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The effect of oregano and vaccination against Glässer's disease and pathogenic *Escherichia coli* on postweaning performance of pigs

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The objective of this field trial was to determine if vaccination against *Haemophilus parasuis* serovar 5 (HPS 5) and *Escherichia coli* and/or the phytogenic feed additive oregano would improve the performance of pigs in the nursery. The trial unit was concurrently infected by HPS 5 and with different serotypes of *E. coli*. All pigs were born to sows vaccinated against HPS 5.
The trial was carried out in four groups. Group 1 (n=312): Piglets were vaccinated intramuscularly at one week of age with inactivated VT2e-toxin and on days 27 and 41 after weaning they were vaccinated against HPS 5. They were fed a diet supplemented with 1,000ppm oregano. Group 2 (n=309): Piglets did not receive the vaccine for *E. coli*. After weaning they received the vaccine against HPS 5 and, also, the oregano feed supplement. Group 3 (n=308): The animals were given both vaccines but without oregano as a feed supplement. Group 4 (n=301): Piglets were neither vaccinated nor given oregano supplement.
The following parameters were evaluated: average daily nursery weight gain (ADG), feed efficiency (FE), and weaner mortality.
Both groups of weaners fed the oregano supplement (Groups 1 and 2) were from day 35 on significantly ($p<0.05$) heavier than the groups that did not receive the supplement (Groups 3 and 4). At day 56, the mean nursery-end-weight of Group 2 piglets (vaccinated against HPS 5 and fed oregano) was 17.8% higher ($P=0.0024$) than that of the control pigs (Group 4). During the last four weeks of the nursery period the ADG and FE were significantly better ($p<0.05$) in the two groups fed oregano than in Group 3 (given both vaccines but without oregano). Control animals suffered significantly ($p<0.05$) higher mortality (10.63%) than did the other groups (less than 2%).

Key words
Pigs,
Haemophilus parasuis,
Escherichia coli,
Weaning,
Glässer's disease,
Vaccination,
Weight gain,
Food conversion,
Postweaning mortality,
Oregano.

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Introduction

Weaning is one of the most stressful challenges in the life of a piglet (Bölcskei *et al.*, 1995). Post-weaning problems are often caused by diseases such as Glässer's disease and post-weaning colibacillosis.

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Porcine polyserositis and arthritis (Glässer`s disease) is associated with *Haemophilus parasuis* (HPS) infection (Little, 1970). HPS is commonly found in the nasopharynx of healthy pigs. It has a large number of serovars (Kielstein and Rapp–Gabrielson, 1992); there seems to be an association between serovars, environmental conditions, concurrent post-weaning colibacillosis, and severity of the disease (Karg and Bilkei, 2002). Infected pigs may develop fever, pneumonia, shifting lameness (joints become hot and swollen) and neurological signs (Nielsen and Danielsen, 1975). Maternal and natural immunity are critical factors for controlling the disease (Nielsen and Danielsen, 1975). Vaccination of gilts resulted in protective maternal immunity for up to four weeks when piglets were

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challenged with the same serovar contained in the bacterin (Solano-Aguilar *et al.*, 1999). In large breeding units of our Hungarian clients, HPS serovars 2, 3, and 5 are often present concurrently with pathogenic *E. coli* serotypes (Bilkei and Biro, 1998).

Post-weaning colibacillosis is one of the major causes of death, reduced health and depressed economic performance in pigs (Francis, 1999). From the practical point of view (Bölcskei *et al.*, 1995), "post-weaning coli complex" (PWCC) can be manifest as post-weaning diarrhoea (PWD), oedema disease (ED), post-weaning wasting (PWW) or haemorrhagic gastroenteritis (HGE).

Oedema disease occurs usually during the first two weeks after weaning (Awad Masalmeh *et al.*, 1989). Pigs suffering from a chronic form of ED develop progressive vascular necrosis and post-weaning wasting, causing enormous economic losses (Bilkei *et al.*, 1996b). In Eastern Europe, ED and PWW are often caused by *E. coli* O139 that produce verotoxin 2e (VT2e; Moon *et al.*, 1999). Verotoxigenic *E. coli* colonise the intestine via F18 pilus (Wilson and Francis, 1986). If *E. coli* O139 has the ability to produce heat-labile toxin (LT), heat-stable toxins (STa, STb), enterotoxin (VT2e), both post-weaning diarrhoea and oedema disease can develop (Bilkei *et al.*, 1995).

Measures to control ED and PWW include:

- Immunoprophylaxis using verotoxin-toxoids (Awad-Masalmeh *et al.*, 1989; Johansen *et al.*, 1997)
- Improving social and environmental conditions at weaning (Bilkei *et al.*, 1996b)
- Nutrition of the weaned pigs (Bilkei *et al.*, 1996b)
- Post-weaning zootechnique and biotechnique, including the application of antibiotics, probiotics, tripellenamin, melperone, amperozide, clorpromazine, central nervous stimulants, prednisolone, zinc-oxide and phytogenic feed additives (Bilkei *et al.*, 1995; Bilkei *et al.*, 1996a, b).

An inactivated VT2e vaccine has been proven to be effective against the ED (Awad Masalmeh *et al.*, 1989). Johansen *et al.* (1997) successfully prevented the clinical and microscopical lesions typical of the disease by using a VT2e-toxoid vaccine.

In Europe, social pressure for reduction in the use of anti-microbial agents in pig production stimulated researchers to seek environment-friendly natural alternatives, which may exert similar therapeutic effects as in-feed antimicrobials. Phytogenic feed additives are not new in veterinary medicine. The essential oils derived from the plant *Origanum vulgare* have *in vitro* antimicrobial activities against various bacteria (Sivropoulou *et al.*, 1996). Its effectiveness has been proven against PWCC (Kyriakis *et al.*, 1998; Gertenbach and Bilkei, 2001), growth retardation of fattening pigs (Bilkei and Gertenbach, 2001) and porcine proliferative enteropathy (Tsinas *et al.*, 1998 a, b). It has been stated that oregano stimulates organic and micro-biotical digestion (Wim H. De Koning *et al.*, 1999). Oregano supports digestion and regulation of gastrointestinal metabolism (Günter and Bossow, 1998) and exerts antibacterial properties by hindering dysbiotic processes in the digestive tract of pigs (Sivropoulou *et al.*, 1996; Kyriakis *et al.*, 1998). It was reported that 1,000ppm oregano feed supplementation during the post-weaning period significantly improved weight gain and health of the pigs (Bilkei and Gertenbach, 2001).

TABLE 1: ***Ad libitum*** **weaner diet formula**

43%	corn,
24%	soyabean meal,
10.4%	cooked rice,
15%	corn starch,
4%	sunflower oil,
3.6%	mineral-vitamin-trace element mix.

Analysis: *Crude protein: 19%; lysine: 1.1%; calcium: 0.66%; phosphorus: 6.2%; fibre: 3.1%*

The purpose of this study was to determine if, under field conditions, pre-weaning vaccination with a VT2e–toxoid vaccine followed by post-weaning vaccination against HPS 5 and oregano as a feed supplement would improve post-weaning pig performance in a unit concurrently infected with pathogenic *E. coli* and HPS 5.

Material and methods

The study was conducted on a large Hungarian pig production unit of 1,000 sows (F1 of Landrace x Large White sows mated to Duroc boars) that suffered low production levels and high post-weaning mortality due to post-weaning coli complex and Glässer's disease.

Pre-trial procedures

In this herd enterotoxigenic *E. coli* (ETEC, presenting pilus types: F4, F5, F41, F18, respectively) and verotoxigenic *E. coli* (VTEC) having pilus F18, heat-labile toxin (LT) and/or heat-stable toxins (STa, STb) were repeatedly found by PCR technique in dead weaned pigs. The same animals tested positive for HPS 5. The herd was free from *Actinobacillus pleuropneumoniae*, toxin-producing *Mannheimia* (*Pasteurella*) *multocida* and *Brachyspira hyodysenteriae*. Before the trial, 16 weaned piglets (24 to 42 days of age, weaned at day 21 of lactation) that had died after showing the clinical symptoms of ED or PWW and Glässer`s disease were subjected to gross pathologic-anatomical and PCR assay investigations ("pre-trial screening"). The principal gross lesions were seen in the lungs (suppurative bronchopneumonia with fibrous structure) and joints (swelling, hyperaemia and cloudy thickening of the synovial membranes, accompanied by increased amount of serosanguineous synovial fluid, without cartilage erosions or degenerative changes on the articular surfaces).

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TABLE 2: Weights (kg) of vaccinated and/or oregano fed and control pigs

Group	day 21	day 28	day 35	day 42	day 49	day 56
1	5.89±0.42	8.09±0.51	11.63±0.67[a]	13.52±0.98[a]	17.59±1.83[a]	21.61±2.07[a]
2	5.61±0.37	7.99±0.55	11.32±0.72[a]	13.54±1.04[a]	17.93±1.78[a]	22.62±1.84[a]
3	6.66±0.46	7.89±0.49	9.36±0.82[b]	11.71±1.08[b]	15.24±1.05[b]	18.99±1.98[b]
4	6.58±0.44	7.72±0.53	9.64±0.85[b]	11.82±1.14[b]	15.23±1.42[b]	18.57±2.09[b]

Group 1	HPS 5 and *E. coli* vaccinated and oregano fed group of pigs
Group 2	HPS 5 vaccinated and oregano fed group of pigs
Group 3	*E.coli* vaccinated, HPS 5 vaccinated group of pigs
Group 4	Untreated control
a,b	difference is significant (p<0.05)
b,c	difference is significant (p<0.05)
a,c	difference is significant (p<0.01)

TABLE 3: Average daily gain (g) of vaccinated and/or oregano fed and control pigs

Group	Week 5	Week 6	Week 7	Week 8
1	372.83±37[a]	471.75±36[a]	571.72±37[a]	674.03±33[a]
2	359.53±29[a]	461.62±45[a]	580.83±49[a]	672.73±52[a]
3	271.53±36[b]	373.52±37[b]	452.55±37[b]	540.73±41[b]
4	221.74±58[b]	302.75±77[b]	442.88±47[b]	462.23±97[c]

TABLE 4: Feed efficiency (kg food/g weight gain) of vaccinated and/or oregano fed and control pigs

Group	Week 5	Week 6	Week 7	Week 8
1	1.32±0.12[a]	1.48±0.14[a]	1.51±0.11[a]	1.52±0.15[a]
2	1.31±0.12[a]	1.49±0.15[a]	1.51±0.17[a]	1.51±0.14[a]
3	1.52±0.16[a]	1.73±0.11[a]	1.74±0.14[a]	1.93±0.14[b]
4	1.68±0.18[b]	1.84±0.27[b]	1.85±0.26[b]	2.13±0.24[c]

TABLE 5: Mortality of vaccinated and/or oregano fed and control pigs

Group	No. weaners	No. died	Percent died
1	312	3	0.96[a]
2	308	4	1.29[a]
3	309	6	1.95[a]
4	301	32	10.63[b]

Laboratory procedures

(i) E. coli

Haemolytic *E. coli* isolated from the small intestine of suspected ED or PWW piglets were serotyped by slide agglutination tests. Analyses of the isolates were performed by multiplex PCR assay for detecting VT2e and F18 ab, ac, heat-labile toxin (LT) and heat-stable toxins (STa, STb). Amplified products suspended in 2% agarose gel were subjected to electrophoresis, stained with ethidium bromide and examined under ultraviolet illumination. DNA fragment lengths were verified by a digested λ-DNA standard run simultaneously. A control for DNA reference strains was included in each reaction. An experimental toxoid vaccine was produced (MacLeod and Gyles, 1991). The VT2e-toxoid was inactivated with 15% Emulsigen (MVP Laboratories USA, as described by MacLeod and Gyles, 1991). The toxin was purified by cation exchange chromatography of a polymyxin B extract of *E. coli* carrying a cloned VT2e gene under the control of the tac promoter (Waddell and Lingwood, 1996).

(ii) Glässer's disease: serological testing by ELISA

HPS 5 whole cells were used as ELISA antigen. All steps of ELISA and bacteriological investigation were carried out according to Solano-Aguilar *et al.* (1999).

Management of piglets

Within three days postpartum, 1671 new-born piglets were individually processed (ear tags, iron injection, clipping of tails and teeth). Cross-fostering was not permitted. At three weeks of age the piglets were weaned into a separate nursery unit. The weaners were allotted into four large nursery barns, with wire-mesh floor pens (0.8 m^2 per pig) where the animals spent five weeks in pens of 10 to 12 pigs. Each pen contained two self-feeders and two nipple drinkers. The room had a shallow pit manure storage system and was environmentally controlled with temperature maintained continuously at 22 to 24°C and a relative humidity of 75%. The piglets were fed *ad libitum* (Table 1). Due to pre-weaning mortality and for technical reasons, only 1,230 animals out of the 1,671 new-born piglets were randomly assigned to four groups and were treated as follows:

Group 1 (n=312): piglets were vaccinated intramuscularly at one week old with 12.5mg and at three weeks of age with 25 mg of inactivated VT2e toxin and they were vaccinated against HPS 5 (Respifend HPS, Fort Dodge Animal Health, USA) on days 27 and 41 post-weaning. The animals were fed with 1,000ppm oregano-supplemented weaning diet (Oregpig® Pecs, Hungary). Oregpig® is dried leaf and flower of *Origanum vulgare*, enriched with 500g/kg cold pressed essential oils of the leaf and flower of *Origanum vulgare* (analysis of Oregpig®: 60g carvacrol and 55g thymol/kg).

Group 2 (n=309): piglets did not received *E. coli* vaccination before weaning; after weaning, they were vaccinated with HPS 5 and they received oregano feed supplementation.

Group 3 (n=308): the animals were given both vaccines but oregano was not given.

Group 4 (n=301): piglets were neither vaccinated nor given oregano supplement.

The following parameters were evaluated: average daily weight gain in nursery (ADG); feed efficiency (FE); weaner mortality caused by HPS or PWCC according to gross and laboratory changes as described above in "pre-trial procedure". The effects of vaccination on ADG were analysed separately for the groups. In all groups, weight at weaning was included in the model. The gender (nearly identically distributed in the groups) was not included in the model. Nursery-end-weights were recorded. Piglets of a pen were weighed together weekly. The weights of pigs that died were recorded as well as the weights of the penmates. In order to get individual piglet weights, the pen weights were divided by the number of pigs in the pen. In order to accurately determine weekly feed consumption, feed was weighted when it was placed in the feeders and the feeders were weighted weekly at the same time as the piglets.

Statistical analyses

Data were analysed using ANOVA with the GLM procedure (SAS Cary, North Carolina, USA). ADG and FE were analysed for only the last four weeks of the nursery phase to avoid potentially confounding variables among treatment groups due to different weaning day and stress caused by different weather conditions and "human factor" (different workers) at weaning.

Results

From day 35 on oregano-treated pigs (Groups 1 and 2) were significantly ($p<0.05$) heavier than pigs in Groups 3 and 4 that were not given oregano (Table 2). On day 56 the oregano-treated and HPS-vaccinated animals (Group 2) had a 17.8% higher ($P=0.0024$) mean nursery-end-weight than the untreated control pigs (Group 4).

During the last four weeks of the nursery period, oregano-treated pigs (Groups 1 and 2) had significantly better ($p<0.05$) ADG (Table 3) and FE (Table 4) than the vaccinated pigs that were not fed the oregano supplement (Group 3).

As shown in Tables 3 and 4, the ADG and the FE were significantly better in the oregano-treated pigs (Groups 1 and 2) than in the untreated controls (Group 4) during weeks 6 ($p<0.05$), 7 ($p<0.05$) and 8 ($p<0.01$).

The control group suffered significantly higher ($p<0.05$) mortality (10.63%) while in the nursery than did the other groups (Table 5).

Discussion

In this study the effects on weaned piglets of a phytogenic feed additive and vaccinations against HPS and *E. coli*, singly or in combination, were evaluated under field conditions in an unit in which both HPS 5 and pathogenic *E. coli* infections were endemic (Bilkei and Biro, 1998).

In pigs, immunity to "early colonisers" (Pijoan, 1995), such as HPS, is good in early lactation but declines progressively. The piglet develops its own active immunity from about three weeks of age (Pijoan, 1995). Maternal vaccination is an effective way of preventing outbreaks of Glässer's disease (Miniats and Smart, 1988; Baumann and Bilkei, 2002b; Hoffmann and Bilkei, 2002). Vaccinated pigs from vaccinated gilts were protected against systemic lesions when challenged with virulent strains of HPS (Solano-Aguilar *et al.*, 1999). Following vaccination, sow immunity – and hence transfer of passive immunity via colostral immunoglobulins – is serovar specific (Rapp-Gabrielson *et al.*, 1997).

Consistent with reports by Awad Masalmeh *et al.* (1988), Bosworth and Casey (1997) and Johansen *et al.* (1997), the present study indicated that vaccination with purified VT2e-toxoid can prevent losses in weaned pigs attributed to post-weaning coli complex.

There was earlier evidence (Kyriakis *et al.*, 1998; Tsinas *et al.*, 1998 a, b) that oregano etheric oils had significantly improved the post-weaning performance of piglets, both in terms of ADG and FE and in coping with gastrointestinal problems associated with *E. coli* infections. The beneficial effects were attributed to the antibacterial properties of oregano. The present results were consistent with that interpretation. The mortality rate was remarkably low amongst the treated piglets.

Thus, under field conditions the combination of oregano as a feed supplement together with VT2e-toxoid and HPS 5 vaccine appeared to provide a satisfactory protective immunity against post-weaning coli complex and post-weaning Glässer's disease.

Further studies are needed to determine the duration of protection afforded by maternal immunity, the optimal time for vaccination, and the efficacy of vaccination against HPS 5 and *E. coli* under field conditions. The protection might be enhanced if farm-specific and geography-specific strains of *H. parasuis* are incorporated into the bacterins used for vaccination of sows.

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