Mucosal vaccines in Veterinary Species

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Why mucosal immunity?

- Protection at mucosal surfaces, the entry site of most pathogens
- Reduces pathogen concentration and spread in the environment by preventing infection instead of preventing disease: production of naïve livestock and danger for epizootic outbreaks
- Animal/human(child) friendly
- Less labour-intensive for mass vaccination
Current mucosal vaccines

Human mucosal vaccines

Holmgren and Svennerholm, 2012. Current Opinion in Immunology

Polio
- Live attenuated vaccine trivalent
  Oral  OPV (Novartis, BIBCOL, Biofarma,)
  Oral  Orimune (Medimmune)
- Live attenuated bivalent
  Oral  Poliomyelitis type 1 & 3 (Sanofi)
- Live attenuated monovalent
  Oral  Poliomyelitis type 1 (Sanofi, Novartis, GSK)

Cholera
- Cholera toxin B subunit (CTB) + inact. V. cholerae
  (10^{11} heat-killed or formalin killed El Tor O1 Inaba & Ogawa biotypes)
  Oral  Dukoral (Crucell)
- inact. V. cholerae (same as above+ 5x10^6 formalin-killed O139, no CTB)
  Oral  Shanchol (Santa Biotechnics)
- CVD 103-HgR live recombinant V. cholerae O1 strain lacking CTA
  Oral  Orochol (Crucell)
- Live attenuated Vibrio cholerae serogroup O1 (CVD 103-HgR)
  Oral  Vaxchora (PaxVax)
- Bivalent inact. V. cholerae (O1 and O139)
  Oral  Euvichol-Plus (EuBiologics)

Typhoid
- Salmonella typhi Ty21a live attenuated vaccine
  Oral  Vivotif (Crucell)
- Salmonella typhi Ty21
  Oral  TypBar TCV (Bharat Biotech)

Rotavirus
- Live attenuated monovalent human rotavirus strain G1P
  Oral  RotaRix (GSK)
- Multivalent human-bovine reassortant (G1-G4 and P1A)
  Oral  RotaTeq (Merck)
- Live attenuated monovalent human rotavirus strain (116E BUK)
  Oral  RotaVac (Bharat Biotech)
- Pentavalent human-bovine reassortant (G1-G4 and G9)
  Oral  BRV-Penta (Serum Inst. Inde)

Influenza
- Live attenuated cold-adapted influenza virus reassortant strains
  Nasal  FluMist (MedImmune)
- H1N1 influenzavirus (swine)
  Nasal  Nasovac (Inst Exp Med)

Adenovirus type 4 and Type 7
- Oral  Teva Women’s Health, Inc

Enterotoxigenic E. coli
- Cholera toxin B subunit (CTB) + inact. V. cholerae
  Oral  Dukoral (Crucell)
Oral vaccines in pigs

**Porcine proliferative enteropathy**
Att. Lawsonia intracellularis MS B3903  Enterisol Ileitis®  Boehringer Ingelheim

**Salmonella choleraesuis**
Avir. live *Salmonella cholerae suis* var. Kurzendorf SC-54  EnterisolSC-54®  Boehringer Ingelheim

**Enterotoxigenic E. coli**
Avirulent live F4 E. coli strain  Coliprotec F4®  Prevtec microbiya
Avirulent live F4 and F18 E. coli strain  Coliprotec F4/F18®  Prevtec microbiya
Avirulent live F4(K88) E. coli strain  Entero vac  Arko Laboratories

**Verotoxigenic E. coli**
Avirulent live F18 E. coli strain  Edema vac F18  Arko Laboratories

**Erysipelothrix Rhusiopathiae**
Avirulent live Er. rhusiopathiae  Ingelvax® ERY-ALC  Boehringer Ingelheim
Avirulent live Er. rhusiopathiae  Suvaxin® E-oral  Zoetis

**Rotavirus**
Bivalent modified live G serotype 5 & 4 serogroup A  ProSystem® Rota  Merck Animal Health

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**dogs**

**Bordetella bronchiseptica**
Recombitek Oral Bordetella (Live attenuated; Merial: one oral dose)
Bronchi-Shield oral (Boehringer Ingelheim)
Vanguard B Oral (Zoetis)

**wildlife**

**Rabies**
Raboral V-RG
Rabitec (live attenuated)
nasal vaccination

Pig

*Porcine influenza*
Live attenuated influenza H1N1 & H3N2  Ingelvac Provenza  Boehringer Ingelheim

Cattle

*Infectious bovine rhinotracheitis*
-Attenuated bovine herpes virus type 1 (DIFIVAC gE-)  Rispoval IBR-marker Vivum  Zoetis
-Attenuated bovine herpes virus type 1 (Stam GK/D gE-)  MSD

Dogs

*Bordetella bronchiseptica + canine parainfluenza virus + canine adenovirus*
-Live attenuated vaccine 3-way  Vanguard Rapid Resp  Zoetis

*Bordetella bronchiseptica + canine parainfluenza virus*
-Live att bivalent  Nobivac BbPi  MSD

*Bordetella bronchiseptica*
-Live att. monovalent  Bronchi-Shield  Zoetis
Mucosal vaccines in birds

Ocular, oculonasal (spray, droplets), oral (drink water)
Live attenuated or deletion mutation

*Newcastle disease virus*
*Infectious bronchitis virus*
*Gumboro disease virus*
*Infectious laryngotracheitis virus*
*Avian rhinotracheitis virus*
*Coccidiose (Eimeria sp.)*
*Avian Escherichia coli*
*Salmonella enteritidis*
*Salmonella typhimurium*
*Mycoplasma synoviae*

Mucosal vaccines in fish

*Nervous Necrosis virus*
Insect larvae containing the virus

Oral
Vaksea
Challenges of mucosal vaccination

• Identification of protective **antigens**
• Efficient **delivery** of protective antigens to mucosa-associated lymphoid tissue
  – delivery systems
• Activation of protective immune mechanisms, often neutralizing **IgA**
  – adjuvants
• **Escaping** maternal (passive ) immunity
  – Passive milk antibodies (lactogenic immunity)
  – Passive serum antibodies (placental-colostral immunity)
Intra-nasal vaccination

- Intra-nasal in mice, is “intra-mouse”: (i.e. also intra-lung, intra-gastrointestinal track)

- Unlike mice, sheep nasal associated lymphoid tissue is similar to human structure.

Vujanic et al., 2012. Vet Immunopath
JP Scheerlinck, University of Melbourne, personal communication
100 µg Alexa 488-labeled Qβ-VLP in 100µl : 50 µl per nostril

Mice lung

Bessa et al., 2008. J. Eur. Imm.
<table>
<thead>
<tr>
<th></th>
<th>5 Lingual tonsil</th>
<th>4 Palatine tonsil</th>
<th>6 Paraepiglottic tonsil</th>
<th>1 Pharyngeal tonsil</th>
<th>2 Tubal tonsil</th>
<th>3 Tonsil of the soft palate</th>
<th>7 NALT</th>
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<tbody>
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</tbody>
</table>

Liebler et al., 2006. Vet Res
G. Kraal, 2005. Mucosal Imm.

Fully developed at 4 to 6 months
Immune responses in lymph following intranasal spray

Most of the antigen is swallowed.

JP Scheerlinck, University of Melbourne, personal communication
Scheerlinck et al, Vaccine 2006
Mucosal adjuvant CTA1-DD in intranasal immunisation

Enhanced efficacy of the M2e-HBc vaccine by combination with the mucosal adjuvant CTA1-DD

Mice (50 µl intranasal)

Pigs (1 ml intranasal)

Filette et al., 2006. Vaccine

Verdonck et al., 2006. Vet. Imm. Immunopath
Immune responses in lymph following intranasal injection

JP Scheerlinck, University of Melbourne, personal communication
Oral vaccination

Virulence
- Too attenuated => no danger
- Too virulent => disease
- Expresses virulence factors

Preferred ROUTE for intestinal immunity

VACCINE
alive ➔ dead

AGE
passive ➔ active

Intestinal Mucosa

Passive immunity
- milk antibodies
- placental (humans) or colostral antibodies (animals)
Gut-associated lymphoid tissue (GALT)

Brandtzaeg and Pabst, 2004

Pig (intestinal length)
0 days
  - total ≈ 7.4 m
  - small intestine ≈ 3.4 m
10 days
  - total ≈ 8.5 m
Adult
  - total ≈ 19 – 25 m
  - small intestine ≈ 15 - 20 m
  - duodenum ≈ 60 cm
  - large intestine ≈ 4-5 m
FOLLICLE-ASSOCIATED EPITHELium
Pathogens
Particulated antigens

Epithelium

Mucosa-associated lymphoid tissue
Immunity

IgA production

MUCOSA
Non-replicating soluble antigens

Immature antigen-presenting cells

Dendritic cells

Draining lymph node
Oral tolerance
Oral vaccination

Follicle-associated epithelium
M-cells

Particulated antigen

Mature DC

Gut-associated lymphoid tissue

Danger

M-cel

Mature DC

Immunity

Enterocytes

Few soluble antigens
Virulence factors

Enterocytes

Lamina propria
Mesenteric Lnd

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F4 as a tool to study the immune response following oral immunization

F4 fimbriae of enterotoxigenic E. coli

Day 4
Peyer’s patches

Van den Broeck et al., 1999. Vaccine

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Binding and uptake of F4 fimbriae

Ligated loops injected with F4

FLUOS-labeled F4

Snoeck et al., 2008. Vet Immunopath.

F4 binds to Aminopeptidase N (APN)

Blotting of brush border proteins and staining with F4

Melkebeek et al., 2012. Mucosal Immunity

Duodenum
Jejunum
Ileum
F4 fimbriae can induce IL17A in naïve PBMCs

Endotoxin-free F4 fimbriae (5 μg/mL) or medium for 72 h.

Concentrations of cytokines in supernatant

*Luo, 2016. PhD UGent*
What is the role of IL-17A?

IPEC-J2 cells stimulated with **IL-17A** (24 h)

- Receptor for IgA
- β-defensin 2
- Mucin 2

**Intestinal barrier becomes fortified**

*Luo, 2016. PhD UGent*
Different strategies are followed

F4 fimbriae
- Carrier (Tiels et al, 2008. Vaccine)
- Aminopeptidase N (APN) is F4R (Melkebeek et al, 2012. Muc Imm.)
- Th17 dominated response (Yu et al., 2015. Vet. Research)

Anti-APN antibodies targeting soluble antigen to APN and the GALT
- Polyclonal Ab Induce IgA (Melkebeek et al, 2012. Muc. Imm.)
- Monoclonal Ab (in development)

Yeast ghosts targeted to the F4 receptor
- Enhanced antibody responses (Baert et al., 2015. J. Contr. Rel.)
- B. Devriendt, Poster 132
Soluble Antigen

Aminopeptidase N targeting antibodies

Antibodies target antigen to APN

Aminopeptidase N (APN) ANPEP targeting antibodies 15 minutes after contact

Antigen reaches antigen-presenting cells in the lamina propria

Oral immunization with 1mg

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Particles carrying antigen targeted to APN

Saccharomyces cerevisiae

Acid/base treatment

Load with antigen complexed with tRNA

E.coli FedF F18 fimbriae

Conjugate APN-targeting antibody

APN targeting antibody-AlexaFluor 64

APN

M-cel

Serum antibody response 14 days after 2 immunisations

Ig

IgA

Mean counted particles per section

Lamina propria

Baert et al., 2015. J Contr Rel
Baert et al., 2016. Int J Nanomedicine
What tissues do you protect if you vaccinate mucosal

The common mucosal immune system is not so common

**Why not so common?**

<table>
<thead>
<tr>
<th>Induction site</th>
<th>Lymph node</th>
<th>Homing receptors</th>
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<tbody>
<tr>
<td>Vitamin A</td>
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<td>Intestine</td>
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<td>Retinoic Acid</td>
<td>IL-6</td>
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<td>TGFβ</td>
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<td>IL-6</td>
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<tr>
<td>Skin</td>
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<td>UVB</td>
<td>IL-4</td>
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<td>1,25(OH) vitD3</td>
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**Tissues**

- CCL = CC Chemokine ligand

**Small intestine**
- CCL25
- MADCAM1
- Small intestine
- Respiratory tract
- Urogenital tract
- Salivary gland
- Inflammatory site
- Skin
- CCL9
- VCAM1
- E-selectin

Modified from Kunkel and Butcher, 2003
**INDUCTOR SITE(S)**

- Upper respiratory tract
- Tonsils
- Digestive tract

**EFFECTIVE SITES**

- NEAR
  - Nose,
  - Trachea, bronchia

- DISTANT
  - Tonsils
  - Dissemination to Mammary Gland
  - Lactating mammary gland

**Blood**

- IgA/IgG
- IgA
Third edition of ECMIS 2019
*E. coli* and the Mucosal Immune System
2 to 5 June 2019
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Acknowledgements

Former PhD students
Prof. dr. Wim Van den Broeck (UGent)
dr. Frank Verdonck (Efsa)
dr. Petra Tiels (VIB-UGent)
dr. Veerle Snoeck (Ablynx)
dr. Kristien Rasschaert
dr. Vesna Melkebeek (B&D)
dr. Philippe Bellot (USG Professionals)
dr. Kim Baert (Anacura)

Lab of Immunology (UGent)
Prof dr. Bruno Goddeeris (KULeuven)
Prof. dr. H. Favoreel (UGent)
dr. Bert Devriendt (post-doc)
Dr Ut Nguyen Van (post-doc)

Lab of Pharmaceutical Technology (UGent)
Prof. dr. Jean-Paul Remon
Prof. dr. Bruno De Geest

Lab of Pharmaceutical Biotechnology (UGent)
Prof dr. Dieter Deforce
dr. Kelly Tilleman

Financial support

Lab of Veterinary Medicine

Vrije Universiteit Brussel (VUB)
Prof. dr. Henri Degreve and Han Remaut