitis of cattle found in blood sera of cows contained predominantly IgG (W h i t m o r e and A r c h b a l d 1977) that was also detected in their follicular fluids. This fact along with the observation of exclusively IgG class antibodies in blood sera and oropharyngeal swabs occurring 3 weeks after vaccination of pigs against AD (R o d á k et al. 1986) directed our effort to demonstration of the IgG in blood sera and follicular fluids as well

Table 4

EIA examination results in sow No. 25

Day 0 of experiment			Day 16 of experiment	Day 27 after revaccination		
blood and ova collection vaccination			blood collection revaccination	blood, ova and follicular fluids collection		
Blood serum: negative			blood serum: titre 900	blood serum: titre 24 300		
ova		follicular fluids		ova		follicular fluids
				titre detected	x 42 554	titre
1	negative	N		1	15 638 310	≙ 13 122*
2	negative			2	15 638 310	
3	negative			3	45 1 914 930	
				4	5 212 770	
				5	45 1 914 930	
				6	45 1 914 930	
				7	45 1 914 930	
				8	negative	

N = no sample collected

* = follicular fluids collected from 2 ovarian follicles (0.3 + 0.5 ml), added 1 ml PBS = 1.8 ml mixed sample

Table 5

EIA examination results in sow No. 26

Day 0 of experiment				Day 21 of experiment	Day 22 after revaccination			
blood, follicular fluids and ova collection vaccination				blood collection revaccination	blood, follicular fluids and ova collection			
blood serum: negative				blood serum: titre 300	blood serum: titre 72 900			
ova		follicular fluids			ova		follicular fluids	
					titre detected	x 42 554	titre	
1	negative	1	negative		1	405 17 234 370	1	>19 683*
2	negative	2	negative		2	negative	2	N
3	N	3	negative		3	negative	3	N
4	N	4	negative		4	negative	4	N
5	N	5	negative		5	N	5	>19 683*
6	N	6	negative		6	1 215 51 703 110	6	>19 683*
7	N	7	negative		7	405 17 234 370	7	>19 683*
8	N	8	negative		8	135 5 744 790	8	>19 683*
9	N	9	negative					
10	negative	10	negative					
11	negative	11	negative					
12	negative	12	negative					

N = no sample collected

*= follicular fluids not diluted further

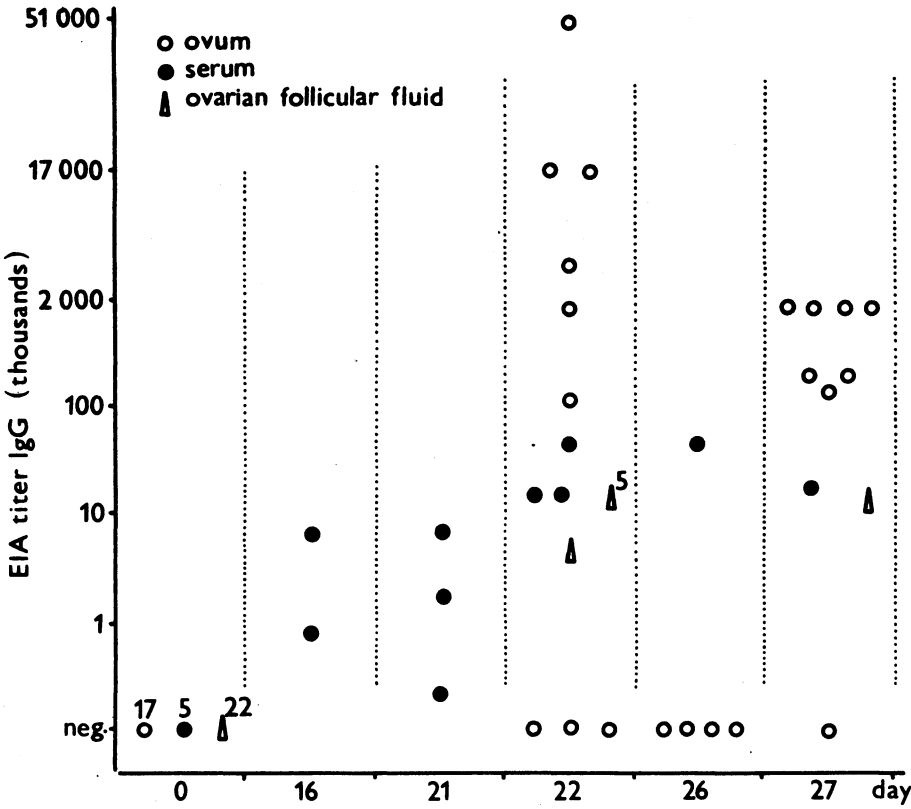

Fig. 1. A schematic presentation of the specific IgG in blood sera, follicular fluids and ova of sows vaccinated and revaccinated against AD. Prior to vaccination (day 0) no IgG was detected in any of the 5 collected blood sera, 22 follicular fluids or 17 ova. In 5 follicular fluids collected on day 22 after revaccination, an IgG titre > 19 683 was detected using the EIA method. In 13 of 21 ova collected after revaccination, the IgG titres were substantially higher than IgG titres in simultaneously collected blood serum samples.

as in the ova of sows vaccinated and revaccinated against AD. As no antibody detection in ova disintegrated in PBS could be expected using the virus neutralization method, a much more sensitive method was selected, namely the EIA, in our experiment the four-layer EIA.

Using this method, the specific antiviral IgG was found in blood sera of all vaccinated and revaccinated sows. Comparison of the results obtained from 2 sows before vaccination and after revaccination revealed that these treatments resulted in occurrence of the IgG also in their follicular fluids. Surprisingly, substantially higher IgG titres (calculated 8.8fold to 709fold) were found in 13 of the 17 collected and disintegrated ova from 4 sows than in their sera collected at the same time. In one sow (No. 7), no IgG was found in her ova. The present data give no answer to the question why in this sow's four ova no IgG was detected despite a high IgG titre of her blood serum

(72 900). Similarly, in sow No. 25 (see Table 4) after revaccination no IgG was found in one ovum but was present in 7 other ova, and in sow No. 26 (Table 5) EIA yielded negative results in 3 ova whereas in 4 other ova from the same sampling, a positive result was obtained. Nevertheless, the data obtained in present work are valuable and stimulating for further research that may enrich our knowledge not only about interaction virus-ovum-embryo (B o l i n et al. 1981; B o l i n et al. 1983; B o l i n and B o l i n 1984) but also provide information on immunity at the earliest stage of ontogenesis.

Průkaz specifického IgG proti viru Aujeszkého choroby v krevních sérech, tekutinách z vaječníkových váčků a ve vajíčkách vakcinovaných prasnic čtyřvrstevnou enzymoimunoanalýzou

U pěti prasnic byla po vakcinaci a revakcinaci inaktivovanou vakcínou proti Aujeszkého chorobě (ACH) zjišťována pomocí čtyřvrstevné enzymoimunoanalýzy (EIA) specifická IgG protilátka proti viru ACH v krevních sérech, dezintegrovaných vajíčkách a u tří ze sledovaných prasnic také v tekutinách z vaječníkových váčků. U všech sledovaných prasnic se po vakcinaci a revakcinaci objevily a stoupaly titry antivirových protilátek třídy IgG. U čtyř prasnic byl specifický IgG prokázán také v dezintegrovaných vajíčkách, kde bylo jeho vypočtené množství výrazně vyšší než v krevních sérech těchto prasnic, získaných souběžně s odběrem vajíček.

Определение специфического IgG против вируса болезни Ауески в сыворотках крови, жидкостях из яичниковых пузырьков и в яйцеклетках вакцинированных свиноматок с помощью четырехслойного энзиммуноанализа

У пяти свиноматок после вакцинации и ревакцинации инактивированной вакциной против болезни Ауески (АСН) устанавливали с помощью четырехслойного энзимоиммуноанализа (EIA) специфическое IgG антитело против вируса болезни Ауески в сыворотках крови, дезинтегрированных яйцеклетках, и у трех из исследуемых свиноматок также в жидкостях из яичниковых пузырков. У всех исследуемых свиноматок после вакцинации и ревакцинации появились и повышались титры противовирусных антител класса IgG. У четырех свиноматок специфический IgG установлен также в дезинтегрированных яйцеклетках, где его рассчитанное количество оказалось значительно выше, чем в сыворотках крови этих свиноматок, полученных параллельно с взятием яйцеклеток.

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