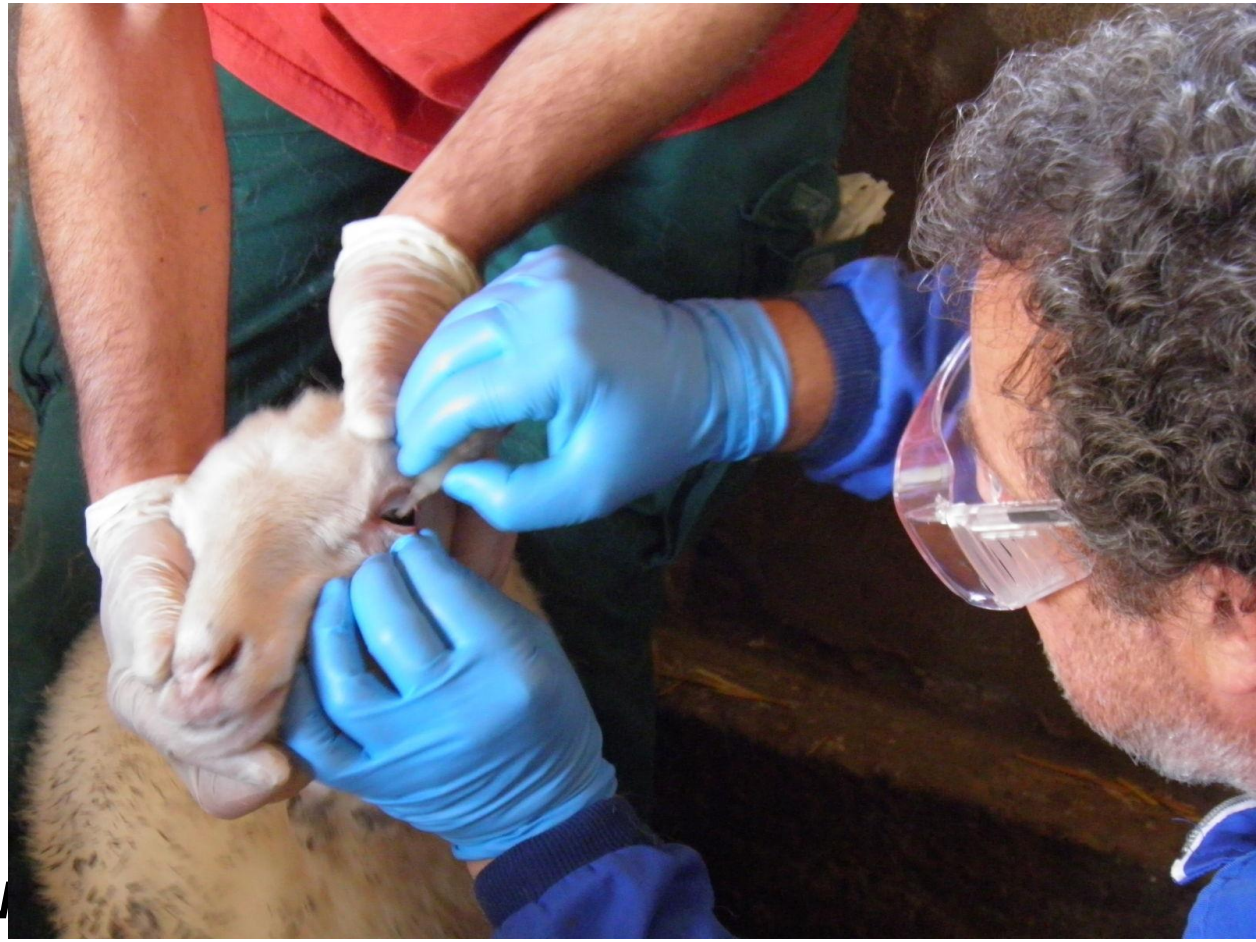
CURRENT BRUCELLOSIS VACCINES (RUMINANTS) AND NEW CANDIDATES



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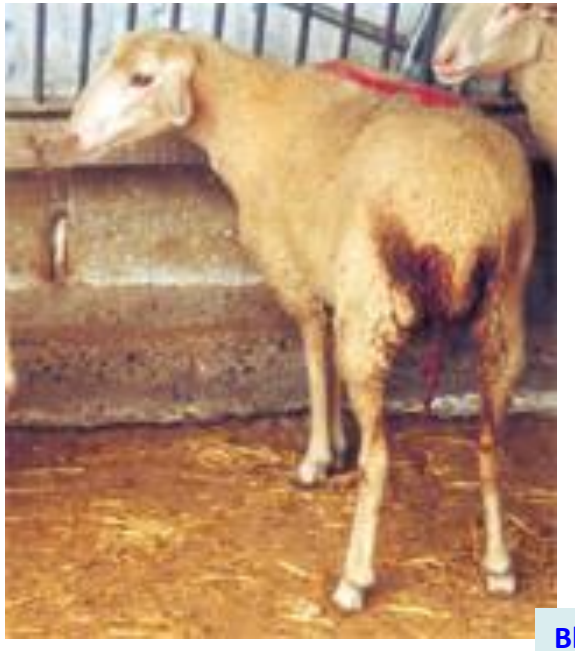
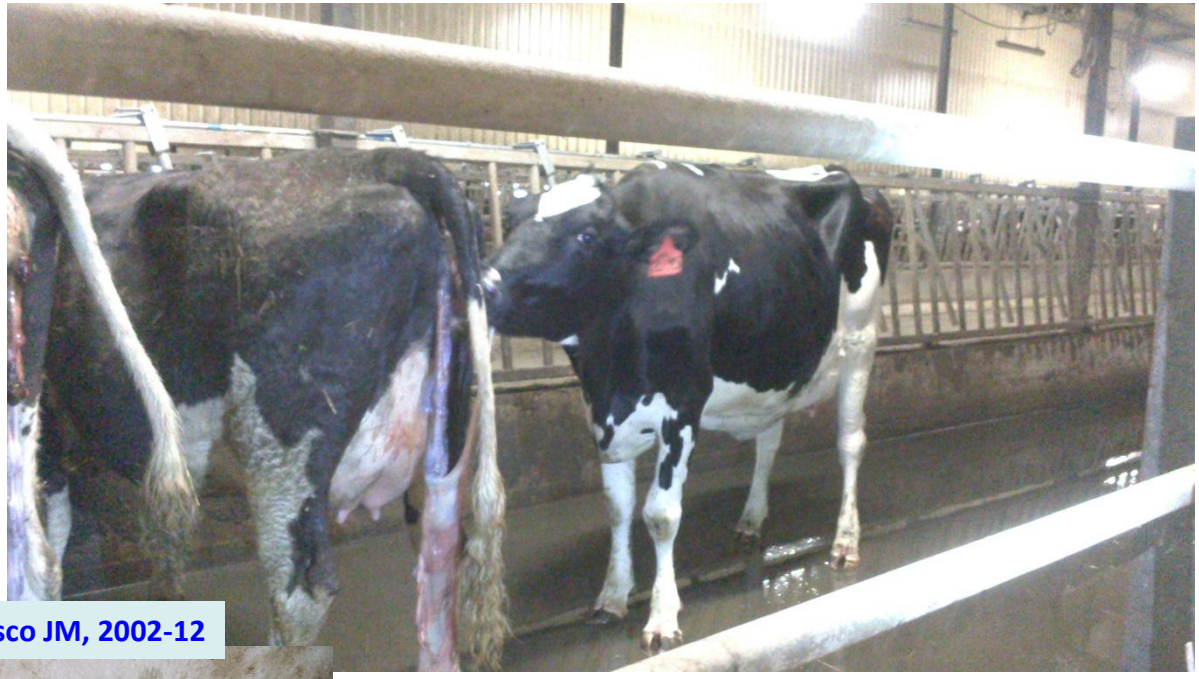
The main infection route





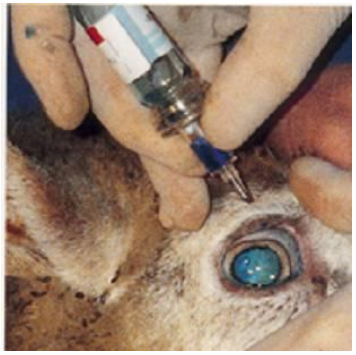
Blasco JM, 2002-12

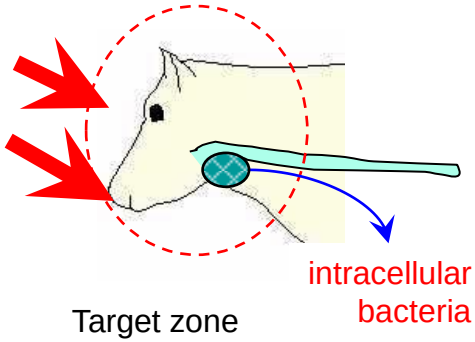
Elzer P, 2010

What science and experience tell us

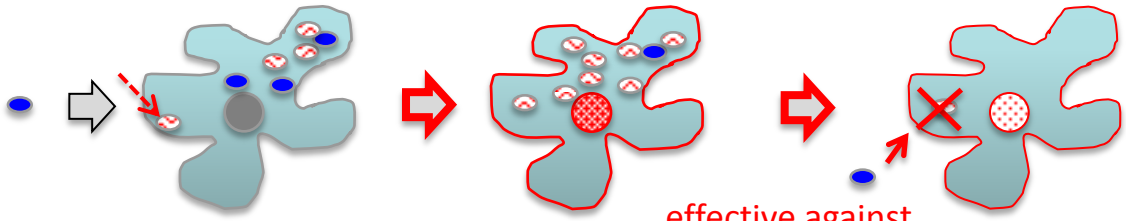
Best vaccination cocktail

Route: conjunctival





Vaccine: live attenuated



Limited multiplication is necessary: because (a), bacterial attenuation delays reaching the intracellular niche and slow down and reduces intracellular multiplication, and (b), these delays give time to immunity to develop

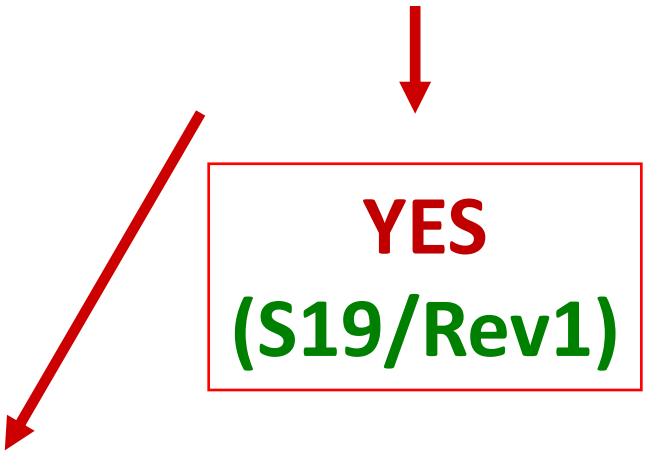
The attenuation/virulence balance is critical for the GOOD vaccines

Do we have GOOD VACCINES?

► Rev 1 excellent for young replacements but RELEVANT safety problems in adults (streptomycin resistance/abortions/milk excretion)

►S19 almost perfect when CONJUNCTIVAL route is used

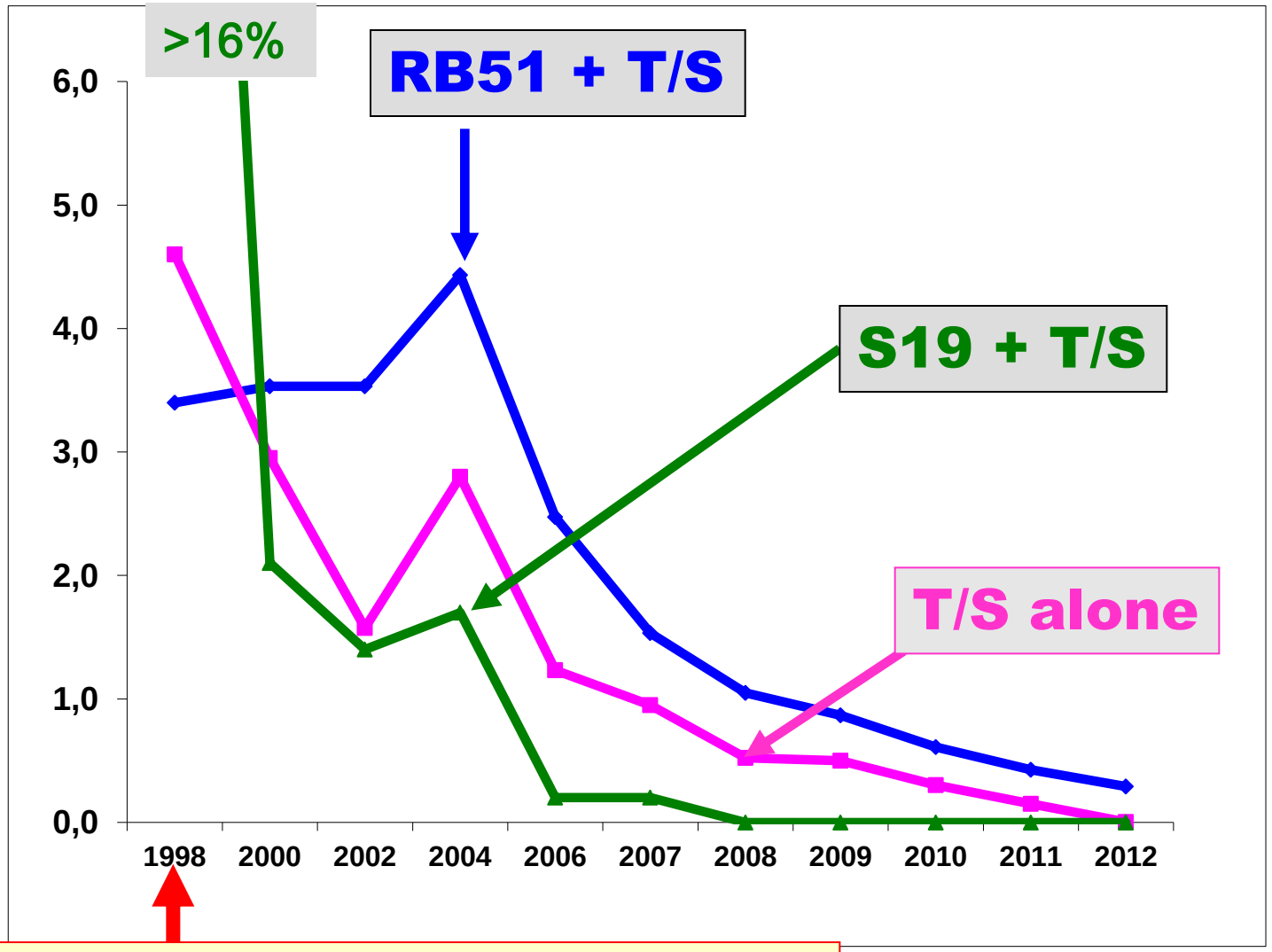
Can be brucellosis eradicated (SHORT TERM) in the DEVELOPED WORLD using the CURRENT vaccines?



YES
(S19/Rev1)

No country has succeeded in eradicating brucellosis using RB51, even after 16 years of intensive use (*ie* Chile)

EVOLUTION OF BOVINE BRUCELLOSIS HERD PREVALENCE IN SPAIN



Can brucellosis be eradicated (SHORT TERM) in LOW INCOME countries?

NO!! → Only CONTROL programs are feasible

VACCINE RESEARCH TARGETS

1 MASS VACCINATION REQUIRED

resolve safety issues in adults

2 DIVA VACCINES

Differentiating **I**nfected from **V**accinated **A**nimals

3. what vaccine(s) should be used here?

CURRENT SITUATION/IMMEDIATE FUTURE RESEARCH

INACTIVATED VACCINES

Subcellular vaccines new generation adjuvants

➔ **(microparticles / nanoparticles)** ***(Murillo et al, 2002 J. Controlled Release 85, 237)***

BUT

- **adyuvants very expensive**
- **need of revaccinations (impractical/expensive)**
- **subcellular antigens: serological interferences**

INACTIVATED VACCINES

DNA vaccination naked DNA or recombinant plasmids either directly or through viral or bacterial vectors → none proven successful in target animals

Glyco-conjugated vaccines

BILL & MELINDA GATES foundation

AHVLA and University of Alberta win Gates Foundation grant for brucellosis research

Improved serodiagnosis of bovine brucellosis by novel synthetic oligosaccharide antigens representing the capping M epitope elements of *Brucella* O-polysaccharide

John McGiven[1#], Laurence Howells[1], Lucy Duncombe[1], Judy Stack[1], N. Vijaya Ganesh[2], Julie Guiard[2], David R. Bundle[2].

JCM Accepted Manuscript Posted Online 4 February 2015
J. Clin. Microbiol. doi:10.1128/JCM.03185-14

Brucella leads to the induction of high antibody titres against the OPS, an unbranched homopolymer of 4,6-dideoxy-4-formamido-D-mannopyranosyl residues (D-Rha4NFo) that are variably α(1→2) and α(1→3) linked. Six D-Rha4NFo homo-oligosaccharides were synthesized - each containing a single α(1→3) link but with a varied number of α(1→2) links. After conjugation to BSA these glycoconjugates **1-6** were used to develop individual iELISAs. The diagnostic capability of these antigens were applied to panels of cattle sera,

the higher the AUC. This culminates in the finding that the disaccharide **1** with only one link, an α(1→3) link, gives the best AUC value of any antigen.

AUC = 0.9928 and optimised DSn and DSp = 100% and 97.78% respectively.

Exploiting the *Campylobacter jejuni* protein glycosylation system for glycoengineering vaccines and diagnostic tools directed against brucellosis

Iwashkiw *et al. Microbial Cell Factories* 2012, **11**:13

of the acceptor protein demonstrated the transfer of a polymer of N-formylperosamine to AcrA *in vivo*. Because *Y. enterocolitica* O9 and *Brucella abortus* share an identical O polysaccharide structure, we explored the application of the resulting glycoprotein in vaccinology and diagnostics of brucellosis, one of the most common zoonotic diseases with over half a million new cases annually. Injection of the glycoprotein into mice generated an IgG response that recognized the O antigen of *Brucella*, although this response was not protective against a challenge with a virulent *B. abortus* strain. The recombinant glycoprotein coated onto magnetic beads was efficient in differentiating between naïve and infected bovine sera.

MOREOVER….

Need of adjuvants (expensive)

Need of revaccinations (impractical/expensive)

Serological interferences?

LIVE DIVA VACCINES

DIVA APPROACH 1: deleting genes coding for relevant diagnostic proteins

Live attenuated NEGATIVELY TAGGED candidates

OBTAINED FROM

- ***B. abortus*** **S19**
- ***B. melitensis*** **Rev 1**

Associated diagnostic tests have to be developed using native or recombinant proteins

CANDIDATES FROM *Rev 1*

B. melitensis* Rev 1 *BP26

- **residual virulence & immunity similar to Rev 1 in mice** ***(Cloeckaert et al 2004, Vaccine 22, 2887)***

- **protective efficacy IN SHEEP similar to Rev 1**

(Jacques et al. 2007. Vaccine 25, 794; Grilló et al. 2009. Vaccine 27: 187).

BUT

moderate/low sensitivity of the associated DIVA test ***(Cloeckaert et al 2001,CDLI, 8, 772; Grilló et al. 2009. Vaccine 27: 187)***

CANDIDATES FROM *B. abortus* S19

B. abortus* S19 *BP26 ***(Broschioli et al. 1997. Infect Immun, 65, 798.)***

- **residual virul. & immunity in mice similar to S19**
- **moderate sensitivity of the DIVA test**

B. abortus* S19 luc:: *BP26 / bmp18* (*B. abortus* INTA 2 vaccine)** ***(Campos et al 2002 Vet Microbiol 87, 1).

Same problems

DIVA APPROACH 2: introducing genes coding for diagnostic proteins (GFP from jellyfish)

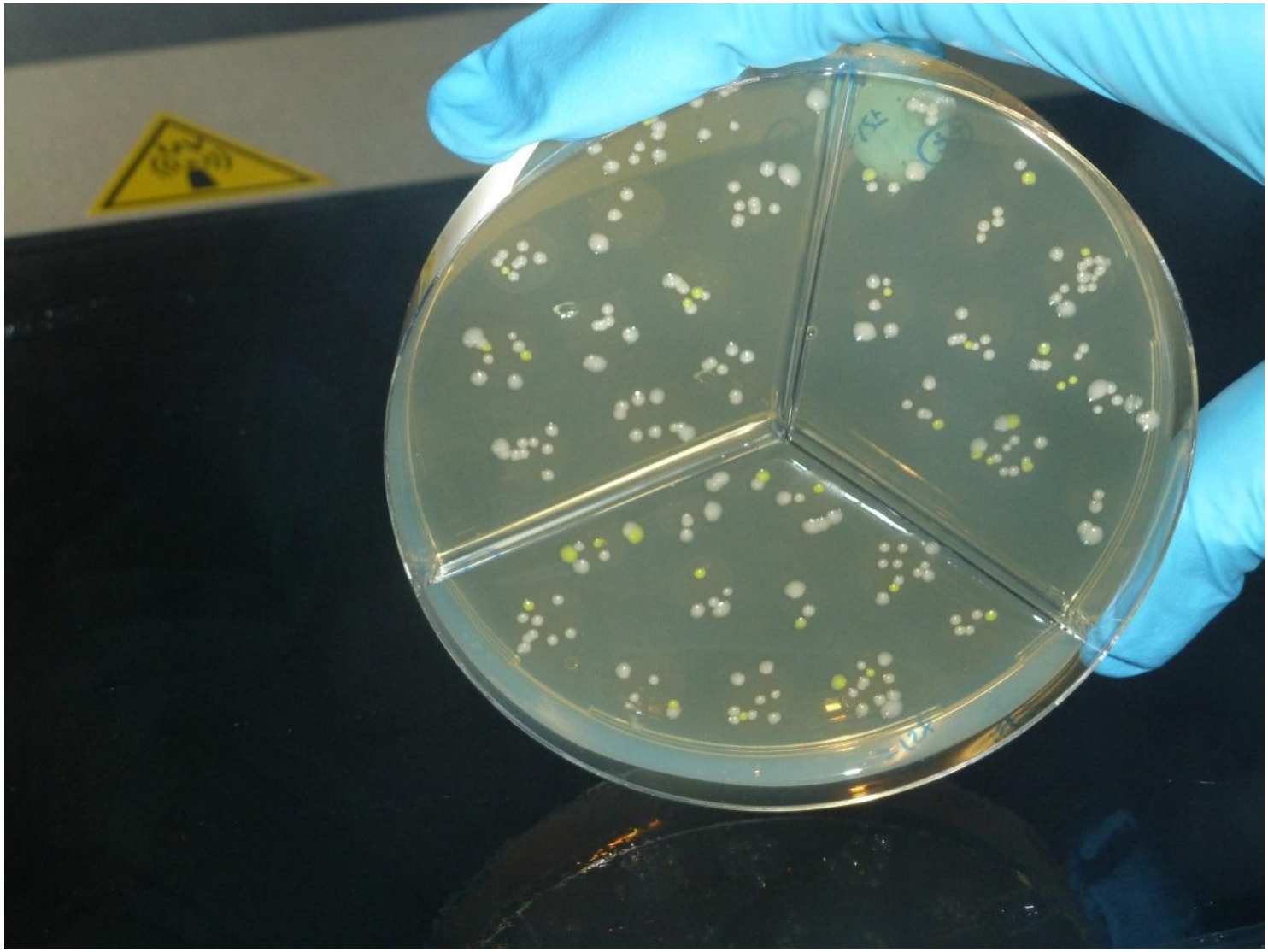
Live attenuated POSITIVELY TAGGED candidates

OBTAINED FROM

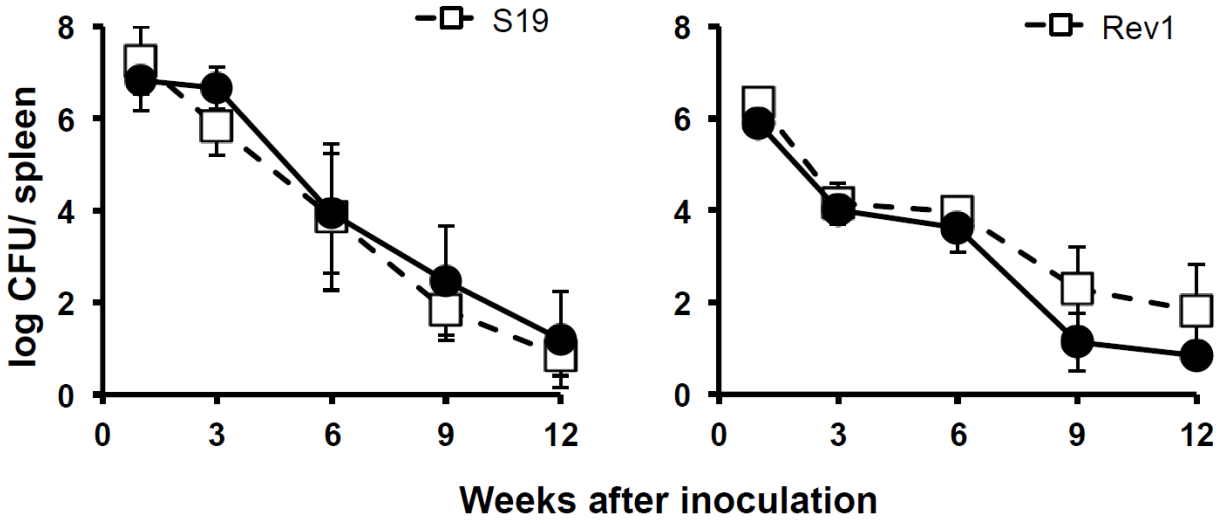
***B. abortus* S19**

***B. melitensis* Rev 1**

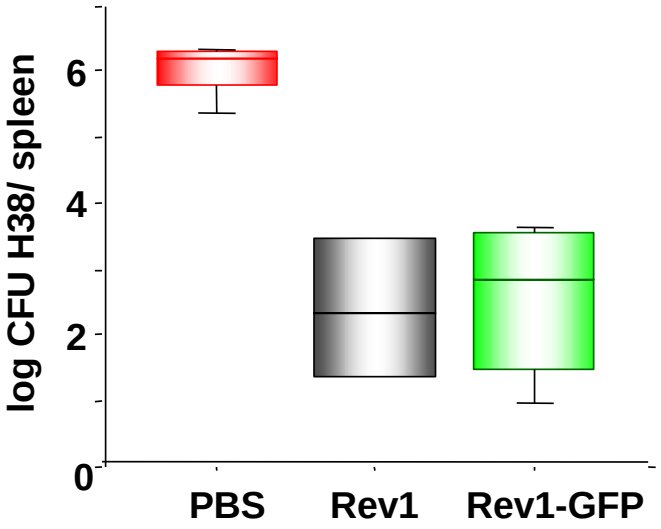
Associated diagnostic tests have to be developed using native or recombinant GFP

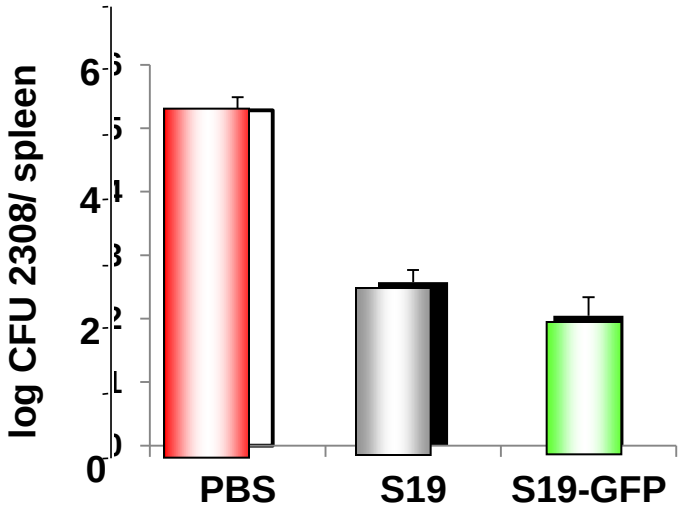


Rev1 and S19::Tn7GFP maintain the same phenotype that wt but are fluorescent

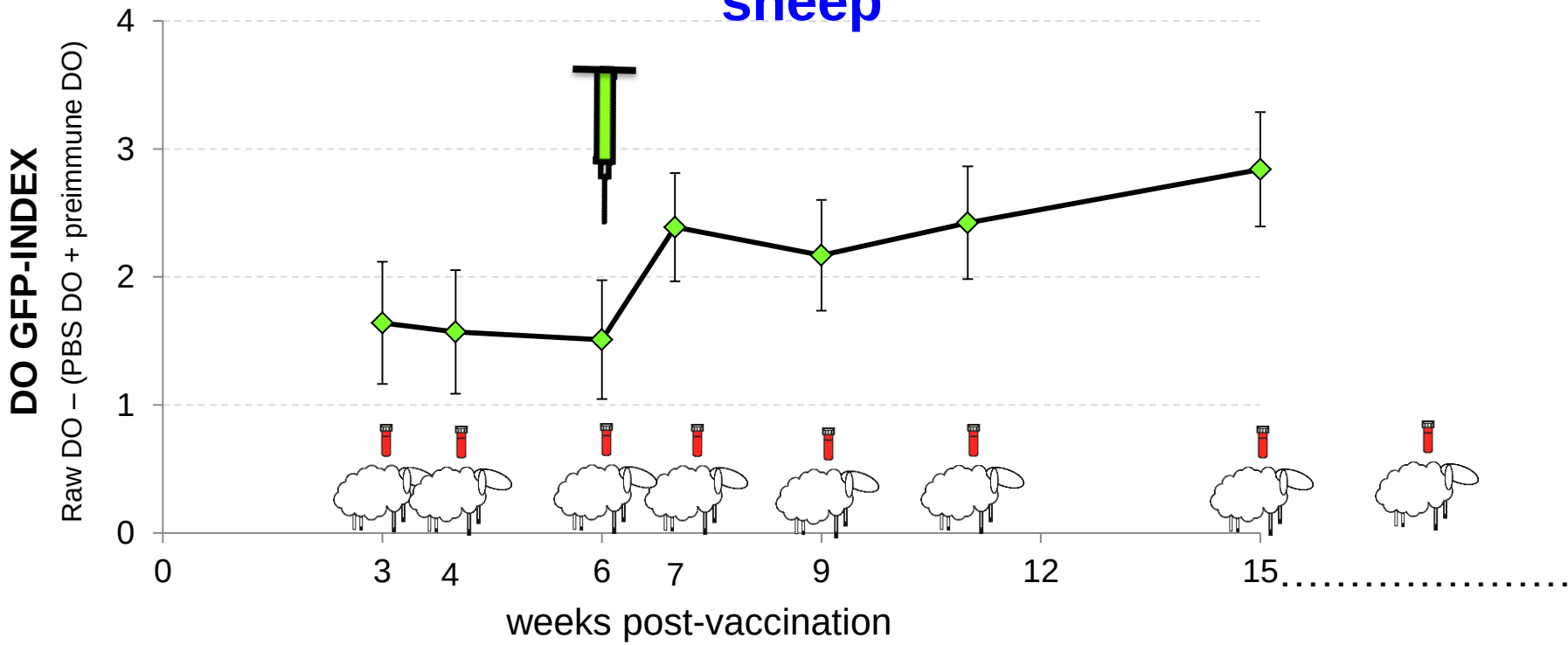


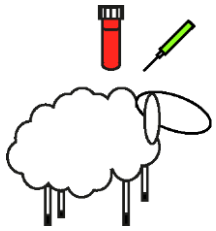
Experiments in mice



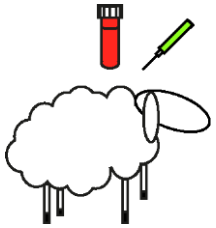


Evolution of ELISA-GFP antibodies in sheep

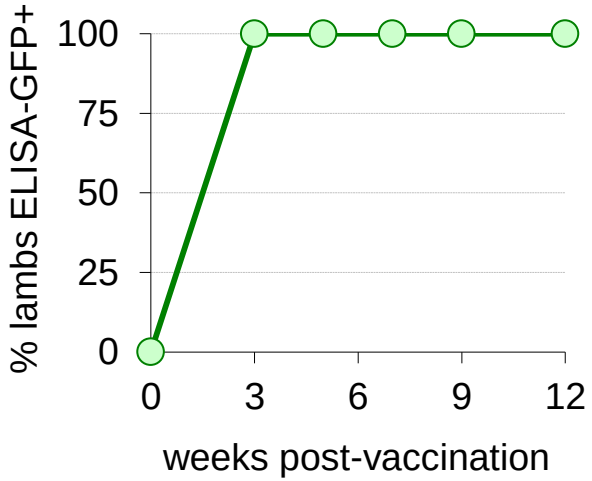




Vaccination (n=8)



GFP/FIA-Booster



UNCERTAINTIES

- **Need of revaccination (only GFP-adjuvant)**

- **DIVA test**

  the serological response against S/LPS and GFP should be parallel…but…what to do with RBT/GFP positive animals?

- **Safety/Efficacy in target species**

DIVA APPROACH 3: deletion in smooth virulent strains of genes involved in O-chain biosynthesis (ROUGH VACCINES)

ie **….searching candidates better than RB51**

ROUGH VACCINE CANDIDATES FROM *B. melitensis*

B. melitensis* mutants:** some tested against ***B. melitensis challenge in sheep *(Barrio et al 2009. Vaccine 27: 1741-1749)*:

Group	Nº of ewes	% abortions	% excretors	% protected
Control	15	100	100	0
Rev 1	12	0	0	100
H38 *wbkfF*	13	38	46	54
H38 *per*	11	64	64	36
16M *wa***	13	54	61	31

Poor protective efficacy and, moreover Serological interferences!!! → **iELISA/S-LPS**

DIVA APPROACH 4: insertion in smooth strains of genes involved in O-PS acetylation

The *Brucella* O-polysaccharide is a homopolymer of N-formyl-perosamine. We have produced *Brucella wbdR* mutants (gene encoding the transferase that acetylates perosamine in the *E. coli* 0:157 O-polysaccharide

↓

U. Navarra patent

Generation of live smooth but S/LPS modified candidates

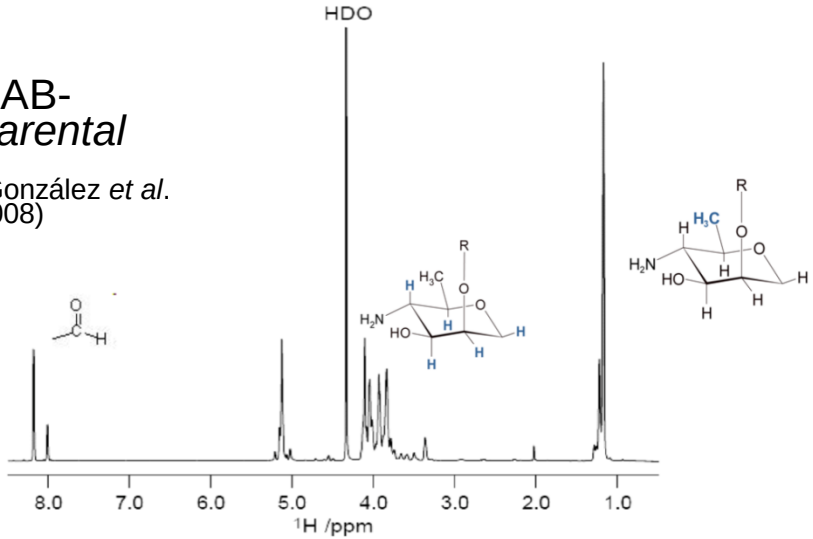
Applicable to S19 and Rev 1 strains

DIVA through S-LPS labelling: NMR profiles

NMR-H^1 (*O-PS*)

BAB-*parental*

(González *et al*. 2008)

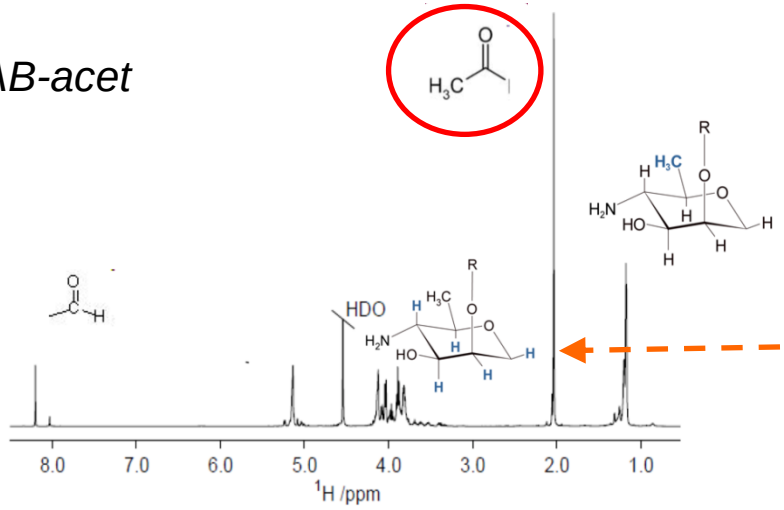


O-PS *B. abortus*:

N-formylation: c.a > 90%

BAB-acet O-PS is a N-acetyl N-formylperosamine heteropolymer

BAB-acet



O-PS *BAB-acet*:

N-formylation: c.a 40%

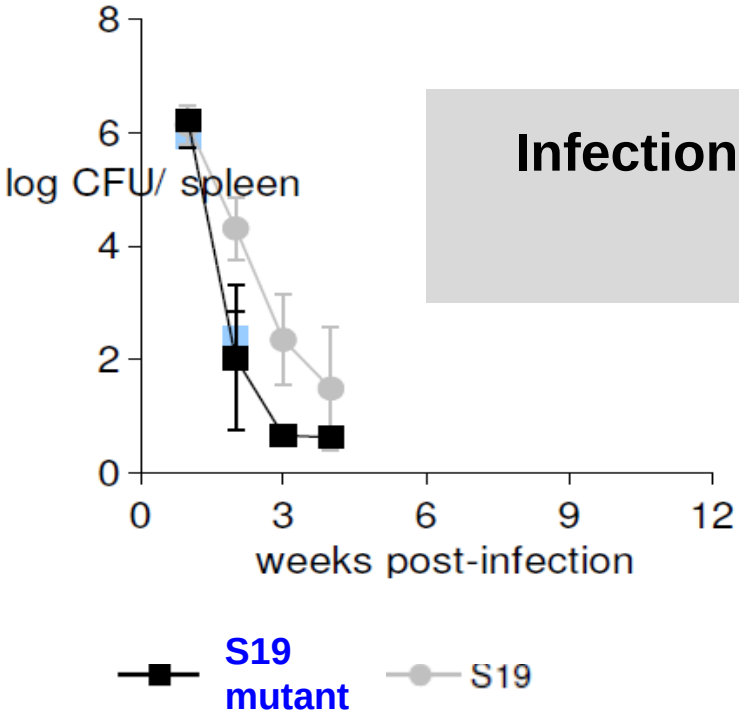
N-acetylation: c.a 60%

UNCERTAINTIES

1. **Serological response and DIVA test(s)?**

2. **Animal research: Virulence/Protection?**

B. abortus S19 wadC (INRA/INSERM) strain



Infection kinetics in mice inoculated with *B. abortus* S19 or S19 *wadC*

Protection against *B. abortus* 2308 in BALB/c

	CFU challenge	
	2 semPI	6 semPI
S19 mutant	2,26 ± 0,78	3,18 ± 1,8
S19	1,69 ± 0,77	3,59 ± 1,40
PBS	6,14 ± 0,25	6,07 ± 0,22

OTHER LIVE CANDIDATES

deletion in smooth strains of genes involved in proinflammatory response

In *Brucella wadC* mutants there is a faster recognition of the mutated LPS by TLR4, that leads to a timely release of inflammatory cytokines, including IL-12, which trigger a protective Th1 response.

INRA/INSERM patent

Applicable to S19 and Rev 1

UNCERTAINTIES

Experiments in target species strictly necessary

- safety/protective efficacy

- Serological interference is produced and a further DIVA mutation is required

MY PERSONAL FEELINGS ON VACCINE RESEARCH

Taking into account that:

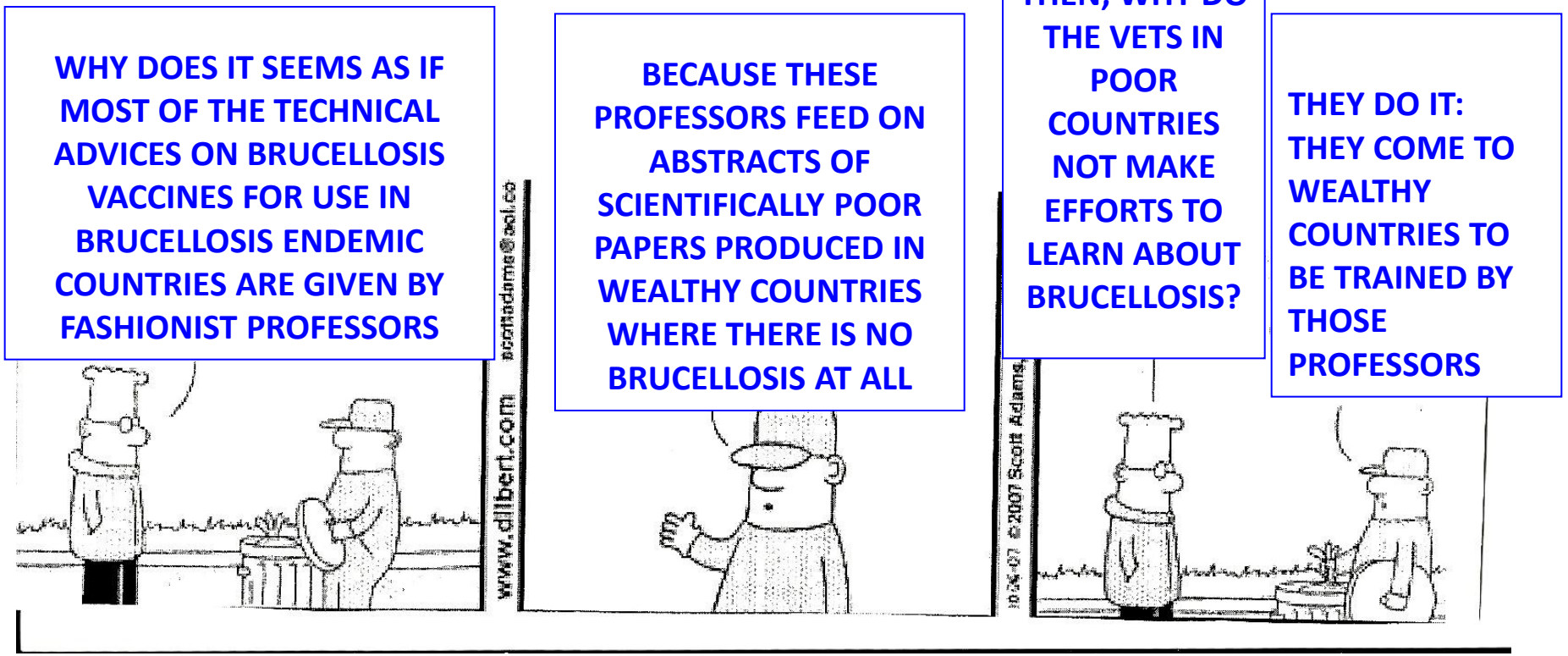
1. research on brucellosis **is waning sharply** in first country economies

LITTER-ATURE

2. biased scientific messages are flooding (as a consequence of the **incontrollable number** of **pay** journals whose peer review system is largely questionable)

3. that ignorance and corruption persist, and that **economic profits pursue without other ethical considerations** in a globalized market,

it is difficult to envision what will be the future of research in brucellosis vaccines, and importantly, if the new vaccines developed **will be really useful for the low-income economies** now affected by the disease


WHY DOES IT SEEMS AS IF MOST OF THE TECHNICAL ADVICES ON BRUCELLOSIS VACCINES FOR USE IN BRUCELLOSIS ENDEMIC COUNTRIES ARE GIVEN BY FASHIONIST PROFESSORS
BECAUSE THESE PROFESSORS FEED ON ABSTRACTS OF SCIENTIFICALLY POOR PAPERS PRODUCED IN WEALTHY COUNTRIES WHERE THERE IS NO BRUCELLOSIS AT ALL
THEN, WHY DO THE VETS IN POOR COUNTRIES NOT MAKE EFFORTS TO LEARN ABOUT BRUCELLOSIS?
THEY DO IT: THEY COME TO WEALTHY COUNTRIES TO BE TRAINED BY THOSE PROFESSORS
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