Update on currently available cholera vaccines:

Inactivated whole cell – B subunit oral cholera vaccine (Dukoral®)

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Fondation Merieux Conference, Annecy 14-17 April, 2009: FOCUS ON NEGLECTED TROPICAL INFECTIOUS DISEASES – INTEGRATING VACCINES INTO GLOBAL CHOLERA CONTROL EFFORTS
The Dukoral® oral cholera vaccine

- Contains inactivated cholera vibrios + rCTB

- Provides 80-90% short-term and 50-60% long-term protection against cholera
  - Added benefit: 50-70% short-term protection against LT+ ETEC diarrhea

- Is licensed in >60 countries world-wide
Composition of Dukoral whole cell-B subunit oral cholera vaccine

- **Inactivated* V. cholerae* O1 bacteria:** 
  \(1 \times 10^{11}\) organisms per dose (≥ 750 EU of O1 LPS/dose), equal numbers of 
  - Classical Inaba (Cairo 48), heat-killed 
  - Classical Ogawa (Cairo 50), heat-killed 
  - Classical Ogawa (Cairo 50), formalin-killed 
  - El Tor Inaba (Phil 6973), formalin-killed

- **Recombinant cholera toxin B subunit, rCTB:** 
  1 mg per dose

Large scale recombinant production of cholera B subunit for vaccine use (Sanchez & Holmgren, PNAS 1989)

Multicopy plasmid directing overexpression of cholera toxin B subunit (CTB)

Cholera toxin gene has been deleted

>1g rCTB per liter (ca ½ million doses per 500-liter fermentor)
Diarrhea

H₂O, Cl⁻

NaCl

Locally produced IgA preventing bacterial colonization and toxin binding

Cholera toxin

GM1 receptor

VACCINE DEVELOPMENT
II. Clarify protective immune mechanisms

Diarrhea
Synergistic protective effect of anti-whole cell (WC) and antitoxic (CTB) cholera immunity

Fold-increase Protection

Fold-increase over controls

WC  CTB  WC+CTB
9     11   100
Cholera
Protective immune mechanisms and protective antigens

- Protection is mediated by *locally produced IgA* antibodies
  - Best stimulated by *oral vaccination*
- Protective antibodies *prevent bacterial colonization and/or toxin action*
  - Antibacterial immunity is mainly directed against O1 LPS
    - TCP is not a primary protective antigen
  - Antitoxic immunity is specific for the B subunit
- Antibacterial and antitoxic antibodies have a *synergistic protective effect*
Vaccine development:
Clinical cholera induces protective immunity against second attacks

Observed vs expected second attacks of cholera in Matlab, Bangladesh 1966-80 (Glass, R. et al, 1982)

<table>
<thead>
<tr>
<th>Number of cases</th>
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<tr>
<td>Observed</td>
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<tr>
<td>3</td>
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GOAL OF VACCINE DEVELOPMENT:
REPLICATE INTESTINAL IMMUNE RESPONSE AND PROTECTIVE EFFICACY BY VACCINE!
Intestinal IgA antibody responses after cholera disease and after immunization with a B-subunit/whole cell vaccine
(0.5 or 2.5 mg CTB + 5x10^{10} killed V. cholerae O1)
Intestinal Immune Responses in Humans

Strong intestinal antibody response and immunological memory after oral (cholera) vaccination – memory lasts for ≥ 5 years
Dukoral®
Clinical Trials Summary

>50 clinical studies have been reported using Dukoral

- Safety, immunogenicity and protection against cholera and ETEC diarrhea + extensive post-marketing experience of safety

- Feasibility and effectiveness in mass vaccination campaigns,

- Mucosal immune responses in humans after immunization by different routes,
  - e.g. oral, sublingual, intragastric, duodenal, colonic, rectal, nasal, vaginal & intramuscular, subcutaneous, intradermal, transcutaneous.
WC-CTB oral cholera vaccine (Dukoral®) is very safe (I)

- Over 240 000 subjects were monitored in clinical studies. Dukoral® showed in all clinical trials an excellent safety profile and was well tolerated clinically in adults and in children.

- The most commonly reported adverse effects (AEs) in association with vaccination were local, e.g. mild abdominal discomfort or pain and loose stools or diarrhea. In all placebo-controlled trials the frequency of AEs were similar in the vaccine and placebo groups.
Oral WC-CTB cholera vaccine (Dukoral®) is very safe (II)

- Post-marketing surveillance in well-reporting countries including 3 million subjects receiving at least two doses of Dukoral® from April 2004 until August 2008 support the excellent safety profile.

- The analysis of all spontaneously reported AEs did not reveal any increased or significant risk with the use of Dukoral®
  
  Total 600 AEs in more than 6 million doses reported. Very few reported serious adverse reactions and not increased over background.

  Dukoral is safe also in pregnancy, HIV infected people, immunodeficiencies, and gastrointestinal and various other diseases
The Bangladesh Field Trial

- 1985-89; PI: John D. Clemens
- 3 doses of oral BS/WC or WC or placebo
- Subjects aged 2-65 years; 63,498 received complete vaccination
- Study protection against cholera (and ETEC)
- Up to 85% protective efficacy against cholera and 67% protection against LT-ETEC diarrhea
  - Similar efficacy after two and three doses!

2. van Loon FPL, Clemens JD, Chakraborty J et al. Field trial of inactivated oral cholera vaccines in Bangladesh: results from 5 years of follow-up. Vaccine 14: 162-166, 1996
<table>
<thead>
<tr>
<th>Follow-up period</th>
<th>CTB-WC vaccine</th>
<th>WC-only vaccine</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Protective efficacy % (95% confidence interval)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>All ages</td>
<td>2-5 y</td>
</tr>
<tr>
<td>6 months</td>
<td>85%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>(56-95%)</td>
<td></td>
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<tr>
<td>1st year</td>
<td>64%</td>
<td>44%</td>
</tr>
<tr>
<td></td>
<td>(50-74%)</td>
<td></td>
</tr>
<tr>
<td>2nd year</td>
<td>52%</td>
<td>33%</td>
</tr>
<tr>
<td></td>
<td>(30-76%)</td>
<td></td>
</tr>
<tr>
<td>3rd year</td>
<td>19%</td>
<td>Nil</td>
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<tr>
<td></td>
<td>(Nil-46%)</td>
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The B subunit added significantly to protection for the first 9 months of follow-up.
Impact of DUKORAL® on Diarrheal Illness in the Bangladesh trial (1st year evaluation)

- 26% reduction in all visits for diarrheal treatment
- 48% protection against fatal or severe diarrhea
- 26% reduction in overall mortality rates of women

Effects were only seen during the cholera season supporting the specificity of findings

_J Clemens et al. Lancet 1988_
Peru Military Trial

- 1994; PI: José L. Sanchez
- 1331 vaccinated volunteers (Adults)
- Two doses of WC/rBS vaccine or placebo; 5 months follow-up
- Objectives: Protection against cholera
  - 85 % protection against cholera
    - El Tor cholera; Two doses; Not endemic population; Blood group O.

Mozambique, Beira

- Demonstration project 2003-2004 – effectiveness trial in a high-endemicity urban slum area.
- Prevalence of HIV infection approaches 30% of the adult population
- 41,000 adults and children were vaccinated with 2 doses under public health conditions

**RESULTS**

- Vaccination was feasible and well accepted
- Vaccination gave 84% protection (after 2\textsuperscript{nd} dose PP)
  - 78% (82% in age 2-4 y) after 1 or 2 doses (per ITT)
  - 89% against severe dehydration (per ITT)

- Conclusion: The vaccine was highly effective against clinically significant cholera in an urban sub-Saharan African population with a high prevalence of HIV infection.

Other mass vaccination campaigns

- **Uganda 1997**
  - “Feasibility study” in refugee camp, 63,220 doses were given, 83% coverage.
  - 2-dose immunization with Dukoral in refugee camp was feasible and well accepted.
  - No cholera cases identified when the effect was evaluated after 1 year.
- **Mayotte 2000**
  - 2-dose vaccination campaign to prevent cholera outbreak.
  - 64% of population was vaccinated - 93,000 persons.
  - 2 cholera cases diagnosed (both defined as “incorrectly vaccinated”).
- **Darfur (Sudan) 2004**
  - Intervention study in a catastrophe area—refugee camp, >40,000 vaccinated.
- **Indonesia 2004**
  - Intervention study in post-tsunami catastrophe area, 137,000 doses given.
- **Zanzibar 2009**

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DUKORAL® - Added benefit by (short-term) protection against diarrhea caused by LT-producing ETEC

ETEC Efficacy trials:

• **Bangladesh 1985-86** (*PI: J Clemens*)
  - 67% PE (2 or 3 doses) against LT⁺ ETEC diarrhea (95% CI: 16-87%)
    - *86%* protection against life-threatening disease

• **Finnish travelers to Morocco 1989** (*PI: H Peltola*)
  - 60% protection against any LT⁺ ETEC
    - *71%* protection against mixed LT⁺ ETEC infections

• **US Students in Mexico** (*PI: H Dupont*)
  - 50% PE in cases occurring ≥ 7 days after 2nd dose
Summary: The whole cell-B subunit oral cholera vaccine (Dukoral)

Strengths

- **Safe and effective:**
  - Gives 80-90% short-term and 50-60% long-term protection against cholera
  - Also short-term protection against ETEC diarrhea

- Two-dose administration is feasible also for mass vaccination campaigns
  - "Very cost effective" by WB criteria for preemptive use in stable refugee settings at high cholera risk

- Public health protective impact can be markedly increased by herd protection

Limitations

- **Costs** (depend on volumes/guaranteed orders)
- **Formulation** — cold chain, buffer and volume
  - Heat-stable dry formulation?

THE VERY HIGH SHORT-TERM EFFICACY ALSO IN UNPRIMED INDIVIDUALS MAKES WC-rCTB OCV ATTRACTIVE FOR USE IN POPULATIONS AT HIGH RISK OF CHOLERA EPIDEMICS!

Also single-dose administration (perhaps with double dose) should be tested.
With thanks

To all co-workers at my own department
&
To many Swedish and international colleagues and organisations

Ann-Mari Svennerholm

B Ivanoff, R Glass, DD Trach, J Clemens
VACCINE DEVELOPMENT

I. Clarify the mechanisms of disease
Dukoral®
Cholera Efficacy Trials

• Bangladesh 1985-89
• Peru Military 1994
  • Peru Pampas 1993-95
Cholera toxin and its receptor

A:B5

Active site

A1

A2

B-subunit

GM1-receptor binding site

Sphingosine

Nervonate (24:1 n-9)

Ganglioside GM1
Oral CTB-WC vaccine protected US volunteers against cholera challenge

<table>
<thead>
<tr>
<th>Any diarrhea</th>
<th>&gt;2 liter diarrhea</th>
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<tbody>
<tr>
<td>Vaccine</td>
<td>Control</td>
</tr>
<tr>
<td>4/11</td>
<td>0/7</td>
</tr>
<tr>
<td>64% protection p&lt;0.01</td>
<td>100% protection p&lt;0.01</td>
</tr>
</tbody>
</table>

Vaccine also reduced *V. cholerae* excretion >30-fold

Black R et al. 1988
Pampas Trial: Cholera results

- 1993-95
- PI: David N. Taylor
- 2 doses primary vaccination
- Booster after 1 year
- 17,800 2 dose recipients (2-65 yrs)
- Active surveillance for cholera cases

RESULTS
- No efficacy first year
- 60% PE second year
- Discrepancy due to
  - Better surveillance documentation during 2nd year?

2 Clemens JD, Sack DA, Ivanoff B. Misleading negative findings in a field trial of killed, oral cholera vaccine in Peru. *J Infect Dis* 2001;183:1306–1308.
Oral cholera vaccines can provide strong "herd protection" in addition to their specific efficacy.

**Herd immunity is conferred by killed oral cholera vaccines in Bangladesh: a reanalysis**

M Ali, M Emch, L von Seidlein, M Yunus, D A Sack, M Rao, J Holmgren, J D Clemens

Vaccine coverage of the targeted population ranged from 4% to 65%. The incidence rates of cholera among placebo recipients were inversely related to the levels of vaccine coverage: 7.01 cases per 1000 in the lowest quintile of coverage vs 1.47 cases per 1000 in the highest quintile; p<0.0001 for trend – this corresponds to 79% "herd protection" between highest and lowest quintile of coverage.
Controlling Endemic Cholera with Oral Vaccines:

Total vaccine impact by a combination of direct (efficacy) and indirect (herd protection) effects may be much bigger than revealed by efficacy only!
Bangladesh trial 1985-86 (J Clemens et al.)

ETEC results

- **67%** PE for 3 months (2 or 3 doses) against diarrhea caused by LT⁺ ETEC (95% CI: 16-87%)
  - **86%** protection against life-threatening disease
- Protection was specific for BS-WCV; no protection by WCV only.
- Protection was short-lasting; after 9 months no protection left.

Protection against ETEC diarrhea in Finnish travellers

- 1989; PI: Heikki Peltola
- Finnish tourists going to Morocco for 1-2 weeks
- 615 randomized, 444 received full vaccination with 2 doses of vaccine or placebo
- Objectives: protection against LT-ETEC-diarrhea, TD

**Results**

- **60%** protection against any LT$^+$ ETEC
  - **71%** protection against mixed LT$^+$ ETEC infections
  - Overall **23%** reduction of TD
  - Borderline statistics, significant depending on method used.
Vaccine protection against ETEC in US Students in Mexico

• 1992; PIs: Herbert L. DuPont and David N. Taylor
• 500 volunteers, US students going to Mexico for up to 5 weeks
• 2 doses of vaccine or buffer after arrival

RESULTS

• Most cases occurred within 16 days after arrival (55 of 74)
• 50% protection counting only cases occurring 7 days after 2nd dose

• Conclusion: Vaccination before departure!