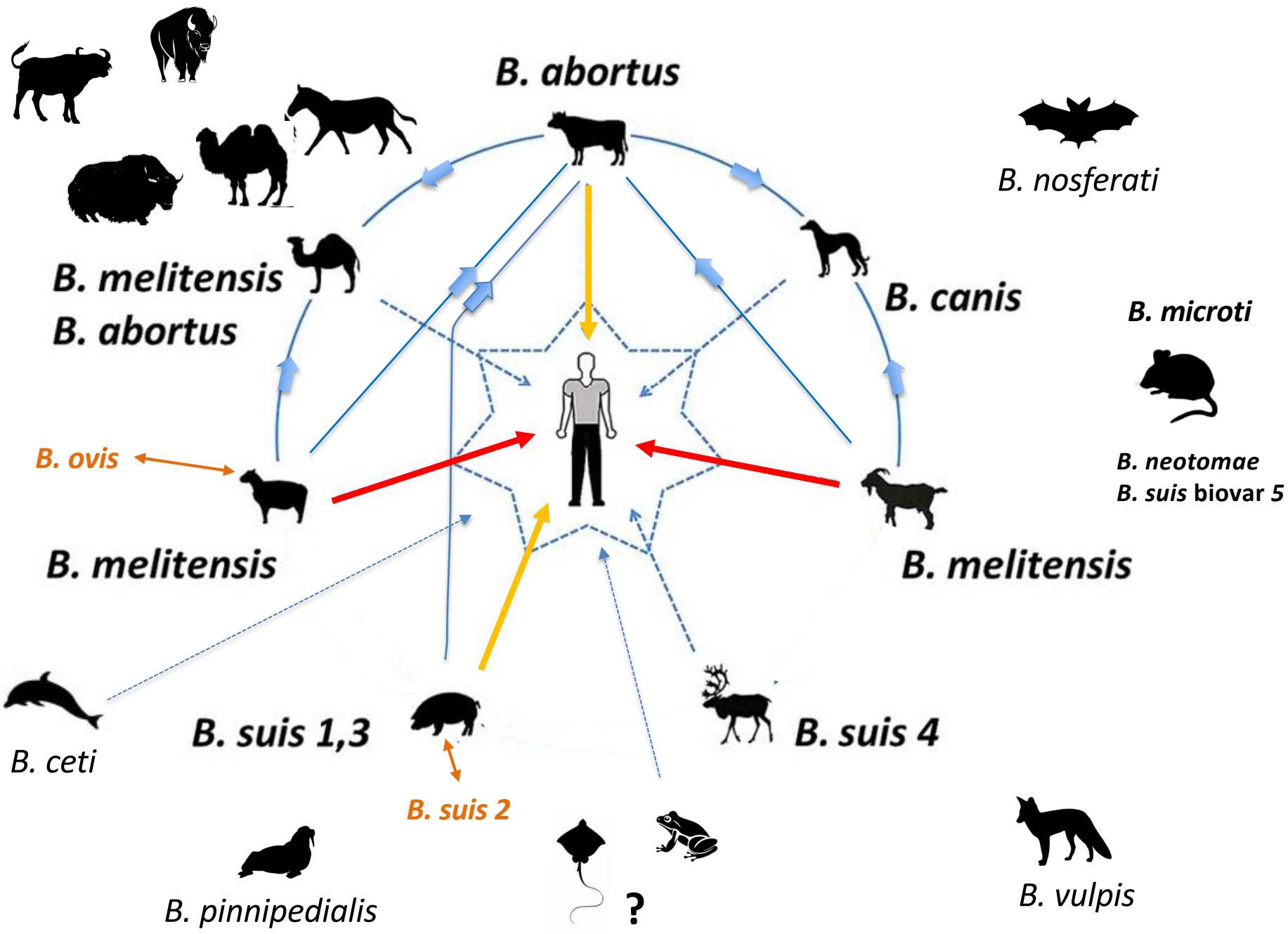
DISCONTOOLS

DISCONTOOLS Project Management Board meeting
December 11th 2024

Brucellosis


B. abortus
B. nosferati
B. melitensis
B. abortus
B. canis
B. microti
B. ovis
B. neotomae
B. suis biovar 5
B. melitensis
B. melitensis
B. ceti
B. suis 1,3
B. suis 4
B. suis 2
B. pinnipedialis
?
B. vulpis

- Delayed recognition of pathogen by innate immunity.
- Facultative intracellular parasite.
- Triggers cellular and antibody-dependent immunity.
- Can affect almost any organ and shows genital tropism.
- The disease lacks pathognomonic signs or symptoms.

↓

Tests and vaccines are essential

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Their use is conditioned by / needs to be adapted to:

- Breeding system: Intensive, mixed, extensive, transhumance.
- Infrastructure & budget.

DP. Socio-Economic Impact & Global Challenge

Zoonosis	Human brucellosis is not a notifiable disease in most endemic countries. For most endemic countries records in the WAHIS are not dependable. A recent conservative estimate: annual global incidence is 2.1 million. Very high morbidity but estimated DALYs per case vary from 0.10 to 7.5 (assessments differ widely in methodology and in assumptions concerning the different clinical forms, duration of the illness and others). Cost of treatment (few analyses). Example (Spain, 1989): €5000/case (treatment, hospitalization and hours of work lost).
Direct impact on production	Not well documented. Impact depends on country, breeding system, etc. A 2024 study in Colombia in cattle estimated $822 USD per animal.
Impact on trade	Very high (international, EU intracommunity and national levels).

Product Gap Analysis Scores for diagnostic tools [1]

Host	Criteria overall score	Comments
Cattle Small ruminants Pigs	All < 0 ("good or very good") but DIVA and stability	DIVA test applicable and/or necessary under most conditions? Cold Chain necessary. DTH antigen for pigs not available
B. ovis (sheep)	All ≤ 0 ("not so good to very good") but DIVA, stability and strategic reserve	Not zoonotic. Strategic reserve?
B. canis (dogs)		Zoonotic. Pets. Strategic reserve?
Yacks, water buffaloes Camelids Wildlife	All ≥ 1 ("poor/not available") but capacity of production	**Huge gaps**
All hosts	DNA amplification tests 0 - 1	Big gap (not homogenized/validated; new technologies to mitigate costs and infrastructure needs).

[1] From 2 ("poor/not available") to -2 ("very good").

DP1. Diagnostics

Worldwide availability	Gap(s) in availability/knowledge
Bacteriological ✓ Basal media. ✓ Antibiotic supplements for Farrell and Thayer's Martin CITA). ✓ PCR kits for *in vitro* identification (including OIE-vaccines).	✓ Antibiotic supplement for CITA medium.
Immunological ✓ Many kits (humans and main domestic livestock). ✓ WOAH and/or EU sera for standardization of most tests. ✓ Most tests in 2023 WOAH Manual.	✓ Most endemic areas: – Costs of iELISA, cELISA, FPA, Brucellacapt. – Validation (iELISA, cELISA, FPA). – Cold storage. ✓ Risk of some standards being discontinued. ✓ Standards for new tests (LFiC, others)? ✓ No kits standardized & validated for camelids, yacks, water buffaloes (etc.). ✓ Few tests validated for wildlife. ✓ DTH tests: No antigens commercially available.
DNA-detection methods. Few	– Available kits/protocols not validated.

Commercial potential

Eradication-surveillance compulsory in the EU in domestic livestock & brucellosis is a priority in many emerging economies. Thus:

- ✓ Very high for serological tests (not available for camels, yacks, water buffalos…)
- ✓ Very high for DNA-detection kits effective in direct diagnosis (not fully developed).
- ✓ High for DTH skin tests in pigs (not commercially available).

Also:

- ✓ Room for *B. ovis*/*B. canis* diagnostic tests (not fully developed).

DIVA tests required and / or available	Gap(s) in availability/problems
✓ An effective DIVA vaccine and diagnostic partnership could encourage eradication. ✓ Some manufactures claim that cELISAs and blocking ELISAs are truly DIVA, but this is not true.	– Required? Census & tagging, repeated access to the animals, laboratories and budget required for implementing any DIVA test. – Feasibility? Current S-LPS tests are highly sensitive, and S vaccines exceedingly difficult to replace (see below).

Product Gap Analysis Scores for Vaccines [1]

Host (vaccine)	Scores	Comments
Cattle (S19)	Immunity/efficacy: -1/-2; others: 1 to -2	Proved usefulness. CJ in bulls?
(RB51)	Immunity/efficacy: 2/2;[2] others 2 to -1	DIVA? Usefulness? Bulls?
Small ruminants (Rev1)	Immunity/efficacy: -2/-1; safety: 2 to 1	**Big gap**: a Rev1 substitute
Pigs	2	Gap (useful in special conditions)
B. ovis (sheep) specific	2	Gap (and non zoonotic).
B. canis (dogs)	2	Gap. Zoonotic. Pets.
Yacks, water buffaloes	2	**Huge gap**
Camelids	2	**Huge gap**
Wildlife	2	Not applicable

[1] From 2 "poor/not available" to -2 "very good".
[2] One panel member estimated a score o 1 for immunity.

DP. Marker Vaccines

Applicability of DIVA vaccine and DIVA test in brucellosis?

Worldwide availability

- Only RB51 combined with the Rose Bengal Test but protection wanes quickly and is not DIVA in infected environments.

Many approaches explored unsuccesfully

DP. Commercial potential

Should be good for:	– Conjunctival S19. – a Rev1 substitute (market? Developing & emerging economies). – Vaccines for water buffaloes, camels, yacks (market? Developing & emerging economies).
Obstacles:	✓ Misconceptions on vaccine & test use, and control & eradication policies. ✓ Lack of budget & infrastructure to implement control. ✓ Regulatory &/or policy challenges to approval. – Some large endemic countries (China, Russia, possibly others) are not open markets (favor their non-WOAH vaccines of questionable usefulness). – Many endemic countries lack effective regulatory/policy rules.
Feasibility (e.g., manufacturing)	✓ For manufacturing attenuated live vaccines, technology & experience gained in Rev 1 and S19 production in the EU.

DP. New or improved vaccines

Time to develop From concept to industrialization and to EU marketing authorization, 10 years or longer	**Main obstacles:** - Human resources and budget. - Few teams remain that have the know-how on evaluation of brucellosis vaccines - Availability of category 3 facilities for large animals. - Genetically Modified Organism legislation in Europe. - Very challenging in animals other than cattle and small ruminants.
Cost of developing and validation	Difficult to estimate but very high.

DP. Obstacles in applying diagnostics, vaccines and pharmaceuticals

1. Non-technical.

Obstacles	Facilitators /Measures/Needs
✓ Lack of awareness (existence, transmission, and zoonotic character, etc.). ✓ Insufficient Vet. services, ref. laboratories, censuses, budget ✓ Laboratory capacity/knowledge for efficient diagnosis (animals and humans). ✓ No or out of context legislation (most developing economies).	✓ Education/awareness (all stakeholders & decision makers). ✓ Capacity building. ✓ Meeting/mitigating infrastructural needs.
✓ Intensification of breeding (makes brucellosis control exceedingly difficult if possible).	✓ Adapting breeding to brucellosis. ✓ More protective vaccines?
✓ Environment & climate (extensive & mixed breeding and animal movements).	✓ Safer (protective) vaccines for mass vaccination.

DP. 2. Technical obstacles in applying diagnostics, vaccines and pharmaceuticals

✓ **Diagnostics**

- Diagnostics in hosts other than cattle, small ruminants and pigs.
- Molecular tests:
  - methods are still expensive & inaccessible to many laboratories.
  - all animal species: lack of validation & harmonization.
  - humans: lack of harmonization; further studies in specific pathologies and upon recovery.

✓ **Vaccines**

- Lack of a safe *B. melitensis* vaccine (small ruminants).
- Safety of S19 vaccine in bulls?
- Lack of vaccine / studies in yacks, water buffaloes, and camelids.
- GMO legislation.

✓ **Pharmaceuticals** (humans)

- Long, expensive treatments.
- Some include non-parenteral administration.
- Streptomycin availability?
- Some use antibiotics of choice for tuberculosis.

DP. Obstacles in applying diagnostics, vaccines and pharmaceuticals

3. Other issues with actual or potentially negative impact.

Research on

- Human incidence and clinical presentations according to *Brucella* species and socioeconomic conditions.
- New *Brucella* species: importance, epidemiology, virulence, antigenic structure.
- Role of wildlife as reservoirs.

Expert group

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Many thanks for your attention

Supplemental Slides

DP. Summary of part 2: Disease Details

Gaps

- ✓ Epidemiology with attention to changes in host-range of “new” brucellae
- ✓ Prevalence and effect of the disease in wildlife animals, and their role as reservoirs
- ✓ Mechanisms of pathogenicity across all brucellae (including “new” species)
- ✓ Virulence for humans of non-classical brucellae
- ✓ Pathology of the agent within each natural host other than humans, ruminants or swine (camelids, yaks, water buffaloes, etc.)
- ✓ Mechanisms for host preference/specificity.
- ✓ Serological tests for atypical brucellae carrying an S-LPS structurally different from that in in core brucellae.

DP. 2. Vaccines

Pros and cons of WOAH brucellosis vaccines

	Cattle		Small ruminant
	Conjunctival S19	RB51	conjunctival Rev 1
Interference in diagnosis *	All tests	ELISAs, FPA, LFiC	All tests
Functional immunity **	Life span	< 4 years	Life span
Usefulness proved in eradication programs	Yes	No	Yes
Cross protection *B. abortus* & *B. melitensis* ***	Yes	No	Yes
Quality control protocol	WOAH	No	WOAH
Side effects in:			
Pregnant animals	Not fully safe	Not fully safe	Highly abortifacient
Male animals	?	?	No
Humans			
Virulence	Low	Low	High
Diagnostic tests	Yes	No	Yes
Antibiotic resistance	No	Rifampin resistant	Streptomycin resistant

* For all vaccines, interference wanes in few months when applied before sexual maturity.

** Protection by any of these vaccines can be overcome by large doses of virulent bacteria.

*** Necessary under extensive & mixed breeding.

DP. Marker Vaccines

Applicability of DIVA vaccine and DIVA test in brucellosis?

Worldwide availability

- Only RB51 combined with the Rose Bengal Test but protection wanes quickly and is not DIVA in infected environments.

Failures

- Other R mutants: no satisfactory protection.
- Rev 1 and S19 deleted in BP26 & associated DIVA test.
- Tagged Rev 1(GFP and genetically modified antigen) & associated DIVA test.
- No subcellular vaccine has been demonstrated to be effective.

DP. Obstacles in applying diagnostics, vaccines and pharmaceuticals

2. Technical (1).

Obstacles	Research
✓ **Vaccines** – Lack of a safe *B. melitensis* vaccine (small ruminants). – Safety of S19 vaccine in bulls? – Lack of vaccine / studies in yacks, water buffaloes, and camelids. – GMO legislation.	✓ On vaccine S19: – Bulls: safety of conjunctival administration. – Yacks, camels and water-buffaloes: safety & efficacy. – New vaccines: ✓ Virulence & mechanisms of pathogenicity – Safe vaccine against *B. melitensis* infection of small ruminants. – *B. ovis* specific vaccine. – *B. suis* vaccine for pigs (may be necessary in special situations) – A vaccine for camels.

DP. Obstacles in applying diagnostics, vaccines and pharmaceuticals

2. Technical (2).

Obstacles	Research
✓ **Diagnostics** – Diagnostics in hosts other than cattle, small ruminants and pigs. – Molecular tests: methods are still expensive & inaccessible to many laboratories. all animal species: lack of validation & harmonization. humans: lack of harmonization; further studies in specific pathologies and upon recovery.	✓ Studies in hosts other than cattle, small ruminants and pigs. ✓ Validated tests for R brucellae. ✓ Molecular tests: new simpler & cheaper & validation & harmonization
✓ **Pharmaceuticals** (humans) – Long, expensive treatments. – Some include non-parenteral administration. – Streptomycin availability? – Some use antibiotics of choice for tuberculosis.	✓ More efficacious/cheaper antibiotics. ✓ Further trials on doxycycline monotherapy. ✓ Further trials on streptomycin replacement by gentamycin.