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**ΜΕΛΕΤΗ ΤΗΣ ΑΠΟΤΕΛΕΣΜΑΤΙΚΟΤΗΤΑΣ ΣΤΗΝ ΠΡΑΞΗ
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ΤΩΝ ΝΕΟΓΕΝΝΗΤΩΝ ΧΟΙΡΙΔΙΩΝ**

ΣΠ. Κ. ΚΥΡΙΑΚΗ**

**FIELD EFFICACY STUDY WITH A NEW 3 STRAINS
ESCHERICHIA COLI BACTERIN
AGAINST NEONATAL PIGLETS DIARRHOEA**

S.C. KYRIAKIS*

SUMMARY

Piglets from sows vaccinated with 3 *E. coli* pilus antigens (09:K35, 99/0157:K88ac and 020, K101:987P) were shown to be protected against natural neonatal diarrhoea. Mortality was less ($P < 0,005$) in the group of piglets from vaccinated sows, up to the age of 35 days, as compared to controls. Also the reduction in scours and the general health status was better in the same group of piglets.

The average live weight gain was improved in the group of piglets from vaccinated sows over a 3 week period (weaning age) and at 35 days ($P < 0,005$). This field efficacy study was carried out in a commercial swine operation with 1.200 sows under production (22.500 piglets/year) and with the involvement of 1546 piglets.

INTRODUCTION

Neonatal diarrhoea due to colibacillosis is still a major problem in the swine industry. Etiology, pathogenesis, clinical signs, immunity diagnosis, treatment and prevention have been thoroughly investigated (Orskov et al., 1977· Kohler, 1978· Moon, 1978· Taylor, 1981· Wilson, 1981).

Threatment is based mainly upon the use of atibacterial agents (Ensley et al., 1979) and in many cases the addition of supporting therapy to combat dehydration due to diarrhoea (Kyriakis, 1981).

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However, the use, in general, of antibacterial agents had varying degree of success associated with: bacterial resistance, residues from improper administration and problems due to the time consuming methods for application of the final formulation of the commercial product, to very young animals such as the neonatal piglets (Kyriakis, 1981· Libal and Gates, 1982).

Prevention is based upon the improvement of the husbandry measures, especially in the farrowing room for both, sows and piglets (Taylor, 1981· Wilson, 1981). Another way of prevention is vaccination. Immunity to pathogenic *E. coli* strains may be stimulated in a number of methods. The efficacy of all biological methods varies. Although mortality of piglets can be reduced and loss of productivity in general is prevented, full protection may not be complete (Taylor, 1981), either because of the possible involvement of other pathogenic agents, or due to the use of the a non efficacious vaccination programme (Taylor, 1981· Wilson, 1981).

In the past, the following methods of stimulating immunity of the piglets, against neonatal colibacillosis, were used:

- a) Vaccination of the sow before farrowing. Killed *E.coli* vaccines given parenterally can, in many cases, induce serum antibody which then is passed to the piglet via colostrum.

In this case, vaccines of mixed pathogenic serotypes, are used or other which contain strains bearing K88 and LT enterotoxin antigens. For all the above cases two injections are given to the sow, one 6 weeks prior to farrowing and a second booster dose 3 weeks prior to farrowing. Killed oral vaccines containing 7 enteropathogenic serotypes, are in use for many years. Autogenous vaccines are also in use in many countries including Greece and given dead or live «Kohler type» (Porter, 1973· Taylor 1981).

Live vaccines are also available but with a strong criticism from the field (Pig Intern., 1982).

- b) Vaccination of the piglet for the control of both neonatal and post weaning diarrhoea due to *E. coli*, using all types of biologicals except the live «Kohler type» (Taylor, 1981) and
- c) Passive immunisation with hyperimmune sera to *E. coli* in piglets either with diarrhoea or at risk from it (Taylor, 1981).

In Greece, neonatal diarrhoea due to colibacillosis is a very important economical problems of the developing swine industry. Losses vary, but overall, the total mortality of the piglets due to the 3 associated clinical conditions covering by colibacillosis (septicaemia, diarrhoea and oedema disease) is up to 25% of the born piglets, until the age of 60 days. Half of these losses are due to neonatal diarrhoea caused mainly by pathogenic *E.coli* strains (Stoforos, 1973· Kyriakis, 1981).

In the past all the above types of vaccines were used in Greece, but non of them gave full solution to the problem. Also, the use of antibacterial agents had varying degrees of success, especially when they were not associated with an parallel improvement of husbandry methods and the use of supporting therapy against dehydration (Kyriakis, 1981).

During the last few years a new type of vaccine was developed for use in pregnant sows and gilts for the prevention of neonatal diarrhoea due to *E.coli*. This vaccine (Porcimune T.M.) contains the pilus antigens from 3 strains of *E.coli* found very common on baby piglets suffering from diarrhoea in the field. The 3 strains are 1439 (serotype: 09:K35,99), P-12 (serotype: 0157: K88ac) and 1413 (serotype: 020, K101:987P). The proportions of each strain in the commercial product is 1459: 1 part, 1413: 1 part and P-12: 2 parts. This trivalent bacterin, adjuvanted with aluminium hydroxide in cell mass, is to be given intramuscularly (IM) to pregnant sows in two 5,0 ml injections. The first 5-6 weeks and the second 3 weeks before the expected farrowing date. Published results indicated that the product is working in the field for the control of the losses due to the neonatal colibacillosis of piglets (Morgan et al., 1978· Nagy et al., 1978· Moon et al., 1980).

In Greece there are not any data or even field experience with this type of 3 (K88, K99 and 978P) porcine *E. coli* strains bacterin.

MATERIALS AND METHODS

Experimental Animals and Farm:

Two hundred sows and their new born piglets were used in the experiment, which was carried out in Greece during 1982, in a commercial swine farm with 1.200 sows under production. All animals (including sows and piglets) on trial had the same genetic potential and lived under the same management conditions (housing, nutrition and hygiene). The farm had always problems of colibacillosis and especially neonatal diarrhoea, with high mortality. The diagnosis of neonatal colibacillosis was based on history of the diseases in the farm, clinical observations, post mortem examinations and laboratory examinations for: enteropathogenic *E. coli* strains (production of ST enterotoxin/the suckling mouse test), *clostridia spp*, *coccidia spp*, TGE, rotavirus and *B. coli*.

Experimental Design: Animals were allocated as follows:

T1 group 100 sows: (age $4 \pm 1,5$ years old) two 5ml IM injections of Porcimune behind the ear, 6 weeks and 3 weeks before expected farrowing dates.

T2 group 100 sows: (age $4 \pm 1,5$ years old) used as control animals, with two 5ml IM placebo injections at the same period as in the case of T1 group. Piglets born from the above 200 sows were under investigation 2 weeks post weaning, until the age of 35 days. Piglets were weaned at 21 days by removing the sow from the farrowing room, and stayed in the same pen for an additional 2 week post weaning period.

This experiment had the following main criteria for evaluation:

- a) Any adverse reactions due to the vaccination of the sows.
- b) Stillbirth rates and mortality of piglets until the age of 35 days.
- c) Any evidence of diarrhoea during the first 2 weeks of life, due to colibacillosis, in all the litters from the 200 sows. The severity of diarrhoea present

was based on fecal consistency and health status; both were scored at the onset of disease on days 1,2,3,5 and 10 on a scale from 0 to 3 (0=normal, 1=feces yellowish/litter quite healthy, 2=feces yellowish and water/liter depressed and 3=severe diarrhoea/litter very depressed).

Total litter weights were recorded on days 1,21 and 35 of their life. Pregnant and lactating sows and litters from both experimental groups (T1 and T2) having any disease problems other than these related to the trial, were removed. Litters with less than 8 born piglets (alive or dead) or more than 11 (also alive or dead) were removed from the trial.

RESULTS

I. Post vaccination observation

No aversive reactions were observed at the injection site of each vaccinated sow (T1 group). No abortions were recorded or any other disease problem related with the vaccination.

II. Stillbirth rates and mortality

The final number of litters that were on trial from both groups, due to problems not related with the trial it self was:

T1 group/positive sows/litters = 82 on trial

T2 group/negative sows/litters = 84 on trial

The difference in stillbirth rates between the vaccinated ad control sows was not statistically significant (table 1)

TABLE 1
Effect of vaccination of stillbirth rates

Group	Number of piglets		Piglets/litter alive
	Dead	Alive	
T1 (vaccinated sows)	58 ^a	762 ^a	762:82=9,29 ^a
T2 (non vaccinated sows)	61 ^a	784 ^a	784:84=9,33 ^a

* Number with the same superscripts are not significantly different («t» test).

During the first 35 days of the life of piglets the mortality caused by diarrhoea due to colibacillosis and any other mortality, except due to accidents was recorded (table 2). There was a significant difference in mortality between the vaccinated and nonvaccinated groups ($P<0,005$).

TABLE 2
Mortality of the piglets up to the age of 35 days.

Group	No deads/No of piglets	Mortality %
T1	51/752*	6,79 ^{a***}
T2	82/755**	10,58 ^{b***}

* 762-752=10 piglets due to accidents

** 784-775=9 piglets due to accidents

*** «t» test statistically significant different (P<0,005).

III. Clinical scores

Any diarrhoea problem, during the first two weeks due to colibacillosis (the diagnosis was based on clinical observations, post mortem and laboratory examinations), was recorded. Treatment was based upon the use of the proper antibacterial agents for both groups, after the results of sensitivity tests. Results of the mean scores of fecal consistency and health status (0-3 score) combined, for trial days 1,2,3,5 and 10, are shown in table 3 and indicate that the group of piglets from vaccinated sows had less clinical problems than the controls and any disease problem disappeared by the 5th day.

TABLE 3.
Effect of vaccination of sows on clinical scores (fecal consistency/health status 0-3)
During neonatal piglet colibacillosis.

Group litters with diarrhoea	Day 1	Day 2	Day 3	Day 5	Day 10
T1/21 out of 82	0,75	0,45	0,12	0,15	0
T2/39 out of 84	1,50	1,76	1,25	0,70	0,2

IV. Average Live Weight (L. W.)

The average piglet weight, according to the total litter weights on day 1,21 and 35 was also recorded (table 4)

In comparison with the controls (T2 group), the immunized piglets (T1 group) had better growth, not only in the critical age of weaning (21 day), but also two weeks later. The difference in both cases was significant (P<0,005).

TABLE 4
Average L.W. of piglets in Kg

Group	Day 1	Day 21	Day 35
T1	1,250 ^{a*}	5,300 ^{a**}	9,600 ^{a**}
T2	1,260 ^a	4,850 ^b	8,700 ^b

* Means with the same superscripts are not significant difference («t» test).

** Statistically significant different ((P < 0,005) «t» test).

DISCUSSION

In the four parameters analysed in this trial the bacterin of the 3 very common *E.coli* strains responsible for the neonatal piglet diarrhoea, was shown to be highly efficacious in reducing mortality (P<0,005), clinical signs of diarrhoea and increasing L.W. not only on day 21 (weaning day), but also 2 weeks later (P<0,005).

This efficacy study which was carry out in a commercial swine farm with 1200 sows under production (average number of piglets/ year 22,500), and with acute problems of neonatal colibacillosis, indicates the validity of this new bacterin under field conditions for the control of this disease. Another factor that gives confidence is the large number of piglets on trial (1546 animals) and the statistical analysis of the data which both provide an extra reliable estimation of the bacterin performance in the field.

ΠΕΡΙΛΗΨΗ

Στην εργασία αυτή περιγράφεται η μελέτη αποτελεσματικότητας στην πράξη, ενός νέου τριδύναμου εμβόλιου, κατά της κολοβακτηριαδικής διάρροιας των νεογέννητων χοιριδίων. Το εμβόλιο αυτό παράγεται από τα ινίδια (pilus) 3 οροτύπων: α) 09:K35,99, β) 0157:K88ac και γ) 020, K10J:987Π, της *E.coli*, που είναι κυρίως υπεύθυνοι για τη αρρώστια αυτή. Το εμβόλιο αυτό κυκλοφόρησε τώρα τελευταία στις Η.Π.Α. (Porcimmune T.N.). Στην Ελλάδα η κολοβακτηριακή διάρροια των νεογέννητων χοιριδίων αποτελεί μεγάλο νοσολογικό πρόβλημα, με ανυπολόγιστες οικονομικές ζημιές. Επίσης δεν υπάρχουν ανάλογες δημοσιεύσεις και εμπειρία από τη χρησιμοποίηση τέτοιων βιολογικών προϊόντων.

Η εργασία αυτή έγινε το 1982, σε χοιροτροφική μονάδα με 1.200 σύες σε αναπαραγωγή και συνολική παραγωγή 22.500 περίπου χοιριδίων το χρόνο με προβλήματα κολοβακτηριδικής διάρροιας στα νεογέννητα. Χρησιμοποιήθηκαν ως πειραματόζωα 1546 χοιρίδια που πέρχονταν από δύο ομάδες σών. Στην ομάδα Τ1 έγιναν, την 6 και την 3 εβδομάδα πριν τον ανα-

μενόμενο τοκετό, δύο ενδομυϊκές εγχύσεις του εμβόλιο και στην T2 έγιναν αντίστοιχα εγχύσεις φυσιολογικού ορού.

Λήφθηκαν τα κατάλληλα μέτρα να μην υπάρχει διαφορά γενετικού δυναμικού και συνθηκών γενικά εκτροφής, των δύο πειραματικών ομάδων των σιών και των χοιριδίων τους.

Από τα αποτελέσματα φαίνεται ότι η θνητότητα, μέχρι της ηλικίας των 35 ημερών μειώθηκε σημαντικά ($P < 0,005$), στην ομάδα των χοιριδίων που πρέρονταν από εμβολιασμένες σύες (table 2).

Επίσης η υγιεινή κατάσταση ήταν καλύτερη στην ομάδα T1 και συνοδεύονταν από μείωση των περιστατικών διάρροιας μέχρι της ηλικίας των 10 ημερών (table 3). Τέλος το μέσο σωματικό βάρος των χοιριδίων, της ομάδας που οι σύες εμβολιάστηκαν παρουσίασε σημαντική διαφορά ($P < 0,005$) και την 21η ημέρα και την 35η μετά τη γέννηση, σε σύγκριση με τα αντίστοιχα χοιρίδια που προέρχονταν από σύες που δεν εμβολιάστηκαν (table 4).

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