Phylogenetic Analysis and Epidemiological Investigation of *Brucella* spp. in a One Health Context

Reflections

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Outline

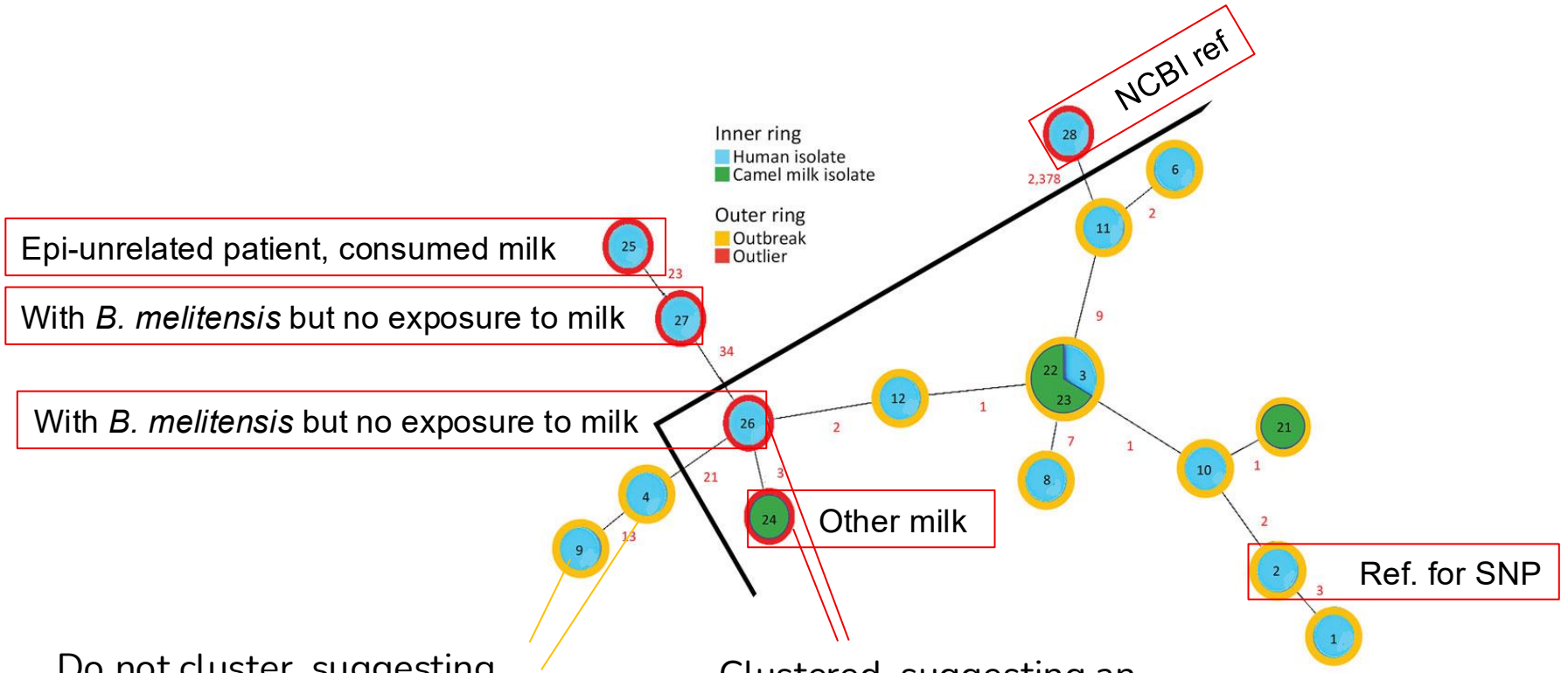
This session consists of the following elements:

1. Real-life examples of WGS-based outbreak investigations to guide reflections on:
    - One Health implications and genomic surveillance strategies
    - Challenges in surveillance of zoonotic diseases
    - Integrating WGS into outbreak investigations

2. Open Q&A session for participants to raise questions and share reflections

B. melitensis in Israel, 2016 (Bardenstein 2021)

- Infection was diagnosed in 19 patients over 4 months
- Epidemiologic investigation noted common exposure: same brand of unpasteurized camel milk, sold through online sales or retail stores
- SNP-analysis included outliers for comparison



Do not cluster, suggesting multiple clones in the outbreak

Clustered, suggesting an unrecognized epidemiologic link

What are the main messages we can take away from this case in Israel? E.g.: Epi-data always leads the conclusions, as isolates with higher SNP are still part of the outbreak.

B. melitensis in Israel, 2016 (Bardenstein 2021)

What are the main messages we can take away from this study? → Examples

Epidemiological perspective	**WGS perspective**	**One Health perspective**
Epi-data always leads the conclusions: isolates with higher SNP are still part of the outbreak!	**Real-life SNP distances** within an outbreak.	Conclusive evidence of **common source.**
WGS data can complement the investigation: outliers were part of the outbreak after all.	Impact of the choice of **reference**.	**Commercial practices** impact the range of the outbreak.
	Potential relevance of **including outliers**.	**Pasteurization** can prevent infection.

B. suis in Germany, 2018 (Zange 2019)

Overview of the case

- One human case of brucellosis in June 2018
- Without: recent travel history, consumption of (unpasteurized) dairy products, animal contacts, hunting experiences, insect bites
- Rest of the family was not sick

- Patient culture was identified as *B. melitensis* via MALDI-TOF
- Later identified as *B. suis* with PCR Bruceladder

↓

Lack of *B. suis* reference spectra in the MALDI-TOF database

B. suis in Germany, 2018 (Zange 2019)

B. suis context in Germany

- **Wild boars** and **hares** in Germany are known carriers of *B. suis* **biovar 2**
- Recently **biovars 1 and 3** had been detected in **wild boars** but no human infections reported

Possible patient exposure

- In the weeks before symptoms patient did a lot of gardening and **saw wild hares**
- **Denied** consumption of **wild boar**

Slido in next slide: Do you have a hypothesis about the source and biovar?

Do you have a hypothesis about the source and biovar?

B. suis in Germany, 2018 (Zange 2019)

WGS analysis

cgMLST tree of 70 *B. suis* strains belonging to different biovars

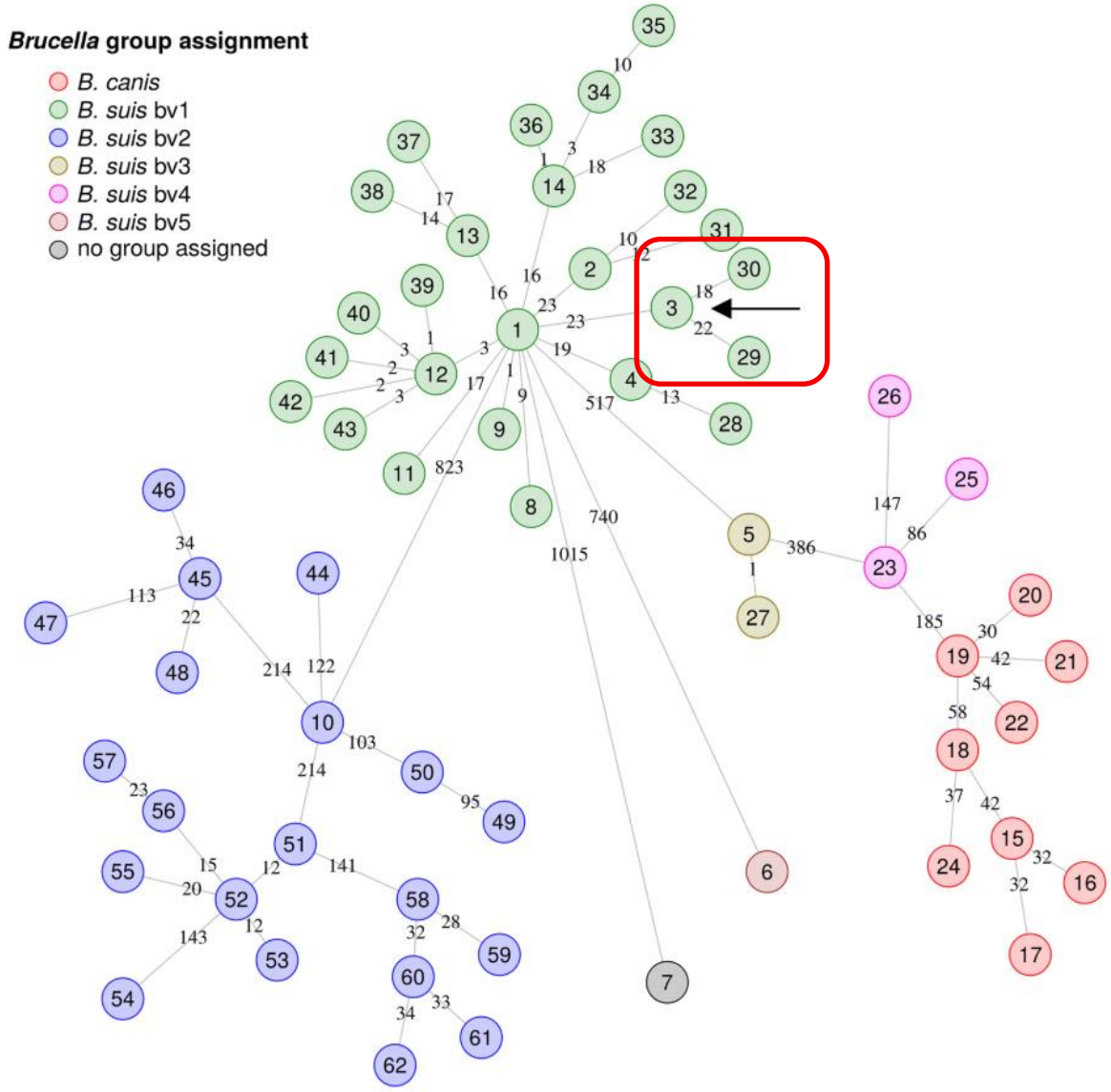
→ *B. suis* biovar 1 clade

First time in humans without travel history in Germany

→ The hares in the garden were not the culprit!
(would be biovar 2)

Closest proximity to strains from a dog fed with contaminated meat from Argentina

So what is happening here?



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FSU_SRR651210
2 ... B06_0059
3 ... R050_36_human_GER
4 ... NZ_07_1559NZ_SRR2058987
5 ... USA_686_ACBL01
6 ... L3_2531_513__inhouse
USSR_513_ACBK01
7 ... suis_S2_30_CHN_ALOS01
8 ... USA_2004000577_CP016981
9 ... 63_227_SRR867711
10 ... 94_11_AQNW
11 ... CNGB_247_HUMAN_ARG
12 ... 1330_NC_017251_1
MX_92_29_AQNU
13 ... CHN_019_CP013963
S2_China_pig
14 ... USA_VBI22_CP003128
15 ... Oliveri
16 ... SCL_LGAQ01
17 ... CNGB_513_AQJZ01
18 ... 2009013648
19 ... 79_122_AQJY01
20 ... HSK_A52141
21 ... USA_2010009751
22 ... ATCC_23365
RM6_66
23 ... 40_ACJK01
BSQ_JMUD01
L3_2530_40__inhouse
24 ... SVA13
25 ... FSU_63_198_AQKY01
26 ... USA_63_252_AQNT
27 ... USA_686_NZ_CP007719
28 ... NZ_08_924NZ_SRR2058989
29 ... ARG_NL_dog2
30 ... ARG_NL_dog1
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32 ... B10_0144
33 ... USA__AR_US1981_CP010850
34 ... USA_B99_1007_SRR2058981
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39 ... ZW043_ZIM_NZ_CP009094_1
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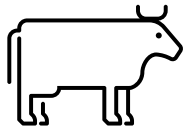
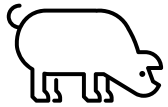
42 ... CHN__BCB025_ALOK0
43 ... NVSL_BS1_1330_SRR2058923
44 ... DK_63_261_AQKZ01
45 ... 06_988_1656_AXNH01
46 ... PRT__PT09143_CP007691
47 ... PRT__PT09172_CP007693
48 ... ESP_Bs143CITA_CP007695
49 ... FR_F5_05_4_AQLC01
50 ... GER_F7_06_1_AQLD01
GER_F7_06_2_AQLE01
51 ... BE_F8_06_3_AQLH01
52 ... FR_97_9757_AXMY
53 ... IT_F9_06_1_AQLI01
54 ... GER_F7_06_5_AQLF01
55 ... BE_F8_06_1_AQLG01
56 ... ESP_Bs364CITA_CP007697
57 ... Bs396CITA
58 ... DK_Thomsen_JMUE01
59 ... DK_F12_02_AQLB01
60 ... SLO_92_63_AQNV
61 ... 04_0115_AXMU01
62 ... FR_F5_05_10_AQIP01

B. suis in Germany, 2018 (Zange 2019)

Interview revealed excessive meat consumption

- Buys batches of meat from wholesale market and processes the meat in his garage

Approx 2 months before symptoms:

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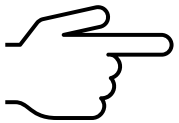
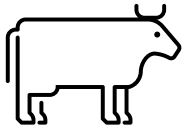


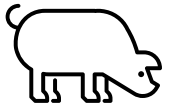
Cut his finger preparing minced meat

Meat **not available** for sampling

Meat in the freezer: all negative for *Brucella*



Botswana



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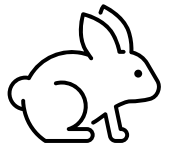


Argentina

Had eaten another leg before

Slido in next slide: Do you have a new hypothesis?

Do you have a new hypothesis?

B. suis in Germany, 2018 (Zange 2019)

Conclusions

- Origin of the minced meat is unknown and *B. suis* biovar 1 is distributed globally → pork meat is a possible source of infection
- Route of transmission is unclear → most likely during preparation of minced meat (cut his finger)
- No samples from the minced meat → evidence-based source attribution was not possible
- Limited number of *B. suis* biovar 1 genome sequences in the databases with sparse/erroneous metadata → spatial attribution was not possible
- Limited genomic data in the databases → epidemiological link with Argentinian genomes is speculative

What are the main messages we can take away from this case in Germany?

B. suis in Germany, 2018 (Zange 2019)

What are the main messages we can take away from this study? → Examples

Epi / Lab perspective	WGS perspective	One Health perspective
Assumptions can be wrong: Hares/biovar 2	**Real-life cgMLST** distances	**Global distribution and spread** of isolates
Epi investigation is essential: Patient exposure pointed to the meat	Importance of comprehensive **metadata**: poor metadata limited the conclusions of the investigation	**Uncommon biovars** can affect humans
Understanding the principles behind test methods is crucial: MALDI-TOF was wrong	Results made **no sense without epi data** supporting their interpretation: no travel to Argentina	**Food hygiene** (or lack of it) has real consequences

Bovine brucellosis in Northern Ireland (Abernethy 2011)

- Eradication scheme started in 1962

  - Annual serological testing of cattle
  - Short-interval testing of contact herds (1-6 mos.)
  - Depopulation of most culture-positive outbreaks
  - **Computerised tracing of contact animals**
  - Monthly ELISA testing of bulk milk
  - Post-abortion testing
  - Pre-movement testing of breeding cattle
  - Testing of cows >30 months at slaughter
  - **Creating lists of contiguous herds after each outbreak**

- By the early 1980s most outbreaks comprised singleton reactors, potentially false-positives
- Recrudescence occurred in 1997
- In 2006 there were a series of outbreaks

Bovine brucellosis in Northern Ireland (Abernethy 2011)

The epidemiological investigation of the 2006 outbreaks

Data sources

- Department's Animal and Public Health Information System (APHIS)

Centralised database with data on between-herd movement and brucellosis test history since 1995.

- Following diagnosis of brucellosis in a herd, technical officers in the local veterinary office identify contiguous herds using a combination of paper-based and digitised farm maps

Depending on the outbreak, up to two "rings" of herds surrounding each outbreak are identified.

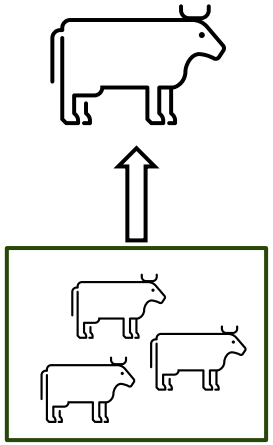
- Veterinary officers interview the owner of each infected herd

Using pre-tested epidemiology questionnaires.

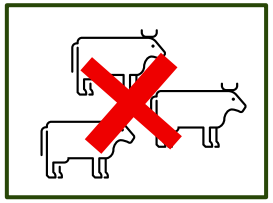
Bovine brucellosis in Northern Ireland (Abernethy 2011)

Epidemiological links

First case was a **recently purchased bull** that tested positive at slaughter.

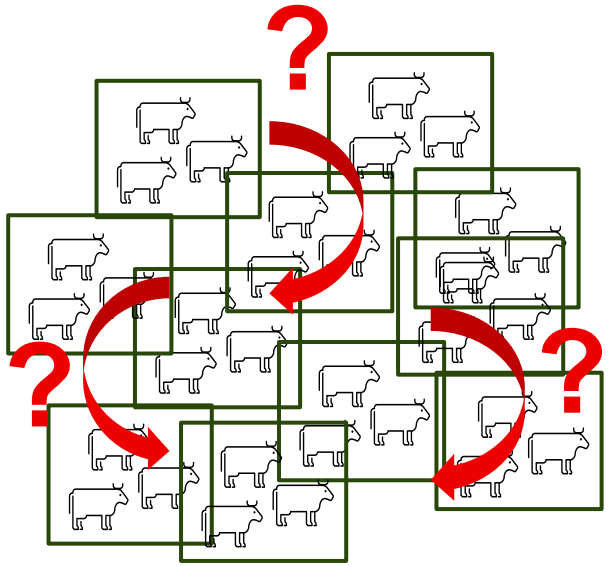


The original herd where the bull came from was negative (vendor herd).



A neighboring herd to the vendor was positive.

All females and bulls were culled.



In total **41 outbreaks** were detected.

Most of these (71%) were detected by **testing contiguous herds.**

There was a flow diagram of **postulated** spread incl. contiguous spread, purchases, fomites.

No definitive source of infection was determined.

Bovine brucellosis in Northern Ireland (Abernethy 2011)

Conclusions

- No credible routes of infection through lateral spread, purchase of cattle, cross-border contact or lack of compliance with legislation

- Failure to identify sources may arise from
  - ineffective investigation
  - inadequate information from herd keepers or from factors such as latent infection that may obscure epidemiological links due to the long interval between infection and disclosure

- **Proposed explanations:** Latent infection in 2002-2003 (uncommon); discrepancies in reporting abortions in 2005

- No further outbreaks from this cluster were detected by the publication date (2011)

What are the main messages we can take away from this case in Northern Ireland?

Bovine brucellosis in Northern Ireland (Abernethy 2011)

What are the main messages we can take away from this study? → Examples

Epidemiological perspective	**One Health perspective**
Control efforts are effective: general decline in cases, excluding the outbreak **Re-emergence** is possible and **surveillance** must be in place to detect and solve the problem In this case the **data collected** was not enough to find the source but it allowed to very quickly identify other affected herds	High **economic losses** from outbreak: high nr. of affected herds, culling

Bovine brucellosis in Northern Ireland (Allen 2015)

The same *B. abortus* population was investigated with MLVA

- **Outbreak continued until 2012**
  - Epidemic involving 387 herds 1991-2012
  - Last confirmed case in cattle: March 2012

- **MLVA11** identified **98 genotypes** in the 387 herds involved in the epidemic.
  - Combined with epidemiological information (chronology and geography) it identified **7 clonal complexes** present in the outbreak.

Bovine brucellosis in Northern Ireland (Allen 2020)

The same *B. abortus* population was investigated through WGS

- SNP analysis distributed the isolates into 2 lineages

- In lineage 2, isolates belonging to different MLVA clonal complexes clustered together, suggesting that VNTR-based genetic sub-structure was not supported.

  One justification: *…some VNTR loci are unstable after multiple culture passages…*

- Lineage 2 exhibited very little diversity. All pairs of isolates were separated by average 2.6 SNPs.

Bovine brucellosis in Northern Ireland (Allen 2020)

Conclusions

- Caution when deploying MLVA schemes, to avoid inferences of population structure and unsupported connections.
- Applying genomic methods to this outbreak had limited capacity to inform on disease transmission dynamics due to the limited genetic diversity.
- Older and recent isolates were connected. Genome datasets, over wide temporal scales, can be useful in assessing the likely provenance of such infections.

Bovine brucellosis in Northern Ireland (Allen 2020)

However…. there were still questions!

- *B. abortus* evolutionary rate was estimated a 1.4 x 10^{-7} substitutions per site per year
- For *B. abortus* with genome size 3.3 Mb this would equal to 0.46 SNPs per year

➢ However the authors found on average only 2.6 SNPs over 12 years outbreak

- The authors also found links between earlier isolates (90s) and later isolates (00s)

➢ This indicates that a viable source must have persisted

Do you have any justification for the low genetic diversity and the relatedness of isolates so far apart in time?

Bovine brucellosis in Northern Ireland (Allen 2020)

Authors perspectives

- Survival in dormant state in a limited number of hosts
- Environmental persistence – not likely as it tends to be < 8 months
- Comparatively short sampling window – not supported by evolution rate
- Control measures could have curtailed transmission and prevent pathogen differentiation
- **Criminal activity**

Anything can happen!

5 October 2010

Criminal and fraudulent activity is preventing DARD from eradicating the disease …

… unable to track down the criminals who were behind a number of sinister incidents, including **leaving an infected calf foetus** at the home of a DARD official.

https://www.belfasttelegraph.co.uk/news/northern-ireland/top-level-talks-on-criminals-behind-cattle-disease/28562730.html

4 March 2011

A **calf's leg was found in silage** being used to feed a group of heifers.

…

DARD investigated a previous incident in Armagh at the end of 2009, when an **infected foetus was deliberately placed in a field of cattle** in an attempt to infect them.

…

"There have been several incidents in Armagh in the last year that have led to local hot-spots of disease. This has cost the taxpayer and farmers millions of pounds."

https://www.bbc.com/news/uk-northern-ireland-12650613

References

Bardenstein S, et al. **Brucellosis Outbreak Traced to Commercially Sold Camel Milk through Whole-Genome Sequencing, Israel.** Emerg Infect Dis. 2021;27(6):1728-1731. doi:10.3201/eid2706.204902.

Zange S, et al. **A headache with surprising outcome: first case of brucellosis caused by Brucella suis biovar 1 in Germany.** Infection. 2019 Oct;47(5):863-868. doi: 10.1007/s15010-019-01312-7.

Abernethy DA, et al. **Epidemiology and management of a bovine brucellosis cluster in Northern Ireland.** Prev Vet Med. 2011 Mar 1;98(4):223-9. doi: 10.1016/j.prevetmed.2010.11.002.

Allen A, et al. **Molecular Epidemiology of Brucella abortus in Northern Ireland-1991 to 2012.** PLoS One. 2015 Sep 1;10(9):e0136721. doi: 10.1371/journal.pone.0136721.

Allen AR, et al. **Genomic epizootiology of a Brucella abortus outbreak in Northern Ireland (1997-2012).** Infect Genet Evol. 2020 Jul:81:104235. doi: 10.1016/j.meegid.2020.104235.

Moriyón I, et al. **Brucellosis and One Health: Inherited and Future Challenges.** Microorganisms. 2023 Aug 11;11(8):2070. doi: 10.3390/microorganisms11082070.

Acknowledgements

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Open Q&A session

Do you have any questions, comments, insights?