Technology offer :
COLIGO,
a novel alternative to antibiotics to protect piglets against diarrhea

Table of contents

Target market and value

With COLIGO we target the market of alternatives to antibiotics to control diarrhoea in piglets.

In young pigs, diarrhoea and oedema disease are extremely important causes of illness with growth retardation and mortality, resulting in severe economical losses due to mortality, decrease growth rate and costs of medication. World-wide approximately 11 % of all post-weaning mortality is due to diarrhoea, which is around 10 million piglets per year. Approximately 50 % of this mortality is due to *E. coli*. Other causes are rotavirus, Salmonellosis and *Brachyspira hyodysenteriae*.

There are currently two vaccines on the market against these pathogens: an oral avirulent F4+ *E. coli* vaccine and a toxoid vaccine to prevent disease caused by the VTx2e toxin. Neither of both vaccines can however protect piglets against diarrhoea caused by F18+ *E coli*. The only alternative to control diarrhoea caused by F18+ *E. coli* is the use of antibiotics. Their use as growth promoter is however banned in most of Europe since 2006 and is increasingly being banned in many countries globally due to the problem with drug resistant bacteria. Also in the US the government is implementing control measures to reduce prophylactic use of antibiotics in pig production. The American Association of Swine Veterinarians (AASV) suggests that if pig growers in the US stopped using antimicrobials for non-therapeutic purposes the annual loss to the sector could be as much as $700 million. The ban on prophylactic use of antibiotics already motivated several feed additive companies to propose alternatives to antibiotics to the market. Today several alternatives such as pro-biotic/pre-biotic feed additives, organic acids, plant and yeast extracts are used. They all claim to be effective against (*E. coli*) diarrhea. In reality however, none of these alternatives specifically targets ETEC infection post-weaning and their effect *in vivo* is often hard to demonstrate. The use of plasma protein against ETEC-related PWD is much more expensive and therefore much less applied.

With COLIGO we could position us between the cheaper (and less effective) alternatives and the more expensive products like plasma powder.

Thirty (EU15) to forty (EU27) million piglets are produced yearly in Europe. At an average feed intake of 32kg per piglet during the critical period between weaning and 6 weeks after weaning this corresponds to 960.000 ton of feed (or 180000 ton of feed for the first two weeks after weaning). At an inclusion rate of 1kg per ton of feed this would thus correspond to a market potential of 180 ton COLIGO for the EU market only or a turnover of more than 16 million EURO (see Economical feasibility).

Description of COLIGO

Infections causing diarrhoea are established by ingestion of an $F4^+$ or $F18^+$ *E. coli* strain that adheres via its surface appendages, F4 or F18 fimbriae, to specific receptors (F4R or F18R) expressed by the intestinal epithelial cells.

The prophylactic strategy against $F18^+$ *E. coli* is based on the recent identification (1) of the receptor that is involved in binding of $F18^+$ *E. coli* to the porcine intestinal epithelium (*Patent protected EP2344167*). Fundamental knowledge of this receptor enabled us to select molecules analogous to the receptor to inhibit $F18^+$ *E. coli* adherence. Several compounds were screened for inhibitory capacity using an *in vitro* adhesion assay and an intestinal perfusion assay. Next, the selected

compound was supplemented to pig food and tested for its protective effect in a challenge experiment with F18^{+} *E. coli*.

COLIGO is composed of natural/GRAS-components. This together with the low inclusion rate in feed or drinking water lead to in vivo efficacy with low risk of toxicity or side effects.

Proof of concept and status of development

We have set up an in vivo challenge experiment after

- in vitro screening (3) of the capacity of different compounds to **interfere with attachment of F18 E coli to the pig's intestinal epithelium**
- confirmation of the the inhibiting capacity of the most powerful inhibitor in an intestinal perfusion test by demonstration of its **blocking effect on diarrhoea induced by an enterotoxin producing F18 strain** (2)

18 pigs of 3-4 week-old were selected on sensitivity to F18 E coli (genetic marker : FUT1 GG (F18R^{+})) and absence of F18 E coli infection (F18 sero-negative). The pigs were divided at random over the following treatments (6 pigs per treatment):

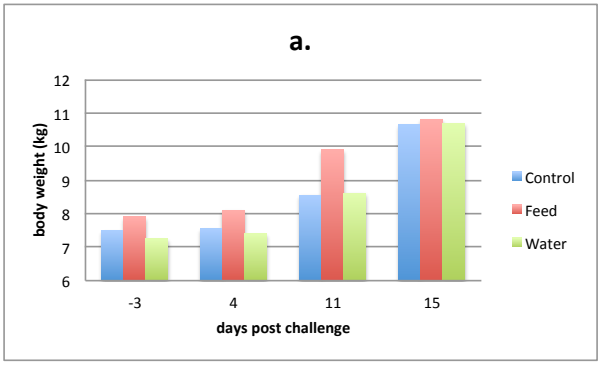
- COLIGO supplied via drinking water: 2.16 mg active ingredient per liter (daily water consumption estimated at 2.5 liter per pig ; ca. 5.4 mg active ingredient per pig per day)
- COLIGO supplied via feed: 11mg active ingredient per kg feed (daily intake estimated at 400 gram per pig ; ca. 4.3 mg active ingredient per pig per day)
- Control: 2.16 mg control ingredient per liter via drinking water and 21.58 mg control ingredient per kg feed

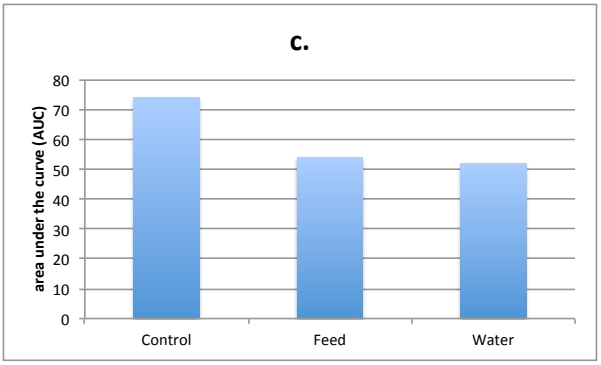
All pigs were orally challenged at two consecutive days with 10^{11} VTEC (F107/86) per pig per day.

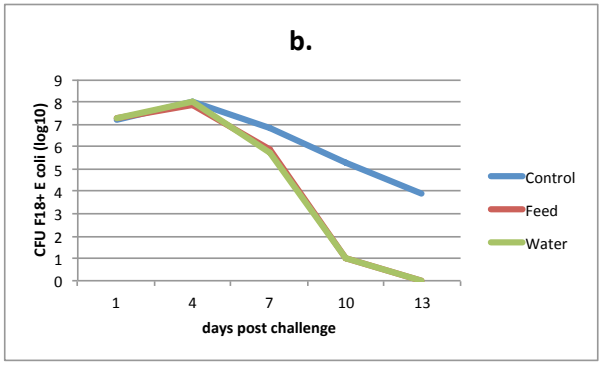
Consecutive events are shown on the following timeline:

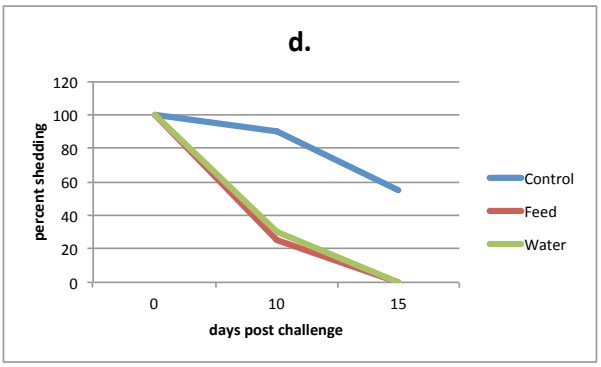
- S: screening was performed at day -13
- Col: colistine, Nu: Nuflor, Ba: Baytril

S	-7	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	day
	Col	Col	Nu/ Ba	Nu/ Ba																	Antibiotics
		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	Products
		W							W							W				W	Weight
		B							B							B				B	Blood
					F	F	F	F	F	F	F	F	F	F	F	F	F	F		F	Feces
					C	C															Challenge









Results : **a.** body weight before and after challenge ; **b.** shedding of F18+ E coli in feces (CFU (log10) of F18+ E coli per gram feces ; **c.** area under the shedding curve. The area (log10 number of $F18^+$ E coli excretion per gram x 15 days) represents the effect of the treatment on the colonization of $F18^+$ E coli in the gut during the whole 15-day duration. ; **d.** Percentage of animals per group that were shedding F18+ E coli post challenge.

Only in the control group clinical signs of oedema disease were observed with one pig that died 5 days after infection. These results clearly demonstrate that, while COLIGO does not completely inhibit colonization of F18 E coli, **it significantly reduces both duration of shedding and the quantity of bacteria that are colonizing and being shed in the environment**.

Thus, critical levels of F18 E coli to induce diarrhea/oedema are not reached while **the contact of the pig's immune system with the bacteria is still sufficient to build up protective immunity**.

Economical feasibility

The economical feasibility of COLIGO has been evaluated based on the following parameters:

Treatment:

- Duration: first 2 weeks after weaning
- Inclusion rate in feed (active ingredient): 10g/T (this could be supplied for instance via a feed additive (COLIGO-FA), containing a carrier and the active ingredient, at an inclusion of 1kg/T)
- Feed intake: 0.4 kg/day/piglet

EU15Market:

- 30 M piglet/year
- 180 000 tons of feed (first two weeks after weaning)

Table 1 shows an overview of the different alternatives, their usual inclusion rate in the different feeds that are fed during the period between weaning and 6 weeks post-weaning (F1 feed, from weaning to 2 weeks after weaning and F2 feed from 2 weeks to 6 weeks after weaning) and their cost per 1000 piglets.

In the example in table 1 we consider a cost of 90EURO per kg and an inclusion rate of 0,1% (of the product containing 1% active ingredient) in the F1 feed. The inclusion rate of the active ingredient in the feed would then be 0,001%. This dose is based on results from in vitro tests, intestinal perfusion tests and a small scale in vivo challenge test.

A more precise positioning of COLIGO-FA versus the competitive alternatives will be possible when we will have more information on i) the efficacy under field conditions and on ii) the cost of goods when the active ingredient is produced at an industrial scale. The price of 90EURO per kg is an estimation of the cost of goods of the active ingredient at large industrial scale based on current production parameters.

	F1 feed	F2 feed	
Period of treatment (W = weaning, w=week)	W to W+2w	W+2 w to 6 w	
Piglet Body weight (kg)	7 - 10.5	10.5 - 25.5	
Feed Intake (kg)	6	26	
Feed cost (€/ton)	350	275	
Alternative for Antibiotics			**Cost per 1000 piglets (€)**
Organic acids			
Cost (€/kg)	1.5	1.5	
Inclusion rate in feed (%)	0.5	0.5	
Cost for 1 ton of feed (€/ton)	7.5	7.5	
			240
Immunostimulants			
Cost (€/kg)	4	4	
Inclusion rate in feed (%)	0.5	0.1	
Cost for 1 ton of feed (€/ton)	20	4	
			224
COLIGO			
COLIGO-FA Cost (€/kg) (1% of active ingredient)	90	90	
COLIGO-FA Inclusion rate in feed (%)	0.1	0	
(final active ingredient inclusion rate in feed)	*(0.001)*		
COLIGO-FA Cost for 1 ton of feed (€/ton)	90	0	
			540
Plasma powder			
Cost (€/kg)	3	3	
Inclusion rate in feed (%)	4	0	
Cost for 1 ton of feed (€/ton)	120	0	
			720

The prices listed above can be compared to the cost of antibiotic treatment.

Antibiotic Name	**Cost €/1000 piglets**
Apramycine	490
Colistine	100
Néomycine	200
Forcyl	500
Apramycine	300
Marbocyl 2%	300

At 90EURO per ton of feed and a consumption of 180000 tons of feed during the targeted period of 2 weeks after, COLIGO has a market potential of 16,2MEURO.

Intellectual property

- *EP2344167 ; INHIBITORS OF F18^{+} E COLI BINDING* (Ghent University)
- *Know-how on E coli infections including animal models, field trials and related diagnostics*

Partnering

We are looking for partners who are interested

- in collaboration on further validation of the efficacy of COLIGO in large scale field trials
- in preparing the production and supply of COLIGO at industrial scale
- to market COLIGO

We can offer expertise in F18 E coli infection and immunity

- a challenge model for F18 E coli and experience in organizing and evaluating field trials
- diagnostics for monitoring F18 E coli on farm and in infected animals (isolation, quantification)
- diagnostics to determine genetic sensitivity of pigs to F18 E coli and selecting the right farms for the field trial
- diagnostics to evaluate the immune responses to vaccination/infection

References

1. Coddens, A., Diswall, M., Ångström, J., Breimer, M.E., Goddeeris, B.M., Cox, E. and Teneberg, S. (2009) Recognition of blood group ABH Type 1 determinants by the FedF adhesin of F18-fimbriated Escherichia coli. The Journal of Biological Chemistry 284, 15, 9713-9726.

2. Loos, M., Hellemans, A. and Cox, E. (2013) Optimization of a small intestinal segment perfusion model for heat-stable enterotoxin A induced secretion in pigs. Veterinary Immunology and Immunopathology, 152, 1-2, 82-86

3. Van den Broeck, W, Cox, E. and Goddeeris, B. (1999) Receptor-dependent immune responses in pigs after oral immunization with F4 fimbriae. Infection and Immunity, 67, 2, 520-526.