WP 2.2: Anti- and probiotics in preterm and term pigs

1. Related WPs, MG contact: Synergy with WP 1.3a, 1.4b, 1.5, 1.6b, WP3. MG contact: Thomas Thymann

2. Key involved personnel, their institution and mail address (project leader + main study site underlined):

Anders D. Andersen, post doc, Comparative Pediatrics and Nutrition, Univ. Copenhagen, adan@life.ku.dk (10%)
Ann-Sofie Riis Poulsen, PhD stud, Dept. Animal Sci, Aarhus University, Ann-Sofie.RiisPoulsen@agrsci.dk (30%)
Axel Kornerup Hansen, Prof., Dept. Veterinary Disease Biology, Univ. Copenhagen, akh@sund.ku.dk (5%)
Charlotte Lauridsen, Ass. Prof., Microbiology/Immunology, Aarhus Univ., Charlotte.Lauridsen@agrsci.dk (5%)
Dennis Nielsen, Ass. Prof., Dept. Food Science, Univ. Copenhagen, dn@life.ku.dk (10%)
Gorm Greisen, Prof., Copenhagen University Hospital, Gorm.Greisen@regionh.dk (5%)
Hanne Frøkiær, Prof., Dept. Veterinary Disease Biology, Univ. Copenhagen, hafr@sund.ku.dk (5%)
Lars Dragsted, Prof., Preventive Nutr., NEXS, Univ. Copenhagen, ldra@life.ku.dk (5%)
Lena Martin, post.doc., Univ. Copenhagen/DAAD Germany, Lena.Martin@fu-berlin.de (70%)
Malene Birck, post doc, Comparative Pediatrics and Nutrition, Univ. Copenhagen, mbirck@life.ku.dk (20%)
Malene Cilieborg, post doc, Comparative Pediatrics and Nutrition, Univ. Copenhagen, macilie@life.ku.dk (5%)
Mette Bach Christensen, Arla Foods, mette.bach.christensen@arlafoods.com (5%)
Ninh Duc, PhD stud., Dept Food Science, Univ, Copenhagen, ndninh28@food.ku.dk (15%)
Nuria Canibe, Ass. Prof. Aarhus University, Nuria.Canibe@agrsci.dk (5%)
Päivi S. Takkunen, PhD stud., Comparative Pediatrics and Nutrition, IKVH paivi@life.ku.dk (50%)
Per Sangild, Prof., Comp. Pediatrics and Nutrition, Univ. Copenhagen, psa@life.ku.dk (10%)
Ping Ping Jiang, Comp. Pediatrics and Nutrition, Univ. Copenhagen, pipi@life.ku.dk (10%)
Preben Bodstrup Rasmussen, company, ARLA Foods amba, preben.bodstrup.rasmussen@arlafoods.com (5%)
Thomas Thymann, Ass. Prof, Comparative Pediatrics and Nutrition, Univ. Copenhagen,
Thomas.thymann@sund.ku.dk (20%)

3. Main aim and sub-aims:

The main aims are: 1) to assess the effect of route of administration of antibiotics (oral vs. systemic), 2) probiotic intervention to preterm neonatal piglets given as a fecal transplant from a healthy donor, 3) antibiotic and probiotic effects in neonatal piglets born at term.

4. Background and a central hypothesis:

Postnatal gut colonization starts immediately after birth and is influenced by environmental bacteria. Probiotics supplementation may be a way to manipulate the early colonization to prevent gut disease. Addition of probiotics to milk feedings has been speculated to promote colonization of beneficial gut bacteria, suppress pathogens and stimulate immune development. However, the current level of evidence limits widespread use of probiotics, especially for vulnerable newborn infants as their potential positive effects are currently not predictable enough. We speculate that the unpredictable effects of probiotics are in part due to the highly variable use of neonatal antibiotics that may hamper the probiotic effect. Also the timing of probiotics inoculation after birth may influence how well they establish and display their beneficial effects. A major factor that influences the gut microbiota is the use of antibiotics. Antibiotics are essential to prevent and treat infections, especially for the weakest newborn infants. Preventive antibiotics treatment around birth may prevent neonatal infections, but the effects on immune, gut and brain maturation are not well known. Both positive and negative effects have been demonstrated. The interaction between antibiotics and probiotics in newborn (preterm and term) infants remains unknown despite its potential high importance for clinical outcome for infants. *We hypothesize that antibiotics will initially benefit the immature immunity, gut, and brain via the reduced bacterial load, and that subsequent probiotics used at the optimal time, dose and strain combination will improve maturation.*

5. Key analyses and methods:

In experiment 1, preterm piglets are derived by cesarean section and reared in incubators. All pigs are provided with total parenteral nutrition for 2-3 days via the umbilical artery. After this they are gradually transitioned to full enteral nutrition with milk formula. During the entire period we prevent NEC out break by providing antibiotics (either oral or systemic administration).

In experiment 2, we collect colon content from healthy suckling piglets, and supply preterm newborn piglets with daily doses of this material. This fecal transplant is assumed to exert probiotic effects in the newborn intestine. Endpoints include clinical assessment, growth velocity, diarrhea scoring and NEC symptoms. Tissue samples and samples from the gut luminal content are collected and analyzed (proinflammatory cytokines, gene expression, mucosal digestive function, microbial composition, SCFA, metabolomics, antibiotic resistance).

In Experiment 3, antibiotic intervention is studied in term pigs in collaboration with Aarhus University, Foulum, based on experiences from experiment 1.

In Experiment 4, antibiotics and probiotics intervention are studied in term suckling piglets in collaboration with Aarhus University, Foulum. Clinical and laboratory endpoints as mentioned above.

6. Expected results:

We expect to determine how profound manipulation of the gastrointestinal microbiota during early postnatal colonization can impact on gut and brain development. Secondly we expect to determine if probiotics given as fecal transplant influences early clinical and paraclinical parameters.

7. Estimated time frame

Task for exp 1, 2, 3, 4 (see point 5)	2013				2014				2015				2016				2017			
Planning, protocol			1			2				3		4								
Sample collection				1	1		2	2			3	3	4	4						
Clinical/ behaviour				1	1		2	2			3	3	4	4						
Gut tissue analyses						1	1		2	2			3	3	4	4				
Microbial comp						1	1		2	2			3	3	4	4				
Brain endpoints (preterm)						1	1													
Publication(s)								1	1	1	2	2	2		3	3	3	4	4	4

8. Estimated budget from NEOMUNE:

2.5 mio DKK, derived from the KU-SCIENCE NEOMUNE budget (Thomas Thymann).

0.5 mio DKK derived from the AU NEOMUNE budget (Charlotte Lauridsen).

9. Estimated budget from elsewhere:

0.5 mio DKK is expected by self-financing (i.e. university funded salaries) plus industrial co-financing

1.0 mio, post doc stipend for Dr. Lena Martin from German Research council

10. Additional comments:

- This WP relates closely to WP2.1 and WP3 on the experimental side. All these WPs represent profound experimental manipulation of the gut flora just after birth. Relative to WP2.1 (germ free conditions), WP2.2 represents a more clinically relevant model. All the animal studies are meant to rely on clinically relevant situations for infants (e.g. WPs 1.2b, 1.4b, 1.5) and the possible interventions for infants (WPs 1.3a,1.6b).
- It will be a challenge to choose the optimal product(s), timing and doses of both antibiotics and probiotics. Clearly it is impossible to test all clinically-relevant combinations. We will focus on products, timing of administration and doses that are currently used in the neonatal clinics around the world. The results from WP 1.4b (data base work) will help to determine this.