

Vaccine Protection Against Experimental Challenge Infection with a PPV-27a Genotype Virus in Pregnant Gilts

This article was published in the following Dove Press journal:
Veterinary Medicine: Research and Reports

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Background/Introduction: Porcine parvovirus (PPV), the causative agent of severe reproductive failures in pigs, is present worldwide. The witnessed spread of the virulent 27a type PPV strains since its recognition raised concerns about the efficacy of the available commercial vaccines.

Methods: To address this question, vaccinated pregnant gilts were challenged with a PPV-27a-like virus strain and parameters related to vaccine efficacy were compared.

Results: The K22 vaccine strain of Parvovax[®] (PVX) was characterized as “Kresse-like” based on the epitope mapping data. Vaccination of the gilts induced a low level of antibody responses. Based on foetal mortality, the number of sows which had challenge virus-affected foetuses, the percent of PPV positive piglets/litters plus their PPV genome and viral load PVX outscored the other vaccinated groups.

Conclusion: Stronger protection was provided by the “Kresse-like” K22 PPV strain-based vaccine than by the NADL-2 and NADL-like strain-based commercial vaccines against a PPV-27a cluster strain challenge. Vaccine-induced antibody levels as measured pre-challenge were not found to be an accurate indicator of protection.

Keywords: PPV, K22 strain, efficacy, PPV-27a

Introduction

Porcine parvovirus (PPV; Ungulate Parvovirus 1, Protoparvovirus genus, Parvoviridae family) is the major causative agent of SMEDI (stillbirth, mummification, embryonic death, and infertility) syndrome, first described in the late 1960s.¹ The outcome of PPV infection depends on the virulence and the amount of the virus, and the stage of gestation.² Infection after 70 days of gestation may result in the late death of some foetuses, others will survive and be born weak or normal, but most piglets born alive develop an antibody response to PPV.³ PPV strains of lower virulence require a higher amount of virus for productive infection and reach lower levels of replication in the different organs/tissues of the host.^{4,5}

Until approximately 20 years ago PPV was considered very stable genetically and antigenically.^{6,7} However, more recent studies revealed considerable genetic diversity among PPVs, comprising at least 7 clusters.^{8–10} Despite the overall diversity of PPVs, a particular genotype has become widespread lately, PPV-27a being the prototype of this cluster.¹¹ Based on experimental data with recent German field isolates of PPV-27a, PPV-143a, and two vaccine viruses (PPV-NADL-2 and PPV-IDT) it was concluded that PPV-27a cluster strains may influence effective vaccination against PPV.^{12–15}

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The aim of this study was to compare the efficacy of Parvoruvax[®] (PVX), an inactivated PPV (strain K22) and *Erysipelothrix rhusiopathiae* (serotype 2) bacterium-based vaccine, with a range of widely used commercial Parvo-Erysipelothrix vaccines (all containing genotype 1 PPV) against the effect of challenge of pregnant gilts with a PPV-27a type strain.

Materials and Methods

Authorization of the trial was provided by the Government Office of Baranya County Foodchain-Safety and Animal Health Department, Hungary, under the BAI/35/56-94/2017 registration number.

Challenge Virus

The challenge virus used, designated as PPV1-HUN, member of the PPV-27a cluster, was isolated from a newborn piglet's organs (heart, lung, spleen, liver, and kidneys) on swine testicle (STE) cells during the fall of 2012, from a Hungarian farrow-to-finish herd, suffering significant losses due to high incidence of weak born piglets with impaired vitality and increased mortality.^{16,17}

Vaccination Experiment

Five groups of each 6–7 DanBred gilts, six-month old and demonstrated PPV ELISA negative (see below) were allocated into four different vaccine test groups and one non-vaccinated control group (Table 1).

All tested vaccines were used according to their SPCs (Summary of Product Characteristics), ie Vaccine C two weeks prior to mating; priming at ~6 weeks prior to mating: PVX, Vaccine B, and Vaccine D; booster 2 weeks prior to mating (PVX, and Vaccine D), or 3 weeks prior to mating (Vaccine B).

Thirty-eight days after the first vaccinations, and following synchronisation with Altresyn[®], the gilts were mated. Pregnancy was confirmed by ultrasound at 30 days of expected gestation. Unreceptive gilts and those having failed to conceive were excluded from the study, resulting in 5–7 animals per each group.

At day ~40 of pregnancy, all gilts in all groups at the same day were challenged intranasally and intramuscularly (2–2 mL) with a dose of 5.5 log TCID₅₀/mL PPV1-HUN virus.

At day 90 of pregnancy, all pregnant gilts were euthanized and autopsied; all foetuses were aseptically removed from the uterus and euthanized. According to the European Pharmacopoeia 9.0 (01/2017:0965) monograph, the size, weight and position of foetuses in the uterus, as well as their general condition, were recorded.

The gilts were blood sampled at D0 (prior to 1st vaccination), D38 (prior to mating), D86 (prior to challenge), and D137 (prior to euthanasia) for serology.

Umbilical blood and tissue samples (of lungs and kidneys) were collected from all live-euthanized foetuses for serology and PCR, respectively. Only tissue samples were possible to collect from already dead (except one foetus in G5, where blood still could be taken) and mummified foetuses for PCR and virus isolation.

Serology

For serological testing, the INgezim PPV Compac 11.PPV.K.3 ELISA and virus neutralisation assay was used. For virus neutralisation beta type test was implemented, each sera was tested in duplicates at a dilution of 1:10–1:1280 on ST (swine testis) cells, using PPV1-HUN virus at appr. 100 TCID₅₀/50μL titer. After six days of incubation, the neutralising titers were determined by vital staining with tetrazolium dye.

PCR

Quantitative PCR (qPCR) of the foetal organ samples was done by SCG Diagnosztika Ltd., following published protocols.^{18,19} Sample preparation for PCR was performed as follows: approximately 0.5 g of foetal organs (kidney and lung together or tissues of mummified foetuses) were homogenized in 4.5 mL PBS, then dispersed by ULTRA-SONIC homogenizer (Sartorius Labsonic M) for 30 seconds, followed by centrifugation at 3000rpm for 10 min. The viral DNA from 200μL amount of the supernatants was purified

Table 1 List and Composition of the Vaccines Used in This Study

Group/Vaccine	PPV Strain	Other Component	Adjuvant
G1. Parvoruvax	K22 ("Kresse-like")	Inactivated <i>E. rhusiopathiae</i>	AlOH, thiomersal
G2. Vaccine B	NADL-2	Inactivated <i>E. rhusiopathiae</i>	AlOH, DEAE-dextran, ginseng
G3. Vaccine C	"NADL-like" strain 014	Inactivated <i>E. rhusiopathiae</i>	α-tocoferylacetat; Polysorbate 80, Simethicone
G4. Vaccine D	"NADL-like" strain 014	Inactivated <i>E. rhusiopathiae</i> ; <i>L. interrogans</i> serovars	d1-α-tocoferylacetat

with the help of High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions. The extracted DNA was eluted in 50 μ L elution buffer. 2 μ L of the eluted DNA was used in the subsequent PCR tests.

Virus Isolation

Out of the PCR positive samples 115 were submitted for virus isolation, representing low/high PPV content, according to the qPCR results, as follows: 20 μ L of the afore-mentioned tissue homogenate supernatant was put onto a 60–80% confluent RPL2 cells ($\sim 2.1 \times 10^5$ cells/well) on a 24 well TC plate (Corning), containing 1 mL MEM-H medium per well. The plates were investigated daily for the presence of potential toxic and cytopathic effects and were evaluated on the sixth day post-inoculation.

Genetic Analysis

In order to determine the nucleotide sequence coding for the major structural protein VP2 of K22 vaccine strain, viral nucleic acid was extracted from cell-culture supernatant using the ZiXpress32 Viral Nucleic Acid Extraction Kit and ZiXpress32 robot (Zinexts Life Science). Nucleic acids were amplified by a random-primed PCR assay and used as template for next-generation sequencing (NGS) using the Ion Torrent PGM platform as described in detail elsewhere.^{20,21} The sequence data were evaluated by the CLC Genomic Workbench (version 7). Contigs were prepared by combining de novo assembly and reference mapping steps.

The three-dimensional presentation of the PPV capsid was done by Viper program and visualized by Poliview.^{22,23}

Statistical Analysis

The analysis of variance of the serological data was performed by the STATGARCHICS Centurion XVI (version 16.2.04) program. The foetus mortality dataset was evaluated with mixed-effect logistic regression using treatment groups as a fixed factor and sow ID as a random factor, the p-values in the pairwise comparisons are adjusted with Holm-Bonferroni correction of p-values for ten multiple comparisons.

Results

Empty gilts and those failed to conceive were removed from the study.

Vaccination induced low-level antibody responses as measured by ELISA: titers surpassing the kit's positivity

limit were detected in approximately 15 percent of the vaccinated animals altogether, while it was ~ 43 percent in G2, the NADL-2 vaccine group (Figure 2). The vaccine induced humoral immune response that was measurable at D38, prior to mating, waned by D86, day of challenge, unanimously. After challenge, a significant rise was measured for all groups, with approximately the same group mean values (102–104 S/P%).

Post vaccination, virus neutralizing titers were also low and also decreased between the date of mating and challenge (D38 and D86, respectively) in all except Vaccine B group (Table 2).

Following challenge, no clinical signs were observed among the sows throughout the trial. The mortality rate and degree of foetal development impairment data are summarized in Table 3. Based on foetal mortality, PVX and Vaccine B proved to be significantly better than the non-vaccinated control group (at 95% confidence interval) and PVX outscoring all other vaccinated groups.

Table 2 Serum Virus Neutralization Titers of the Gilts After Vaccination and Pre-Challenge Against 100 TCID₅₀ PPV1-HUN Strain

Group	Gilt ID	D38 Log Titer	D86 Log Titer
Parvoruvax	51	1.3	0
	55	0	0
	56	2.35	1.15
	70	1.9	0
	74	1.6	0
	75	1	0
	78	0	0
Vaccine B	46	1.76	1.45
	48	1.15	1.6
	54	1.3	1.15
	81	1.9	1.76
	84	0	0
	87	1.76	1.15
	88	1.3	0
Vaccine C	11	1.15	0
	17	0	0
	58	0	1.15
	63	1	0
	89	1	0
Vaccine D	1	1.3	0
	12	0	1.76
	18	1.15	0
	23	0	0
	34	1.6	0

Table 3 Physiological Parameters of the Litters

Group (# of Sows)	% Sows with PPV Affected Litter (Dead or Mummified)	Average Live Litter Size	Number (% of Live Foetuses)	Number (% of Dead Foetuses)	Number (%) Mummified of Foetuses	Average Live Foetus Weight (g)	Average Live Foetus Length (cm)	% Ingezim PPV ELISA Positive Foetuses
Parvoruvax (7)	0	13.7	96 (100)	0 (0)	0 (0)	600.1	29.4	0
Vaccine B (7)	43	11.4	80 (89)	0 (0)	10 (11)	609.9	29.4	0
Vaccine C (5)	60	11.0	55 (92)	0 (0)	5 (8)	719.8	31.0	0
Vaccine D (5)	80	9.4	47 (64)	0 (0)	26 (36)	555.1	28.8	4
No vaccine control (6)	100	7.6	46 (40)	5 (4)	65 (56)	663.4	30.4	26

Altogether 192 samples proved positive by PCR; the calculated virus titers varied between 0.3 and 8.1 log TCID₅₀/mg tissue (Table 4). Out of the PCR positive samples, 115 was submitted for virus isolation, which resulted in 49 positive samples, 13 in G4 and 36 in G5, the rest was negative by this test. The lowest calculated titer from the qPCR results, which was positive by virus isolation was 3 log₁₀ TCID₅₀/mg tissue.

There was a clear negative correlation between litter size and PPV genome positivity of piglets ($R^2=0,8153$) as calculated in MS Excel.

ELISA seropositive foetuses were found only in G4 and G5 groups, 2 and 11, respectively. One of them was a dead foetus in G5. All seropositive foetuses were also PCR positive, except two. One live foetus in G4 and one dead in G5 were negative by virus isolation.

Epitope Mapping

PPV1-HUN had identical amino acid (aa) sequences with PPV-27a for VP1 and VP2 ORFs. The VP2 amino acid sequence of K22 was identical with that of the virulent PPV-Challenge, UK 1986 strain (GenBank accession number KF049426), and further, it showed a close relationship to the highly virulent Kresse strain (Figure 1), with a total of

only four aa differences (L145I, G192S, I215T, P238L) from which two (I215T, P238L) represent neighbouring surface-exposed aas (Figure 1A). Four additional surface-exposed aa changes were found between K22 and PPV-27a (Q228E, A414S, E419Q, P436T) (Figure 1B). When NADL-2 sequences were compared to PPV-27a (Figure 1C) nine aa differences were revealed (S45T, T215I, E228Q, G378D, Q383H, S414A, Q419E, T436S K565R) and only one of them (S45T) was not in surface-exposed position. These data suggest that NADL-2 is antigenically more distinct from PPV-27a cluster strains than K22. It contains quantitatively more (8 versus 6) and qualitatively different mutations on its surface than K22 does. Only four aa changes are common between the K22 and NADL-2 strains compared to PPV-27a (I215T, Q228E, A414S, E419Q), and NADL2 contains additional mutations in the two-fold depression (D378G, H383Q, R565K) and on the spike (S436T). Based on these important features, we characterized K22 strain as highly virulent “Kresse-like”.

Discussion

Most commercially available PPV vaccines are supposedly based on the NADL-2 strain;²⁴ however, the sequence analysis of the K22 strain of PVX vaccine revealed its distinct

Table 4 Viral Genome Copy Load and Virus Isolation Measurements Results

Group (# of Sows)	% of PCR Positive Piglets/Group	% of PCR Positive Piglets per Group and Mean Viral Genome Copy Load in the Organs (Kidney/Lung) Calculated log ₁₀ TCID ₅₀ /mg Organ (Min/Max)			# of Virus Isolation Positive Piglets/Group
		Live	Dead	Mummified	
Parvoruvax (7)	3	3% 1.1 (0.7/1.7)	N/A	N/A	0
Vaccine B (7)	17.8	7.5% 1.6 (0.9/3.2)	N/A	100% 1.2 (0.3/2.3)	0
Vaccine C (5)	25	18% 1.9 (0.6/3.0)	N/A	100% 1.8 (1.5/2.2)	0
Vaccine D (5)	60.3	57% 2.4 (1.0/5.9)	N/A	100% 4.7 (1.1/7.6)	13
No vaccine control (6)	90.5	78% 4.0 (1.4/7.0)	100% 6.6 (5.9/7.2)	98% 6.0 (1.5/8.1)	36

Abbreviation: N/A, Not Applicable.

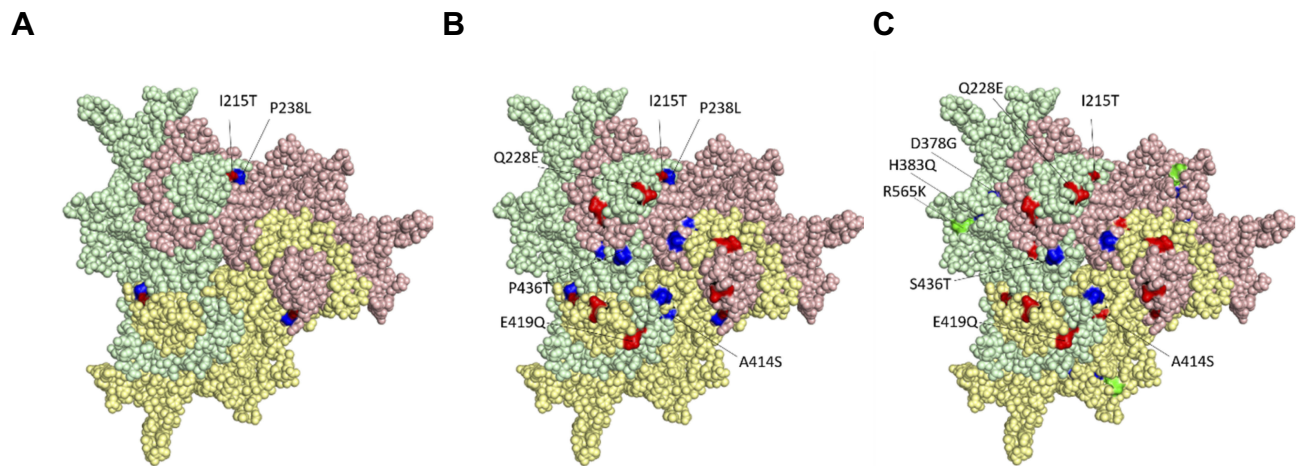


Figure 1 Surface amino acid modifications among different PPV strains. All altered amino acids highlighted on the surface of a capsid trimer (the three inter-linked identical monomers are shown in different colors), but changes only on one chain are annotated. Numbering is indicated according to NADL-2 VP2 positions. **(A)** K22 vs Kresse. Altered neighbouring surface amino acids highlighted with red and blue. **(B)** K22 vs 27a. Surface amino acid changes are indicated with red and blue. **(C)** NADL-2 vs 27a. Surface amino acid changes similar to K22 v. 27a are highlighted with red, other amino acid changes are indicated by blue and green.

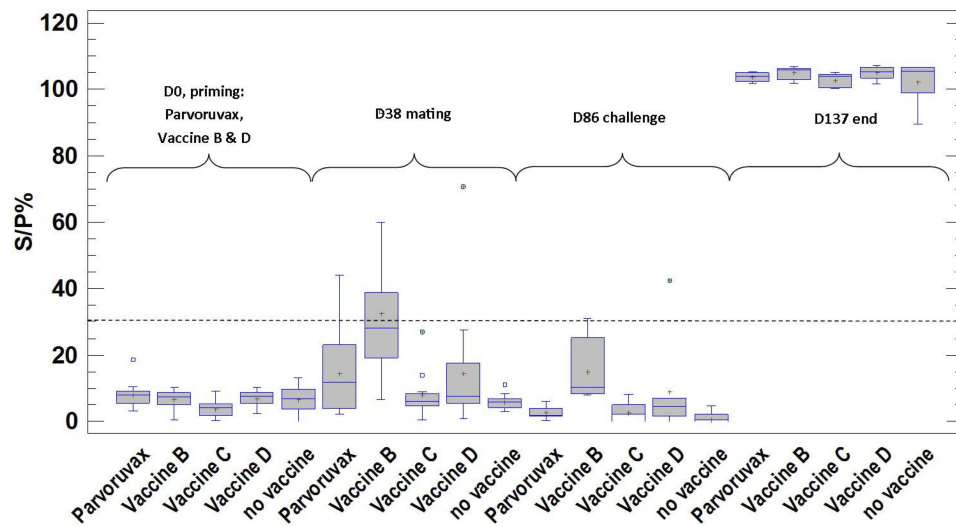


Figure 2 Serological response of the gilts over time to vaccination and challenge measured by a commercial blocking ELISA. The dashed line indicates the cut-off of the ELISA.

nature from the rest of the vaccine strains. Also, information on vaccine protection against the virulent PPV-27a cluster strain viruses is limited in the literature. Studies elaborated on the efficacy of heterologous (IDT, Stendal, NADL-2) and an experimental homologous vaccine against the clinical disease evoked by the PPV-27a challenge virus determined that neither of the vaccine types can prevent virus infection.^{18,25} However, no data were available in this regard on the “Kresse-like” K22 containing PVX vaccine and other highly relevant commercial vaccines used on a daily basis all over the world for PPV prophylaxis.

The result of the used ELISA test showed limited value in evaluating the humoral response to vaccination, although the

group immunized with the vaccine containing the homologue strain to the one used in the ELISA (NADL-2 both) reached higher titers than the rest of the groups. The antibody response to the challenge infection was unanimous as measured by the ELISA, regardless of the vaccination status of the pigs, ie the anamnestic humoral immune response of the vaccinated pigs did not differ from the response of the non-vaccinated PPV naïve/challenged gilts.

With the exception of vaccine B, the drop of the anyway low VN titers of the gilts before challenge was in accordance with previous findings, where homologous vaccine/challenge virus counterparts of PPV-27a were investigated.¹⁸ Further, the level of pre-challenge VN titers did not correlate

with protection in this trial, since the highest clinical protection was seen in the group with a relatively low VN titer right before challenge. These results are questioning the use of serological methods (ie ELISA and VN) for the evaluation of vaccine-induced protection against PPV and suggest that other mechanisms or indicators for immunity should be involved to properly evaluate the protection level of the gilts than serology. In fact, the early IFN- γ and INF- α activation in pigs upon PPV1 infection²⁶ and the role of the memory B cells–helper T cell complex of the cell-mediated immunity (CMI) are proven in the induction of immune response to PPV,²⁷ as also proven for the human adeno-associated viruses.²⁸ This hypothesis, ie PPV-specific cellular immune responses being a major differentiating factor in protective capacity, is further and specifically supported by the already demonstrated development of PPV-specific CMI evoked by PVX; briefly, in a trial where SPF pigs were vaccinated with PVX at D0, D21, and D154, antigen-specific IFN γ ⁺ T cell responses were measured upon vaccination, which was significantly boosted 4 months later,²⁹ similar to that of repeated PPV infection.²⁷

In agreement with previous findings,¹⁸ this study also demonstrated that vaccination with commercially available inactivated vaccines cannot prevent infection since all groups responded to the challenge with antibody titer rise. However, there is a difference between the “readiness” provided by the different vaccines, which is plausibly manifested in the timely activation of memory B-cells and T-cells against infection.

However, in contrast to the aforementioned study, we could detect the challenge virus nucleic acid by PCR even in the foetuses from vaccinated groups, and furthermore, in one vaccinated group (Vaccine D) infectious virus could be recovered from a low number of foetuses. The dose of the challenge viruses was in the same range (5.5 and 6 log TCID₅₀/mL applied at 2+2 and 4+1 inoculum into the nostrils and into the neck muscles, respectively) in the two studies, and the applied PCR was based on the same protocol,¹⁹ though its sensitivity might slightly be different lab by lab. On the other hand, there were differences in the strain of the challenge virus, the breed of the pigs, which all may contribute to such differences. Nevertheless, isolation demonstrated the presence of live PPV in the foetuses only in one of the vaccinated groups (G4) and in the non-vaccinated group (G5). Thus, virus isolation proved to be less sensitive than PCR to demonstrate the presence of PPV genetic material in foetal tissues, or, in other words, PCR detected also non-infectious viral DNA besides the replicative forms of

the virus. Based on the literature data^{12–15} and on the presented serological and virus detecting results the following categories could be established in relation to protection level: i, full protection: seronegative and PCR negative foetuses (and no PPV excretion following challenge or infection, which parameter was not measured in this trial); ii, sufficient protection: seropositive and/or PCR positive, but virus isolation negative foetuses and no reduction in foetal numbers; and iii, insufficient protection: sero-, PCR-, and virus isolation positive foetuses and/or reduction of foetal numbers.

Conclusion

In conclusion, the “Kresse-like” K22 PPV strain-based vaccine provided stronger protection against the recent PPV-27a-type strain challenge than the NADL-2 and NADL-like PPV-based commercial vaccines in terms of reducing the number of sows which had challenge virus-affected foetuses, and the percent of PPV positive piglets/litters, which in turn resulted in the highest number of live piglets/litter in this vaccinated sow group. The exact mechanisms by which PVX acts to protect the foetuses remain to be elucidated. Besides the higher antigenic homology of K22 capsid (VP2) to that of the PPV-27a cluster strains other differentiating components of the vaccine (eg method of antigen preparation/production, adjuvant, *E. rhusiopathiae* strain) might also contribute to make it superior to the other tested vaccines.

The GenBank accession number for K22 VP2 nucleotide sequence is: MN627433.

Ethics Statement

The following guidelines were followed throughout the trial: Hungarian Act No. XXVIII of 1998 on the welfare and humane handling of animals; Hungarian Governmental Decree No. 40/2013. (II. 14.) on animal experiments; Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes; Guide for the Care and Use of Agricultural Animals in Research and Teaching, 3rd ed. 2010, Champaign, IL, Federation of Animal Science Societies.

Acknowledgments

Thanks to Edit Fodor, Andrea Tábi, Krisztián Bányai, and Imre Szabó, for providing an expert contribution to virus isolation and neutralization, ELISA tests, sequencing, and statistical analysis, respectively. Thanks to Han Smits for the critical reading and comments on the manuscript.

Disclosure

Dr Pál Bajnóczi is employed by Prophyl Kft. (Ltd.) and receives personal fees (salary) from Prophyl Kft as an employee. Prophyl Kft. is subcontracted by CEVA Santé Animale S. A. to perform animal studies, and Prophyl Kft. received service fee from CEVA Santé Animale S.A. for the conduct of the study. I. Kiss, E. Kovács, A. Cságola, P. Mortensen, & V. Palya are employed by Ceva. The authors report no other conflicts of interest in this work.

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