Original Article

Pilot study: evaluation of homeopathic treatment of *Escherichia coli* infected swine with identification of virulence factors involved

Cideli de Paula Coelho[1], Erlete Rosalina Vuaden[2],
Francisco Rafael Martins Soto[3], Priscilla Anne Melville[4],
Flavia Carolina Souza de Oliveira[4], Leoni Villano Bonamin[5],
Andrea Micke Moreno[4], Patricia Tahan[1], Nilson Roberti Benites[4]

(1) Santo Amaro University, UNISA, São Paulo, Brazil
(2) Veterinarian Specializing in Homeopathy, Brazil
(3) Federal Institute of Education, Science and Technology, Brazil
(4) University of São Paulo, USP, Brazil
(5) Paulista University, UNIP, Brazil

ABSTRACT

Aim: To evaluate the efficacy of prophylactic homeopathic treatment of swine diarrhea to achieve a satisfactory balance between weight gain and cost. **Methods**: *Escherichia coli (E. coli)* was isolated from 4 piglets with diarrhea, their clinical signs were used to select the adequate homeopathic medicine *China officinalis (Chin*). Newborn piglets were divided in 4 groups (n=11 or 12): control group treated with the routinely used antimicrobial agent (ceftiofur 4 mg 1 ml/piglet for 3 days); one group with homeopathic medicine *Chin* 30 cH; one group treated with biotherapy prepared from locally isolated *E. coli*; and one group was treated with association of *Chin* and biotherapy. All isolated samples of *E. coli* were subjected to polymerase chain reaction (PCR) to identify virulence factors in each group. **Results**: There was no difference in the number of animals with diarrhea between groups treated with homeopathic medicine and control; weight gain was greater in groups treated with homeopathic medicine alone and associated with biotherapy compared to control ($p<0.05$). Cost of homeopathic treatment was lower compared to ceftiofur. Only one *E. coli* sample used to prepare biotherapy medicine tested positive for virulence factors (F41); 3 samples from the *Chin* treated group tested positive for Stb enterotoxins; 1 sample from the homeopathic medicine plus biotherapy treated group and 1 sample from the biotherapy treated group tested positive for F41; 2 samples from the control group tested positive for F41. **Conclusion**: Strain variability was too large in PCR to allow for any conclusion; despite feces tested positive for *E. coli*, weight gain of piglets was greater in groups with homeopathic treatment that thus can be rated more effective in newborn piglets diarrhea.

Keywords: Swine diarrhea, piglets, *E. coli*, homeopathy, biotherapy, PCR.

Introduction

Consumer demands for meat free from chemical residues and the ban on the use of antibiotics and other chemical agents in swine production especially by European countries [1,2,3,4] led technified swine production to search for alternative methods to treat and prevent the major diseases occurring in this context [2,4,5]. Homeopathic veterinary medicine is a relevant alternative approach acknowledged by agro-ecological international legislation; in Brazil the homeopathy has been recognized through the Act 662, of July, 14th, 2000, by the Federal Council of Veterinary Medicine. Besides its specific therapeutic action against definite pathogenic agents, use of homeopathy in swine production also aims at decreasing the herd level of stress and improve immune performance against bacterial, viral ecto and endoparasitic infections [2,5,6,7].

Besides homeopathic medicine, this study also tested nosodes (also known as biotherapy or isotherapy) prepared by way of dilutions of bacterial or parasite organic derivatives, in this case *Escherichia coli (E. coli)*. These products are sterilized and then diluted, thus, they are unable to infect individuals while they keep their potential to induce reaction [7,8,9,10].

Diarrhea is an important setback for intensive swine production systems in technified farms [3,11,12]. Enteritis can appear in three different stages: neonatal diarrhea appears in the first days of life; piglet diarrhea from the first week of life to weaning; and post-weaning diarrhea. *E. coli* is the agent most frequently associated with neonatal and post-weaning diarrhea in swine [14,15,16,17,18,19,20,21,22]. From the clinical point of view, piglets might refuse to suckle and exhibit watery diarrhea leading quickly to dehydration, weakness and eventually death [15,22,23].

Enterotoxigenic Escherichia coli (ETEC) is the importante pathotype in swine, Principally in neonatal diarrhea, and includes strains that elaborate one or several enterotoxins that induce severe diarrhea [24, 25].

The aims of this study were: a) to compare the efficacy of prophylactic treatment against swine diarrhea with homeopathic medicine and *E. coli* biotherapy both single and in association against conventional treatment (control); b) to identify experimental evidence grounding systematic indication of *E. coli* biotherapy in commercial swine production; c) to assess the difference in weight gain of piglets between groups; d) to compare the cost of treatments.

Materials and Methods

This study was carried out in a commercial swine production farm in the State of São Paulo, Brazil. *E. coli* was isolated from the feces of 4 piglets exhibiting diarrhea and was used to prepare biotherapy. Clinical signs observed in piglets were used to select the homeopathic medicine through repertory analysis (Table 1) [26,27] and survey of the homeopathic materia medica [9,28].

Homeopathic medicines were prepared according to the standard procedures described by Brazilian Pharmacopoeia in a pharmacy certified by ANVISA (National Agency for Sanitary Surveillance) [29]. Themedicines was prepared in 30% hydro-alcoholic solution.

The medicines were carried out in 30% hydro-alcoholic solution. The pharmacist in charge is an accredited homeopath. Vials were labeled with codes, which were disclosed only after the end of the study.

Table 1. Repertory analysis of symptoms exhibited by 4 piglets with diarrhea.

Symptoms	***Chin***	***Ars***	***Phos***	***Apis***	***Sec***	***Crot-t***	***Olnd***	***Podo***	***Verat***
Rectum, diarrhea, in nursing children	01	-	-	-	-	-	-	-	02
Rectum, involuntary, evacuation	02	02	03	01	03	01	03	-	03
Stools, watery, yellow	02	01	01	02	01	02	03	03	-
Stools, thin, watery, green	02	-	-	02	-	01	-	02	-
Generals, weakness, due to diarrhea	03	03	03	02	02	01	03	03	03
Generals, emaciation, cachexia	02	03	01	-	01	-	-	-	-
Total	11/6	09/4	08/4	07/4	07/4	05/4	09/3	08/3	08/3

Chin: *China officinalis*; *Ars*: *Arsenicum album*; *Phos*: *Phosphorus*; *Apis*: *Apis mellifica*; *Sec*: *Secale cornutum*; *Crot-t*: *Croton tiglium*; *Olnd*: *Oleander*; *Podo*: *Podophyllum*; *Verat*: *Veratrum album*. 01: Low frequency of symptom; 02: Average frequency of symptom; 03: High frequency of symptom. Total: total grades addition of each remedy/number of symptoms.

Forty-six newborn piglets were divided into 4 groups, which remained with their respective mothers. Births and treatments were simultaneous as follows:

- Group 1: *E. coli* 30cH prepared according to homeopathic standard procedure from strains isolated in the farm;
- Group 2: *China officinalis (Chin)* 30 cH plus *E. coli* 30cH;
- Group 3: *China officinalis* 30 cH (chosen after homeopathic analysis of symptoms exhibited by piglets with diarrhea);
- Group 4: control (antimicrobial agent routinely used in the farm to treat diarrhea: ceftiofur 4g, 1 ml/piglet for 3 days). Since this is a commercial production farm, the owner did not allow employing placebo.

Homeopathic medicines and biotherapy were administered 0.1 ml by oral route to newborn piglets in alternate days for 12 days; feces were collected to investigate *E. coli*.

Ethics and Conflicts of interest

This project was authorized by Bioethic Commission of de School of Veterinary Medicine and Animal Science of São Paulo University (USP), accordingly with the current legislation of animal protection, under the protocol number 1166/2007. The authors declare no conflicts of interest.

Detection of virulence factors in *E. Coli* strains isolated from the bowels content of swine by way of polymerase chain reaction

Investigation of virulence factors in *E. coli* strains isolated from swine was carried out by way of polymerase chain reaction (PCR) at Laboratory of Swine Health and Virology of Faculty of Veterinary Medicine and Zootechnics, University of São Paulo.

Genes codifying thermolabile (LT) and thermostable (Sta and Stb) enteroxins [25] and verotoxin VTe2 (Stx2e) were investigated together with genes codifying F4 (K88), F5 (K99), F6 (987P), F18 and F41 fimbriae to establish potential microorganism changes. Oligonucleotides used and sizes of amplified fragments are described in Table 2.

Table 2. Primers used in PCR to amplify fragments of different genes for enterotoxins (LT, STa and STb), F4 (K88), F18, F41, F5 (K99), F6 (987P), F41, Stx2e [29].

Primer	Forward primer (5' – 3') / Reverse primer	Size of amplified product (bp)
F4 (K88)	tga atg acc tga cca atg gtg gaa cc gcg ttt act ctt tga atc tgt ccg ag	500
F18	tgg cac tgt agg aga tac cat tca gc ggt ttg acc acc ttt cag ttg agc ag	380
F41	tta gca gcg aag atg agt gat ggg gta cta cct gca gaa aca cca gat cc	600
F5 (K99)	gcc agt cta tgc caa gtg gat act tc gtt tgt atc agg att ccc tgt ggt gg	250
F6 (987P)	gcg act acc aat gct tct gcg aat ac gaa cca gac cag tca ata cga gca	400
LT	acg gcg tta cta tcc tgt cta tgt gc ttg gtc tcg gtc aga tat gtg att ct	290
STa	gtc agt caa ctg aat cac ttg act ct cat gga gca cag gca gga tta caa ca	190
STb	gct aca aat gcc tat gca tct aca ca cat gct cca gca gta cca tct cta ac	150
Stx2e	cgg tat cct att ccc agg agt tta cg gtc ttc cgg cgt cat cgt ata aac ag	700

DNA-extraction was carried out by the boiling method [21,30]. Bacterial samples were collected and suspended in 200 µl buffer solution (Tris-EDTA). All samples of bacterial suspension were boiled for 10 minutes [31,32,33], followed by incubation in ice for 15 minutes, centrifugation for 1 minute; the supernatant was used for testing [34].

Statistical analysis was performed by Fisher exact test at 5% significance level [35] with software GRAPHPAD INSTAT 1992-98.

Assessment of cost per piglet per treatment

Cost of treatments was estimated by the following formula: **(A x B) x C = V**, where: A – each piglet daily dose; B – length of treatment in days; C – cost of 1 ml of medicine; V – cost per piglet (in BRL, 1 BRL = 0.622 USD).

Results

There was no statistical difference in the number of animals with diarrhea at the beginning and the end of treatment between groups treated with homeopathy and control, but the number of animals with diarrhea was lower in the group treated with association of homeopathic medicine and biotherapy (Table 3).

Table 3 – Number of animals with diarrhea at the beginning and the end of treatment and percentage and mean of the sample weight in grams at the beginning of treatment and weaning, number of piglets in each group and weight gain of the sample (Fisher test).

		E. coli 30 cH	*China officinalis* 30 cH *plus E. coli* 30 cH	*China officinalis* 30 cH	Control Ceftiofur	Total of piglets
Animals with diarrhea	Day 1 (%)	2 (16,6%)	1 (8.3%)	3 (25,0%)	1 (9,0%)	47 (100%)
	Day 12 (%)	5 (41.6%)	0 (0%)	0 (0%)	2 (18.1%)	43 (100%)
Average weight of the sample	Day 1	1116 (12)	1950 (12)	1107 (12)	1600 (11)	47
	Day 24	3627 (12)	6416 (8)	6100 (12)	6000 (11)	43
	Weight gain	2511**	4466	4993*	4400	

* Fisher test, $p < 0.05$ compared to the remainder of groups.
** Fisher test, $p < 0.05$ compared to control.

At the beginning of treatment and weaning phase there was statistical difference in the weight gain among groups (Table 3). The group treated with *Chin* 30cH exhibited significant ($p<0.05$) greater weight gain than the other 3 groups. Weight gain in the group treated with association of homeopathic medicine and biotherapy was also greater compared to the control group. On the other hand, the group treated with *E. coli* 30cH exhibited significant less weight gain ($p<0.05$) compared to the control group (Table 3).

Four animals that exhibited greater development than the remainder of animals in the group treated with association of homeopathic medicine and biotherapy were removed, this being a routine practice in this farm.

Among the samples used to prepare biotherapy, only one tested positive for virulence factors (F41). No enterotoxin was found in the tested samples.

Three samples positive for Stb enterotoxins were found in the group treated with *Chin* 30cH; one sample in the group treated with association of homeopathic medicine and biotherapy tested positive for virulence factor F41. In both groups, despite the presence of virulence factors in the tested samples, diarrhea did not occur until the end of the study. One sample tested positive for F41 in the group treated with biotherapy alone, 2 samples tested positive for F41 in the control group.

Homeopathic and biotherapy treatments had the lowest cost (USD 0.09 per piglet), association of both had intermediate cost (USD 0.18 per piglet), whereas treatment with ceftioufur 4 g had the highest cost (USD 1.30 per piglet).

Discussion

Results point to a lack of correlation between the clinical evolution induced by treatments and the presence of genes for virulence factors in the samples taken from each group. Further studies are needed to understand better the mechanisms involved and whether treatments exert or not selective pressure on the strains of *E. coli* prevalent in this farm.

Technological improvement in intensive swine production resulted in significant increase of meat production in the last decades; however, previously unknown problems also arose, such as high replacement rate and the need to increase periodically the number of sows. Too frequent introduction of animals in farms is a serious sanitary risk since they might carry or incubate infectious diseases absent in the destination herd [3,36].

Causes of diarrhea in piglets are multifactorial and the simple presence of enteropathogens does not suffice for disease to develop. Morbidity and mortality depend on agent virulence, host resistance and presence of risk factors such as environmental temperature, poor hygiene in facilities and difficult access to colostrum and milk [2,6,7,15,16,23]. In swine, *E. coli* develops resistance easily and is associated with neonatal diarrhea [14,23,37]. Antibiotic resistance in bacteria not only affects pig production, but also has an impact on human health through the transfer of resistant organisms via the food chain [38].

In one blind placebo-controlled trial, where treated sows with *E. coli* 30K for a month before delivery. The frequency of piglet diarrhea was 6 times higher in the placebo group than in the group treated with homeopathic medicine ($p<0.0001$). These authors observed that protection was seemingly higher in the group treated with homeopathy, since diarrhea did not propagate, whereas piglets in the placebo group infected one another quickly [39]. It is noteworthy that in this study sows were treated instead of piglets, which points to the importance of global management together with homeopathic treatment to achieve satisfactory results in animal production. Future studies are needed to compare the performance of different dilutions in a same experimental setting (Hahnemann´s centesimal dilutions –cH- and Korsakov method –K-).

Ceftiofur, which was used in the control group, is rated a good antimicrobial agent against *E. coli* [40]. Nevertheless, broad and sometimes even indiscriminate use of antimicrobial agents results in the selection of resistant bacteria [21,41]. The latter might not only become predominant within the bacteria population but may also transfer genetic materials to susceptible bacteria that thus become also resistant [41]. Resistance to antibiotics and ability to produce enterotoxins are frequently transferred together, therefore it is believed that the use of antibiotics might increase the number of pathogenic *E. coli* strains [42]. For these reasons, alternative and complementary strategies must be found to achieve a rational use of antimicrobial drugs in swine production [43].

The two groups treated with homeopathic medicine (*Chin* 30cH) in our study exhibited the greatest weight gain. This suggests that homeopathic medicines chosen according to the principle of therapeutic similarity might contribute to the weight gain of piglets during their phase of development. It is worth to remind that decision-making in homeopathy is grounded on the *characteristic totality of symptoms* and *individualization of medicines*. This means that individuals exhibiting a same disease might be treated with different homeopathic medicines as a function of their peculiar clinical manifestations (including behavioral, general and particular signs and symptoms) [9,44]. In this study, absence of diarrhea in animals treated with *Chin* 30cH associated or not with biotherapy also correlated with greater weight gain [45].

When compared the use of antibiotics to homeopathic treatment on animals in the fattening stage in intensive swine production farms, zootechnical results were better in the group treated with homeopathy, which agree with the results obtained in our study [46]. Previous studies by our group in piglets during the nursery, growth and finishing phases using homeopathic medicines instead of conventional treatment showed

reduction of the mortality index from 3.2% to 1.7% during the nursery phase (p=0.03) and from 5.9% to 0.3% during the growth phase (p<0.01). Therefore, homeopathy allowed for a higher rate of animal production per womb with an overall annual cost 50% lower compared to conventional treatment [5].

These results agree with this study, where also the association of *Chin* 30cH with biotherapy resulted in significant cost reduction compared to ceftiofur. One sow delivering 12 piglets produces about 30 piglets per year: cost of treatment per sow per year would be USD 2.65 with homeopathy and USD 39.00 with ceftiofur. In a farm with 200 sows, this difference would amount to saving USD 7,226.56 per year.

Besides financial advantages, we still need to remind the requirement to diminish or abolish the use of oral or parenteral antibiotics in animal (swine, bovine and fowl) production to comply with the legislation of meat importing countries, particularly the European community [2,4,46,47].

Conclusion

Use of homeopathic medicine associated or not with *E. coli* biotherapy prepared from samples isolated in the farm was efficient to decrease the number of animals with diarrhea at the end of the study and increase their weight gain. These results together with significant cost reduction highlight the potential role of homeopathy as zootechnical tool in technified swine production. Further studies are needed to optimize management and the techniques to apply this resource.

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