

Investigation and molecular typing of *Brucella* isolated from dairy cattle herd immunized with *Brucella abortus* RB51 vaccine in Egypt.

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Federal Research Institute for Animal Health



NRL for Brucellosis, The Institute for Bacterial
Infections and Zoonoses (IBIZ).



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History of brucellosis



Sir David Bruce
1855-1934
(microbiologist)

- In 1886 a cocco-bacillus isolated from from the spleen of a man who had died of Malta Fever.
- He named it “Micrococcus melitensis”
- Human disease was associated with people who consumed goat milk and had other close contact with goats.



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History of brucellosis

- In 1886 first isolation from human
- In 1897 a similar microbe was isolated from the udder of cows
- In 1914 isolated from swine.
- In about 1920 the genus was renamed *Brucella* and its species became respectively *B. melitensis*, *B. abortus*, and *B. suis*.
- *Brucellosis is a highly contagious major bacterial zoonosis spread worldwide and has different names: Infectious abortion, contagious abortion, enzootic abortion in animals; and Crimean fever, Mediterranean fever, rock fever, undulant fever or Malta fever in humans.*



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Brucellosis

- According to WHO brucellosis is classified among the top seven world neglected zoonotic diseases.
- The true incidence of human brucellosis is not easy to estimate globally but an estimated number of more than 500,000 persons get infected newly every year.
- Genus *Brucella* includes 11 nomo-species:
 - The six “classical” spp. *B. melitensis*, *B. abortus*, *B. suis*, *B. canis*, *B. ovis*, and *B. neotomae*.
 - Two of marine origin: *B. pinnipedialis* and *B. ceti*.
 - *B. microti* isolated from the common vole *Microtus arvalis*.
 - *B. inopinata* isolated from a breast implant wound of a female patient
 - Finally, *B. papionis* was described from baboons (*Papio* spp.)



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Brucellosis in Egypt

1886 In Malta

After 53 years

1939 In Egypt



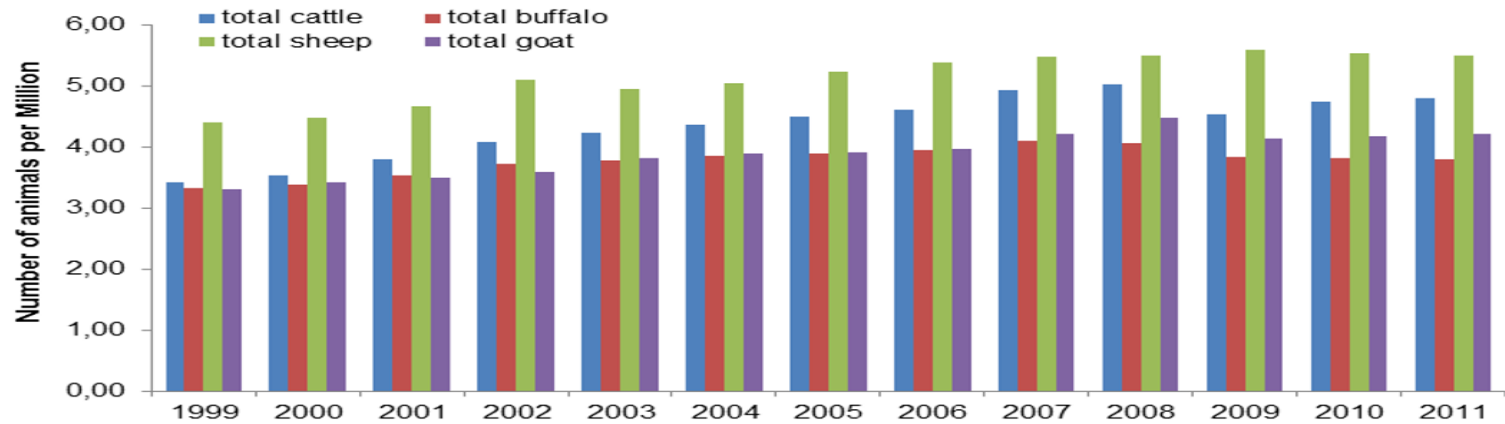
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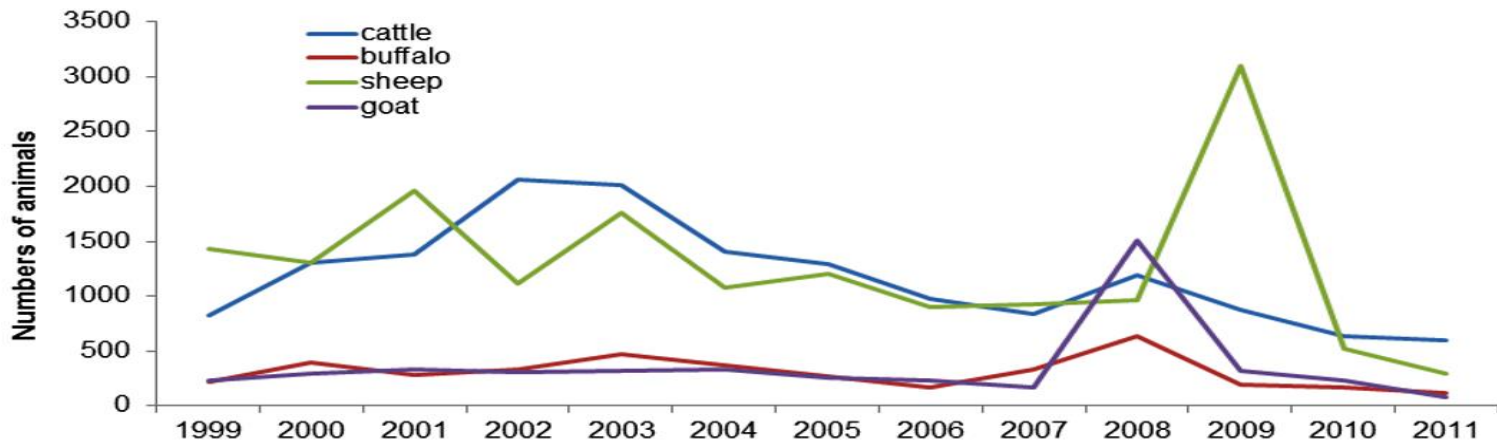
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Animal population and seroprevalence in Egypt



The total number of animals was steadily increasing during the last years in Egypt. 1999-2011 (FAO, 2012).



Number of seropositive animals according to GOVS, Egypt, 2012



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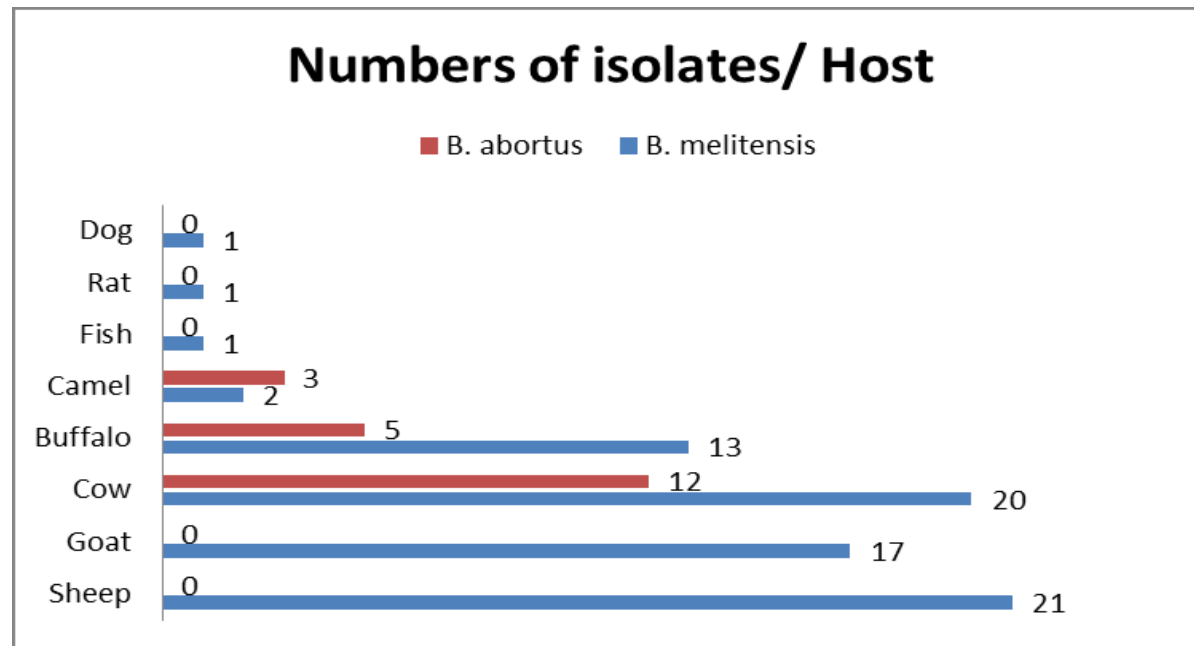
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Brucellosis in Egypt

- ❖ It is likely that brucellosis has been an endemic disease in Egypt for thousands of years
- ❖ In 1939, brucellosis was reported in a scientific report from Egypt for the first time
- ❖ Brucellosis is prevalent nationwide in all farm animal species, in the environment, and in carrier hosts.



Wareth et al., 2014: Animal brucellosis in Egypt_review_ *J Infect Dev Ctries* 2014; 8(11):1365-1373. doi:10.3855/jidc.4872



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Brucellosis in Egypt

Cross species transmission of brucellosis was reported



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What is the Solution?



Men bathing their water buffalo in the Nile Delta Region

<http://muirgildream.tumblr.com/post/27296121225/men-bathing-their-water-buffalo-in-the-nile-delta>



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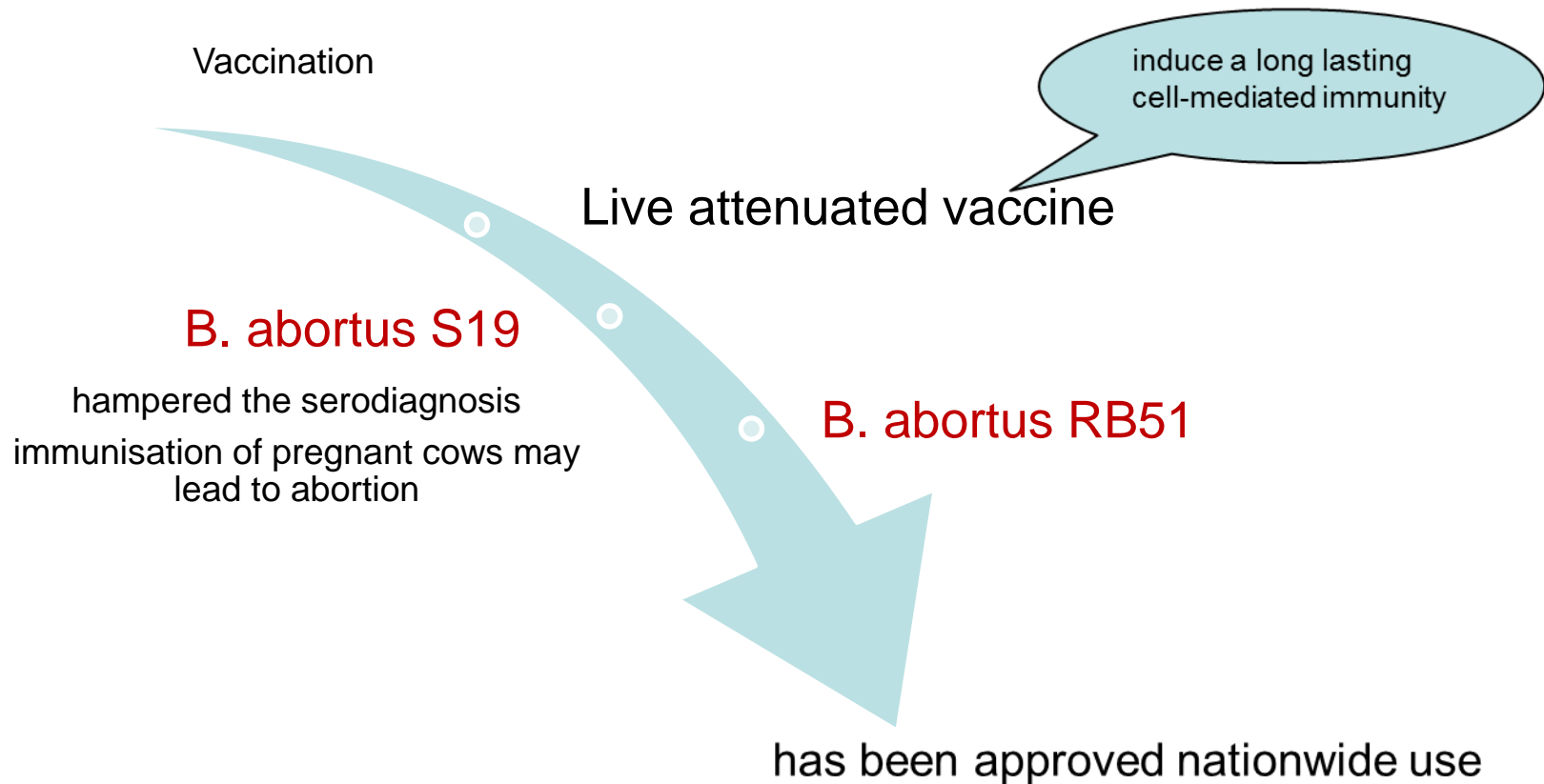
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Control and Surveillance program

Surveillance and control programs in endemic developed or developing countries based on **Test and slaughter strategy** with assistance of **vaccination**.



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The study

Status	Animals	Stable ^a	Serology ante vaccination	No.	Abortion	Time of abortion	Serology post vaccination ^b	Other
vaccinated animals	pregnant cows and heifers ≤ 3 months of pregnancy	A	negative in the past 3years	197	20 cows, 1 heifer	5-6 months post fertilization	20 cows, 1 heifer positive	
	non-pregnant cows and heifers ≥ 8 months	B	negative in the past 3years	93	-	-	negative	
vaccinated total				290				
non-vaccinated animals	Bulls	C	negative in the past 3years	50			negative	kept for meat production
	young calves	D	negative in the past 3years	185			negative	≤ 8 months
	pregnant cows and heifers	E	negative in the past 3years	75	-	-	negative	≥ 3 months of gestation
non-vaccinated total				310				
cattle total				600				



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Results

Bacteriology

- Two *B. abortus* rough strains
- Two *B. abortus* smooth strains
- No RB51 vaccine strain was detected

Genotyping

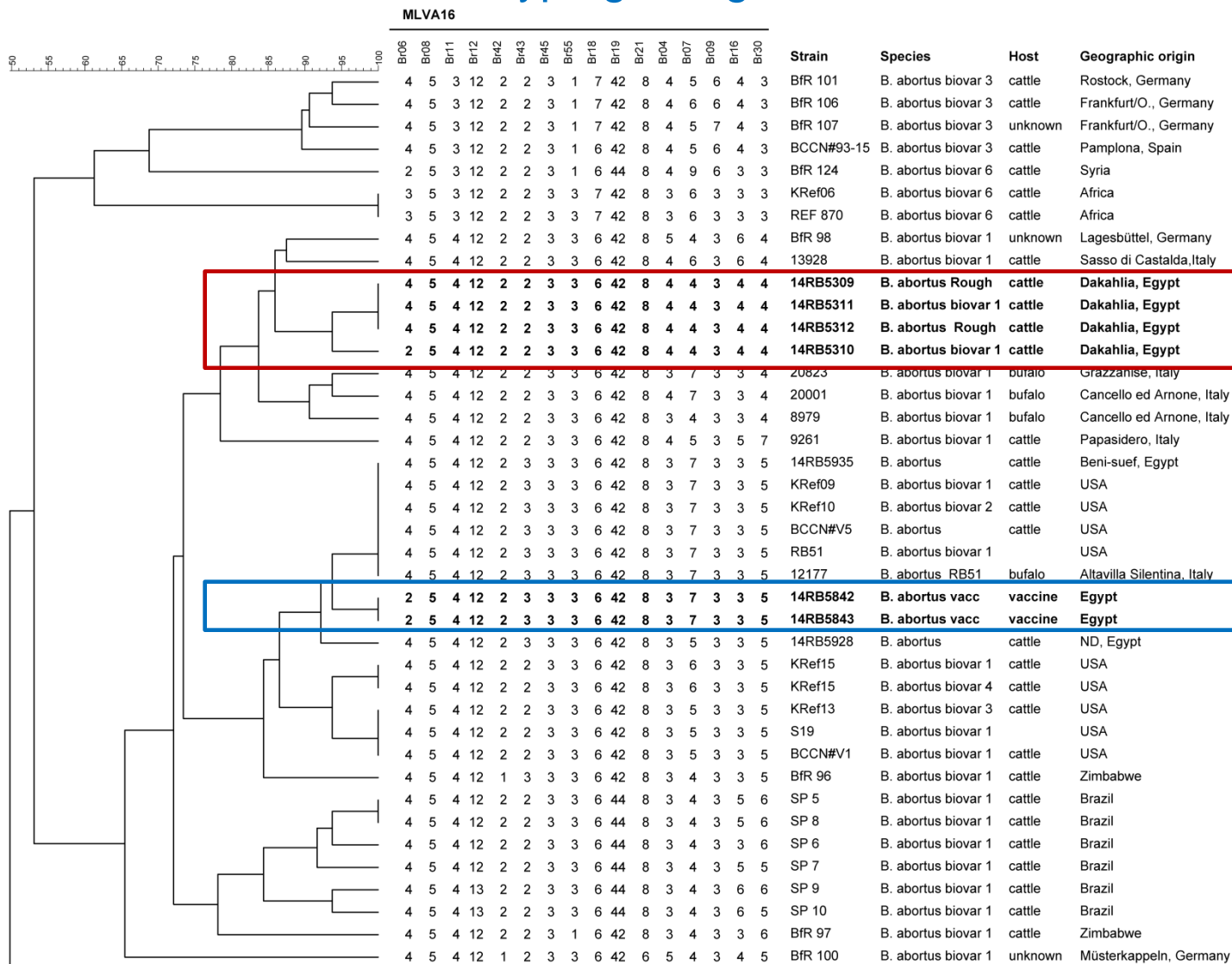
- Two different genotypes among the four isolated *B. abortus* strains with low genetic diversity

Histopathology

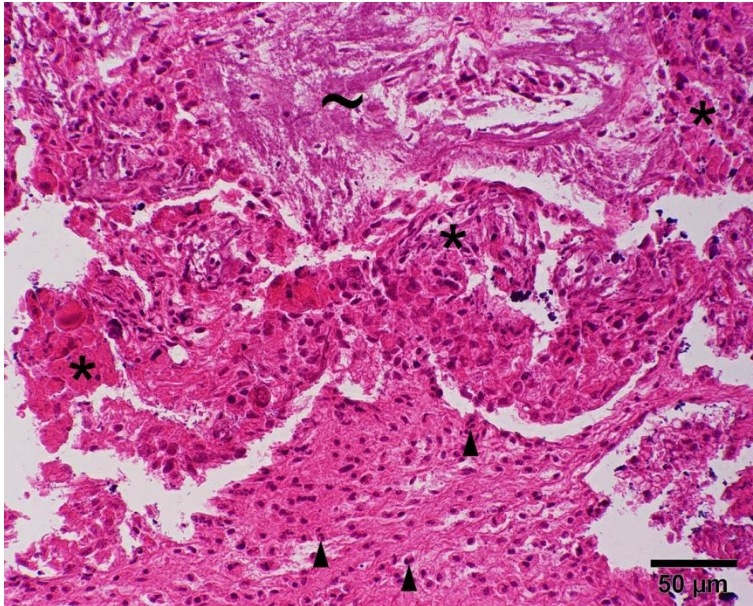
- Extensive necroses accompanied by mild to moderate suppurative inflammation
- Brucella antigen was detectable.



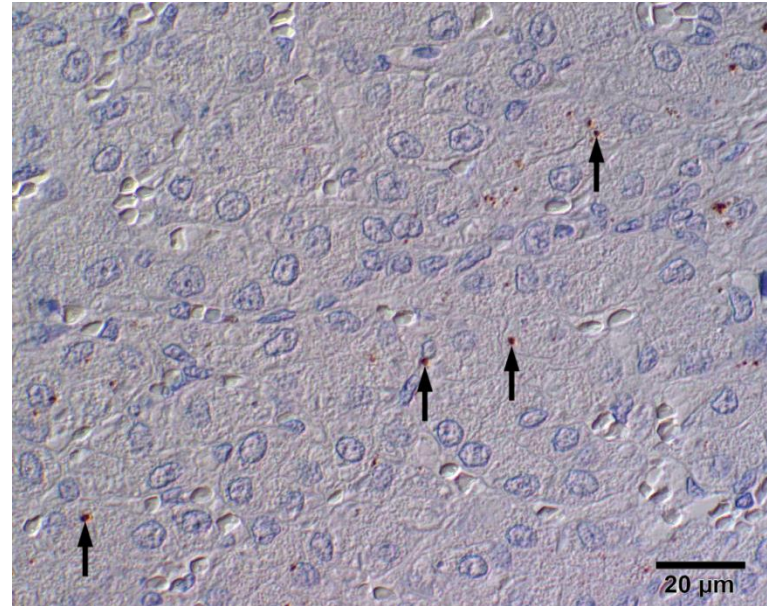
Genotyping using MLVA-16



Histopathology



Fetal placenta. Severe necrosis (asterisks) of placental tissue, accompanied by mild to moderate infiltration of leukocytes (mainly neutrophils, arrowheads) and dystrophic calcification (tilde). HE.



Liver of aborted foetus. Immunohistochemical detection of scattered Brucellae (arrows) within the cytoplasm of hepatocytes



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Summary of the study

- Aborted cows were tested seronegative for brucellosis in the past 3 years.
- 10 % of vaccinated cows aborted after 3 months.
- All aborting cows had previously delivered at least twice healthy calves without difficulties .
- All aborted cows are seropositive for standard agglutination test.
- All isolated *B. abortus* are field strains with low (or no) genetic diversity.
- No RB51 strain was isolated.
- The vaccine offers no complete protection from infection by field strains but it is known to reduce spread of infection



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What is the possible explanation for that result?



The source of infection in the present study could not be clarified

but several scenarios are plausible



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Scenario 1: Artificial insemination

Use of contaminated sperm for artificial insemination
Brucella spp. has been detected in semen of serologically
negative bulls



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Scenario 2:

Introduction of seropositive animals without previous testing of its serological status

A cattle market hold before the Eid al-Adha festival, 10th Oktober 2011, Nile delta province of Menufiya, Egypt



http://news.xinhuanet.com/english2010/photo/2011-10/30/c_131220532_4.htm



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Scenario 3: Latent infection

a latent carrier suckler cow without antibody
production against Brucella but could shed of the
agent and infecting other animals



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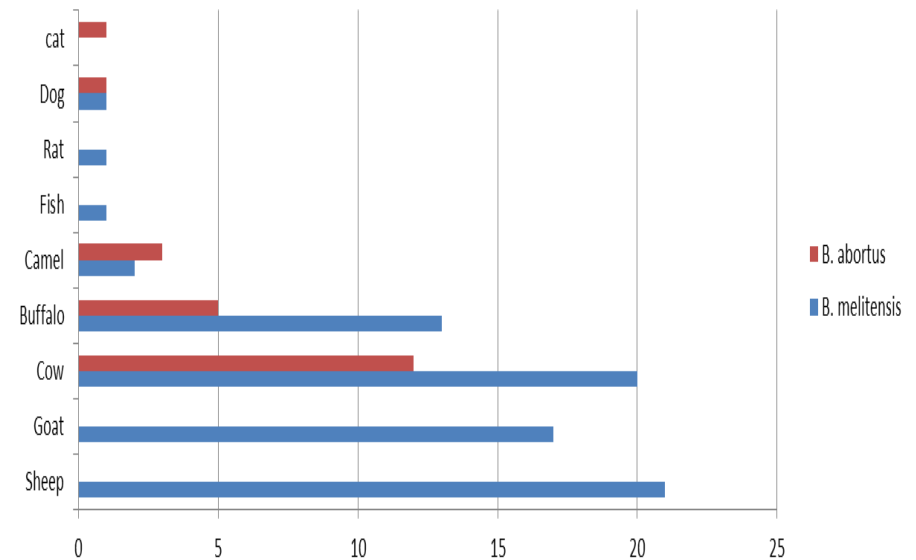
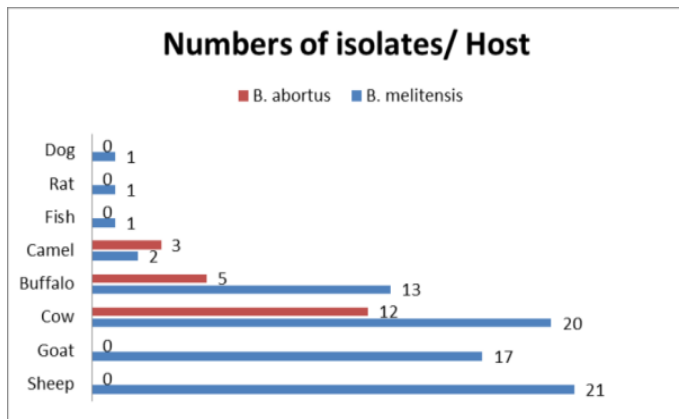
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Scenario 4: Vector

introduction of the agent via vectors such as rats and stray cats and dogs



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Conclusion

- ❖ *B. abortus* bv1 was the source of this outbreak but not the RB51 vaccine applied.
- ❖ RB51 vaccine might reduce the number of abortions caused by field strains but offers no complete protection from infection.
- ❖ The most likely source of infection appears to be the uncontrolled introduction of the agent via vectors or latent carrier.
- ❖ Eradication of brucellosis is a multidirectional process required combination between ordinary control program and high biosecurity level in the farms.



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Special thanks for AGr 130 members



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أشكركم على الاهتمام

Thank you for attention,
Gracias por su atención,
Vielen Dank für die Aufmerksamkeit,
Merci de votre attention,

