



World Organisation
for Animal Health
Founded in 1924

中华人民共和国农业农村部
Ministry of Agriculture and Rural Affairs of the People's Republic of China

Diagnosis and Control Practices of Brucellosis in China

Shufang SUN

China Animal Health and Epidemiology Center

Division of Zoonosis Surveillance

National Animal Brucellosis Para-Reference Laboratory

sunshufang@cahec.cn



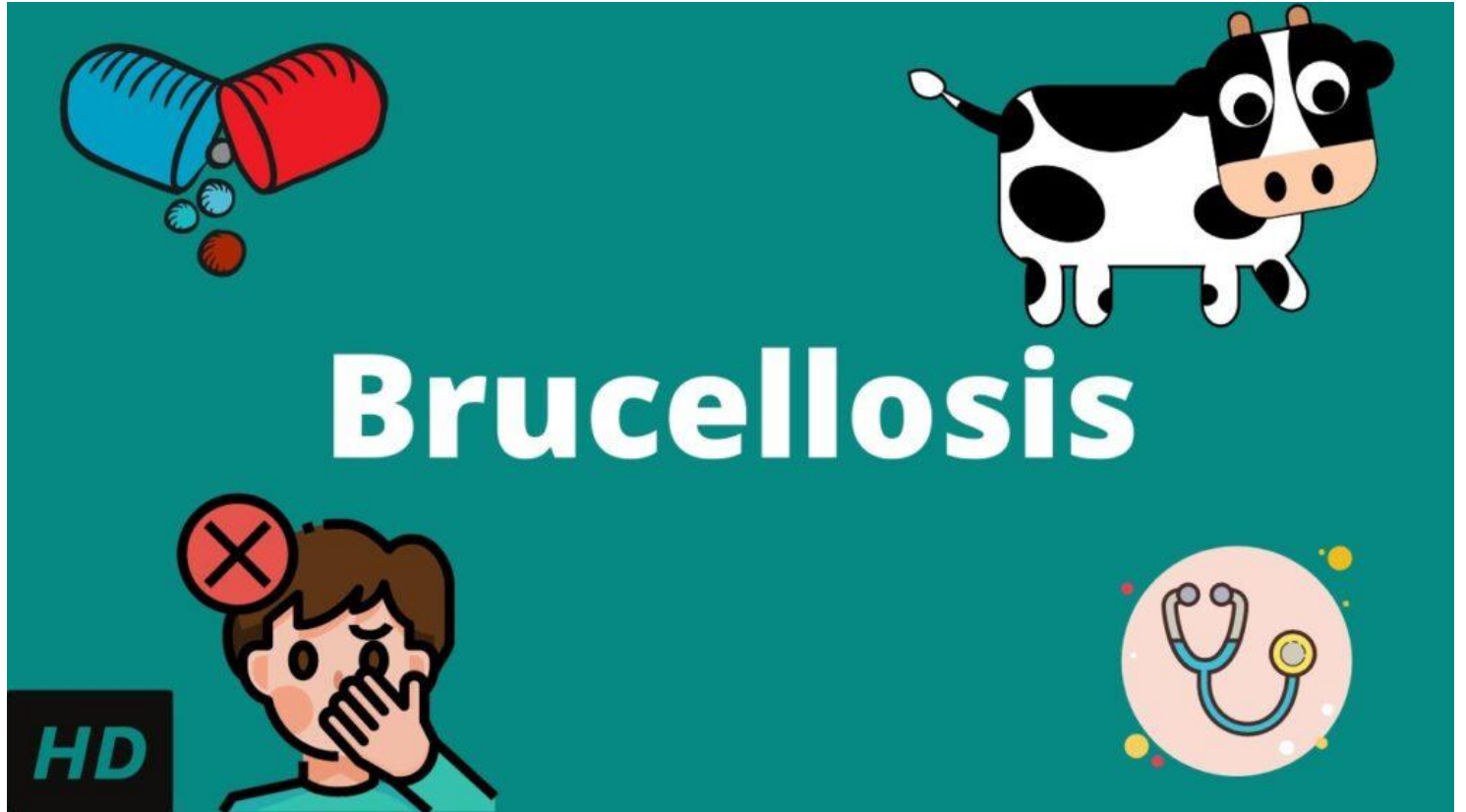


1. Prevalence

2. development of

Diagnostic techniques

3. Policies and Challenges





1

The Prevalence of Brucellosis

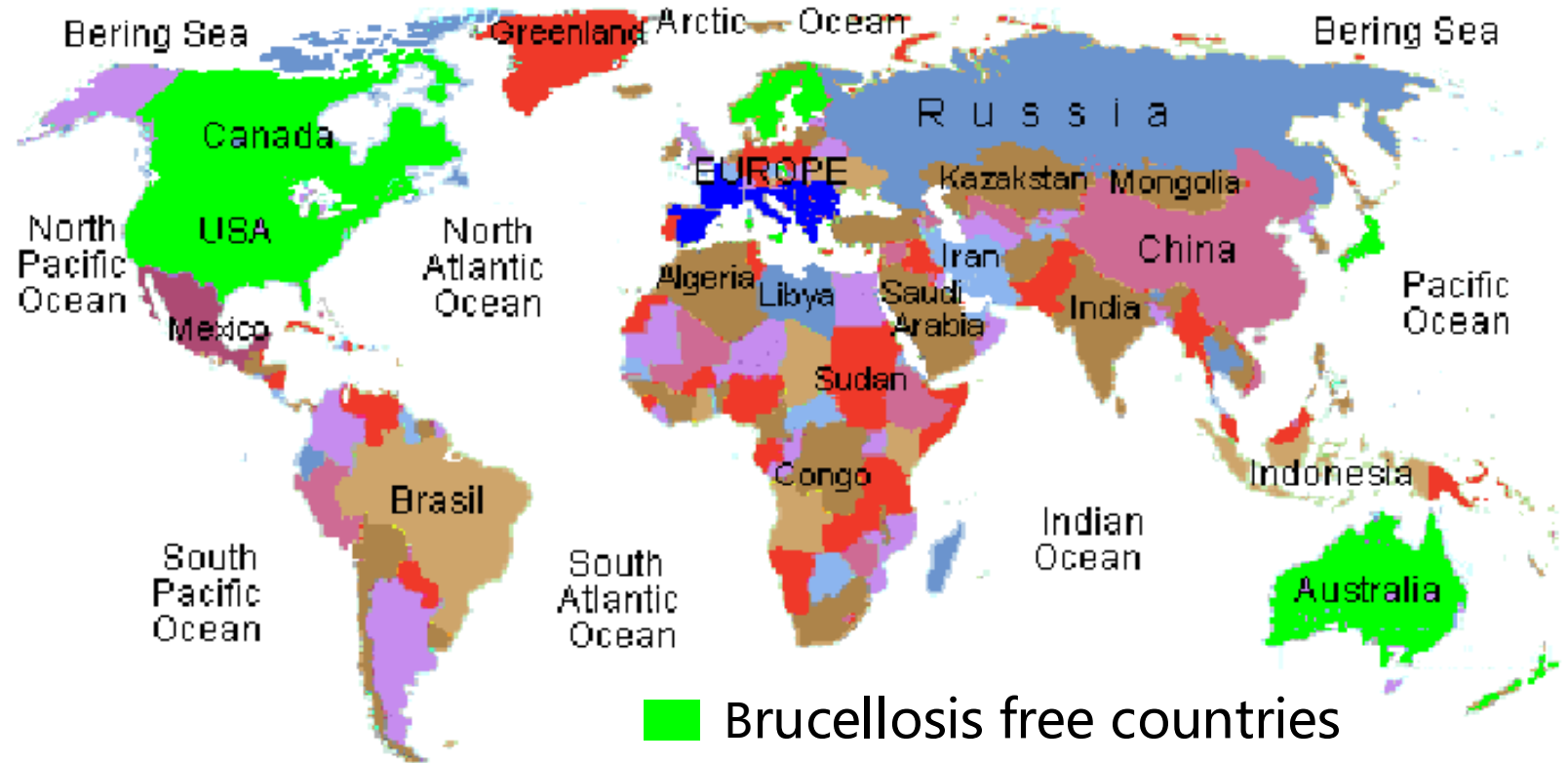


1.1 Global situation

4

Prevalence in 170 countries and regions, mainly in Africa, the Middle East, South America, and Asia

- bovine: 101
- small ruminant: 50
- swine: 33



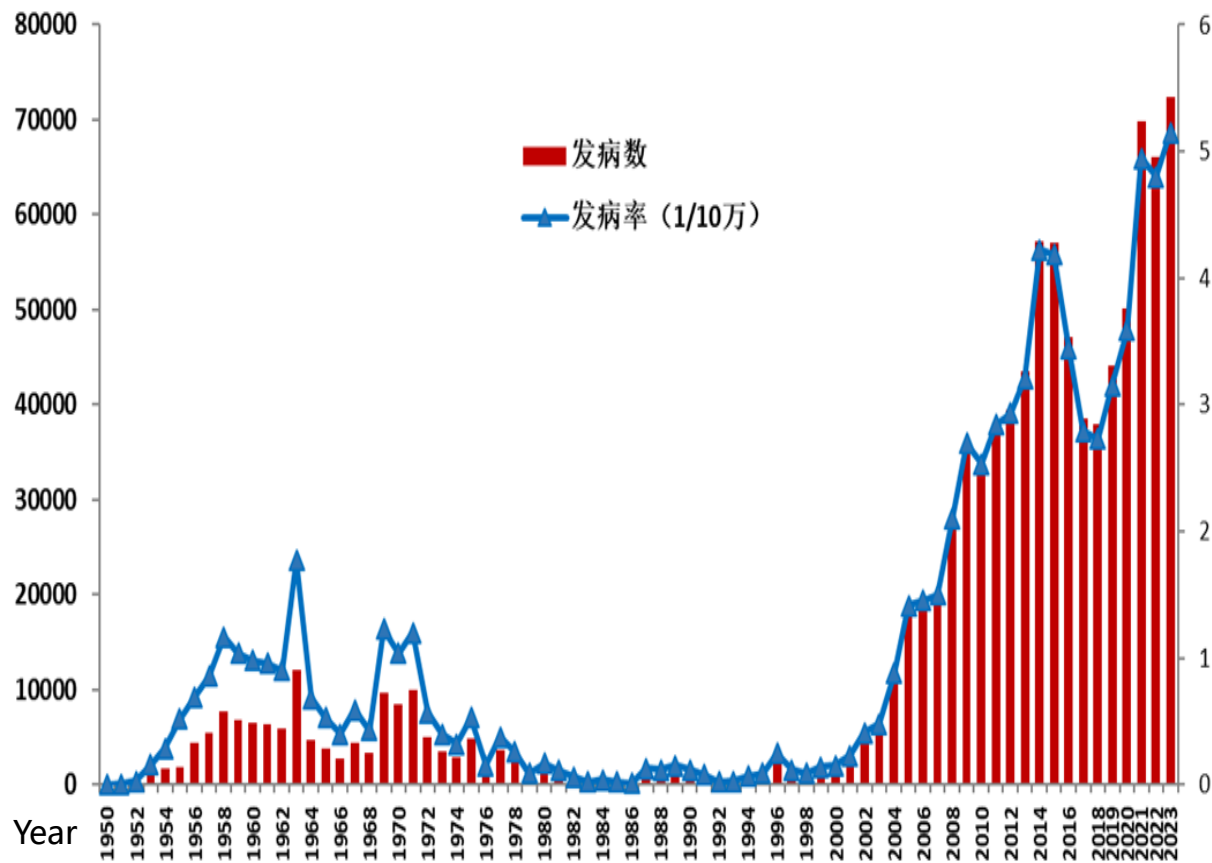


1.2 Human brucellosis in China

5

Incident number (case)

Morbidity (1/lakh)



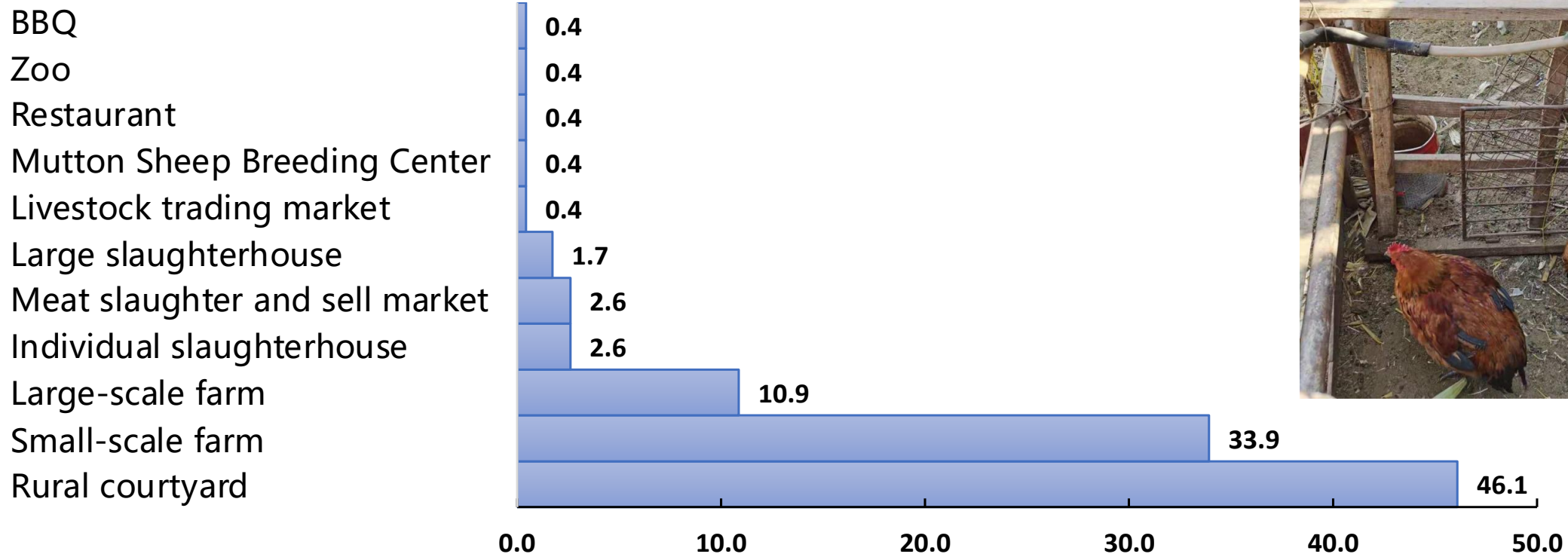
2023: 72344 cases



1.3 The occupational population affected by brucellosis

6

- Human brucellosis cases from rural household (46.1%)
- Small-scale animal breeding farm in rural (33.9%)

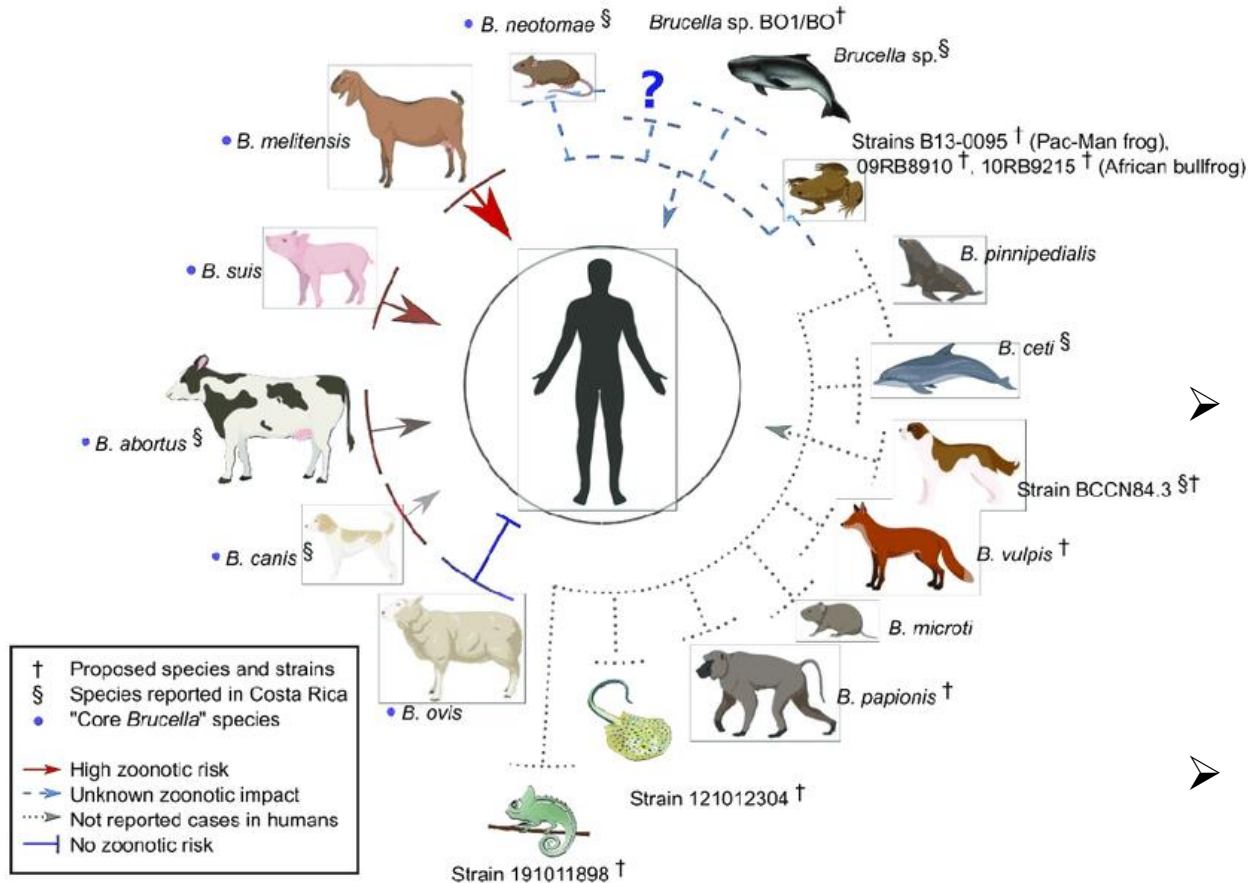


Data source: China CDC



1.4 The species and biotypes of *Brucella* in China

7



➤ *B. abortus*

- biovar 1, a small portion
- biovar 3, 90%
- biovar 4, occasionally found in yak, Tibet
- biovar 9, occasionally found in Inner Mongolia

➤ *B. melitensis*

- biovar 1, 10%
- biovar 2, 8%
- biovar 3, 82%

➤ *B. canis*

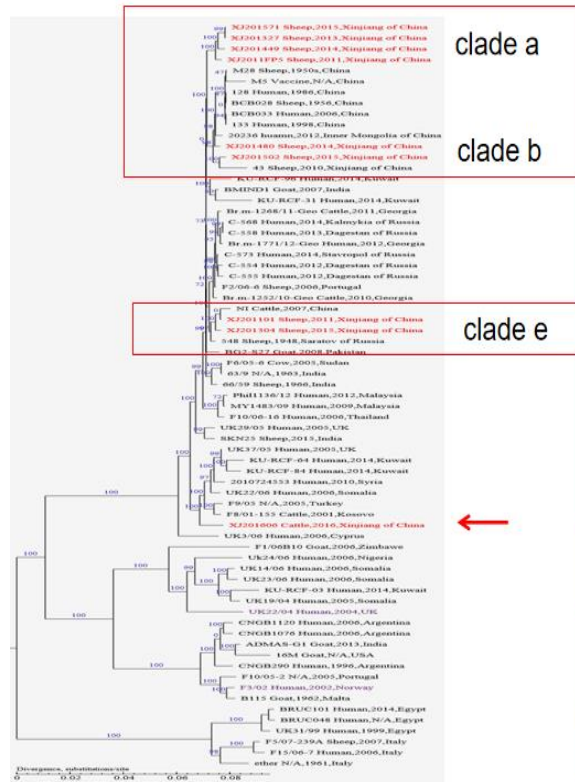
➤ *B. suis*



1.5 The phylogenetic analysis of *B. melitensis* strains

8

At the genomic level, *B. melitensis* strains isolated in China are closely related to those in Kazakhstan, Turkey, and Syria



Group I

亚洲和东地中海国家

Asia and Eastern Mediterranean countries

Group II 非洲

Africa

Group III 美洲和西地中海

Americas and the Western Mediterranean

Group IV 意大利和埃及

Italy and Egypt



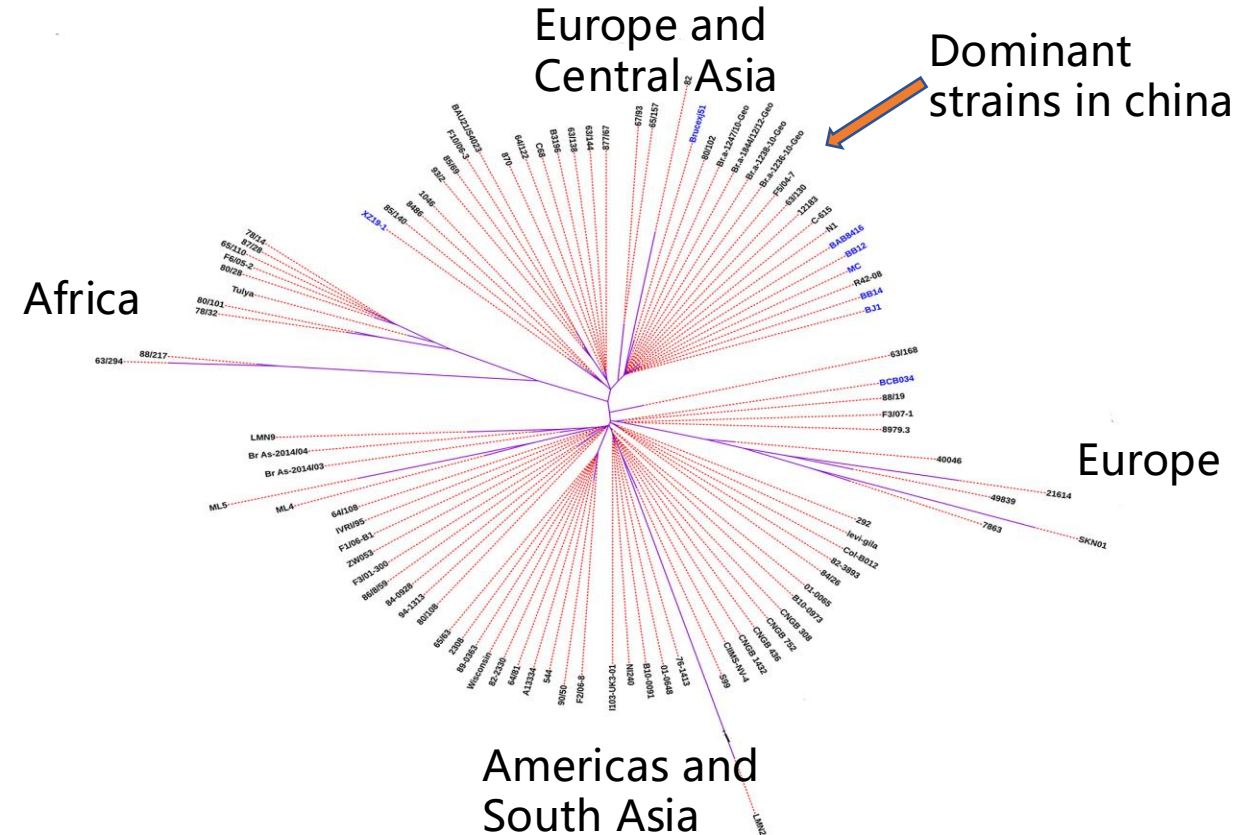
Mingjun Sun, et al. Frontiers in Veterinary Science, 2017



1.6 The phylogenetic analysis of *B. abortus* strains

9

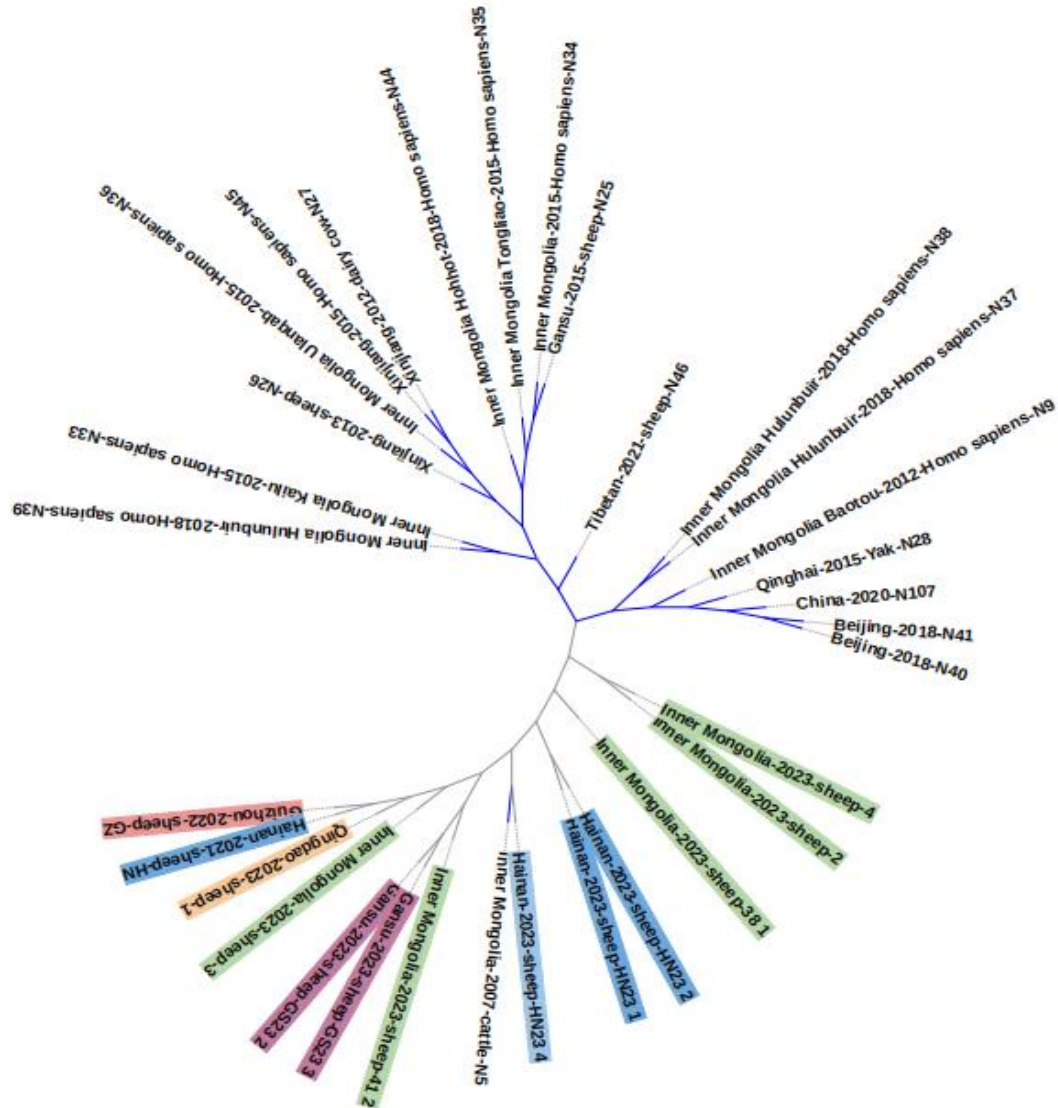
- Similarly, the *B. abortus* isolates in China are also closely related to strains found in European and East Asian countries
- But they have a distinct distance to strains found in Africa and the Americas





1.6 The phylogenetic analysis of *B. abortus* strains

10



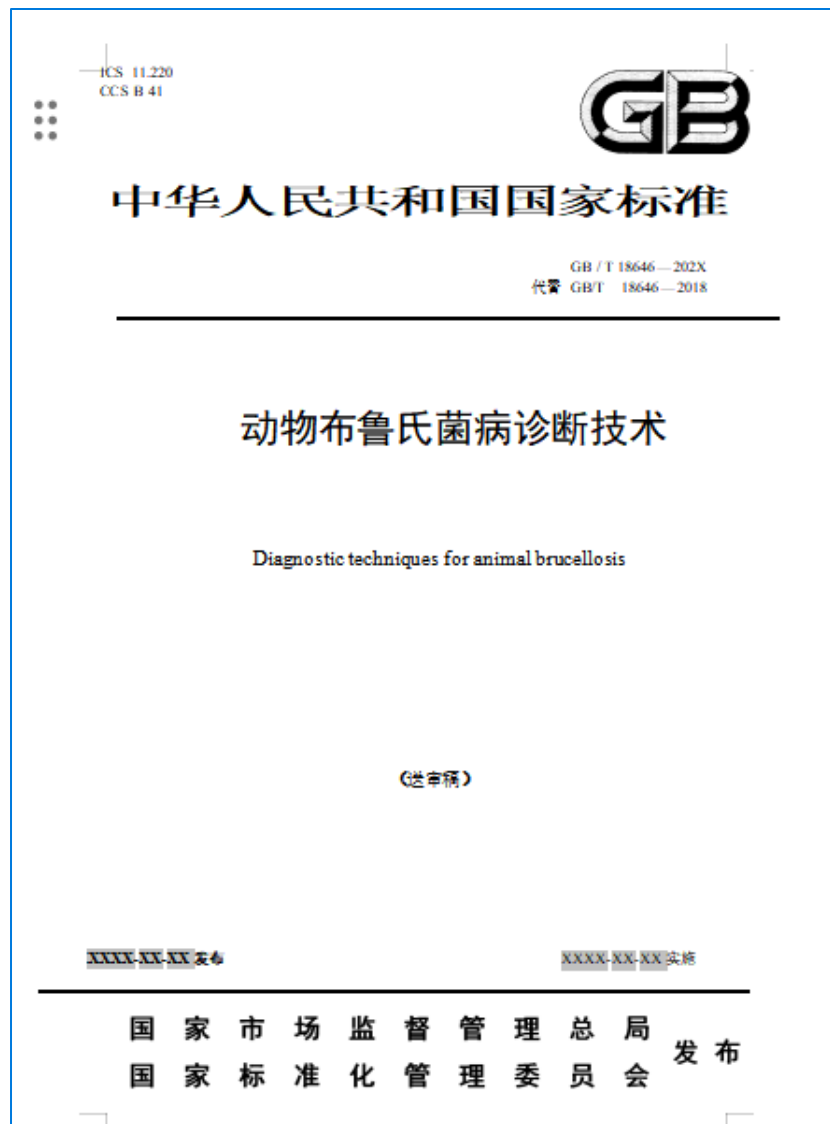
- The *Brucella* strains isolated in recent years are clustered on a distinct branch in Evolutionary tree, different from those isolated around 2010
- The *Brucella* strains in China are constantly evolving

2 Development of Diagnostic Techniques



National standard for animal brucellosis diagnostics

12



B. DIAGNOSTIC TECHNIQUES

Table 1. Test methods available for the diagnosis of infection with *Brucella abortus*, *melitensis* or *suis*

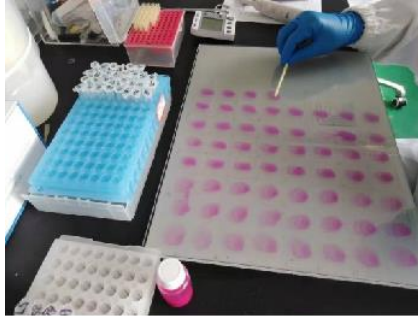
Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection ^(a)	Contribute to eradication policies ^(b)	Confirmation of suspect or clinical cases ^(c)	Herd/flock prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection of the agent						
Staining methods	–	–	–	+	–	–
Culture	–	–	–	+++	–	–
PCR ^(d)	–	–	–	+ / ++	–	–
Detection of immune response						
BBAT (RBT or BPAT)	+++	++	+++	+	+++	–
FPA	++	++	+	++	++	–
CFT	++	++	+++	++	+++	–
I-ELISA	+++	++	+++	++	+++	–
C-ELISA	++	+	+	+	++	–
BST	++	–	+	+++	++	–
SAT	++	+	+	–	+	–
NH and cytosol protein-based tests ^(e)	–	–	+	++	–	–
Bulk milk tests ^(f) Milk I-ELISA or Milk ring-test	+++	–	+++	+	+++	–

Manual of Diagnostic Tests and Vaccines for Terrestrial Animals
(WOAH) 3.01.04 BRUCELLOSIS



2.1 Serological tests

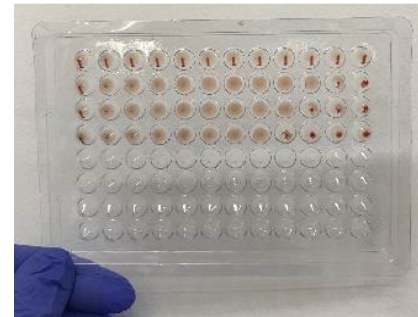
13



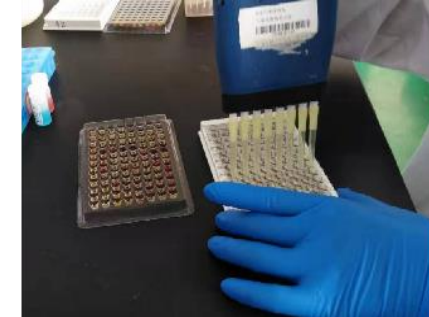
Rose bengal test
(RBT)



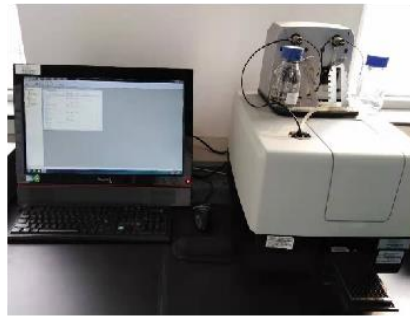
Serum agglutination
test (SAT)



Complement
fixation test (CFT)



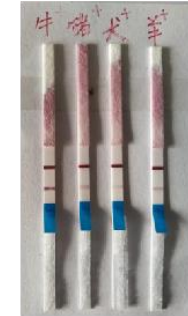
Indirect/competitive
enzyme-linked immunosorbent
assay (I- or C-ELISA)



Fluorescence
polarisation assay (FPA)



Milk ring test
(MRT)

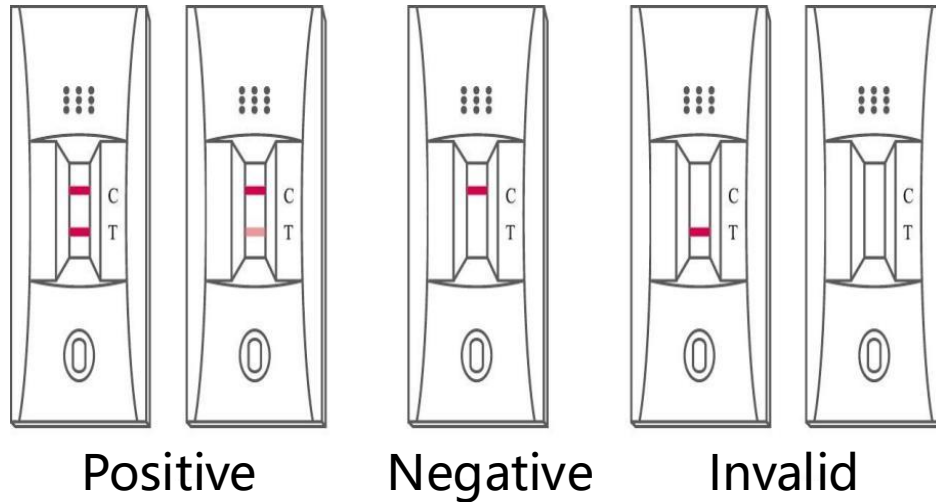


Immunochromatographic
assay (ICA)



2.1 Immunochromatographic test card (ICA)

14



Marker: colloidal gold, color gel, fluorescent microspheres, quantum dot

0.5-8 IU /mL VS RBT 20 IU /mL



Compared with the ID-VET ELISA kit:

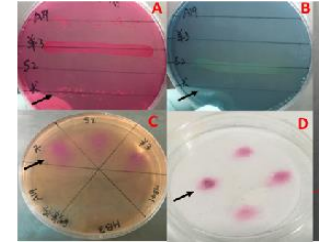
- **Total coincidence rate 96.7% (87/90)**
- **Positive sample coincidence rate 96.7% (58/60)**
- **Negative sample coincidence rate 96.7% (29/30)**



2.2 Bacteriological tests

15

- Ziehl-Neelsen staining
- Bacteria isolation and identification
- Real time PCR
- Recombinase aided amplification (RAA)





2.2.1 Real time PCR-Primers and Taqman probes

16

Target	Forward primer	Reverse primer	Probes
<i>Brucella</i> genus	CGCTCGCGCGGTGGAT	CTTGAAGCTTGCGGACAGTCA CC	ACGACCAAGCTGCATGCTGTT GTCGATG
<i>Brucella abortus</i>	CAGTTCTCGAACAAGCTGACG	CTATAATCATTGGCCGCCGAA AG	CAGCGTGCCAGAAACCCGACA CAGC
<i>Brucella melitensis</i>	AGCGAGATTGGAATAGCTTAC CC	CTGGTTACGTTGAATGCAGAC AC	CGCCCTGCCACCAGCCAATAA CGG
<i>B.canis</i> and <i>B.suis</i>	CCTGCAAAAAGCAGGAACCA	CCTCCGCCAGTCGTGAAA	ATATGGCCGGCTATCCGCGTTC G
A19 (S19) vaccine and wild type strains	GCGGCTTTTCTATCACGGTATT C	CATGCGCTATGATCTGGTTACG	ACACGCCCTAGAACGCCTTTC GGA
	CGGGATTCAAACGTCAAA	GGCTTTTCTATCACGGTATT	TCAATCCACTAGAACGCC#
	TCGATGGCGATGCGGA	GCTGGTCGCCATCGATGA	CGTGTGCGTCTGG#
	TCGATGGCGATGCGGA	GCTGGTCGCCATCGATGA	CGTGTGCTTCTGG#
#MGB Probe, 3' MGB, others Taqman probe, 3' BHQ1			

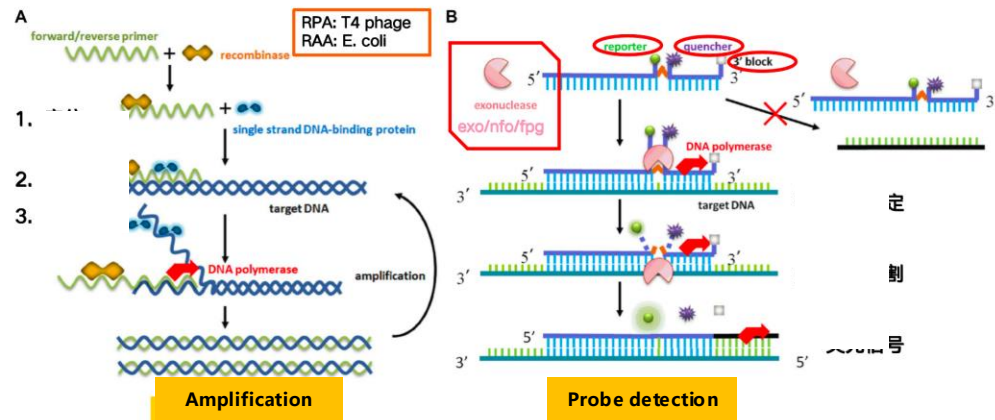
Based on the animal's vaccination background, appropriate real time PCR methods can be used to distinguish vaccine strains from wild-type strains



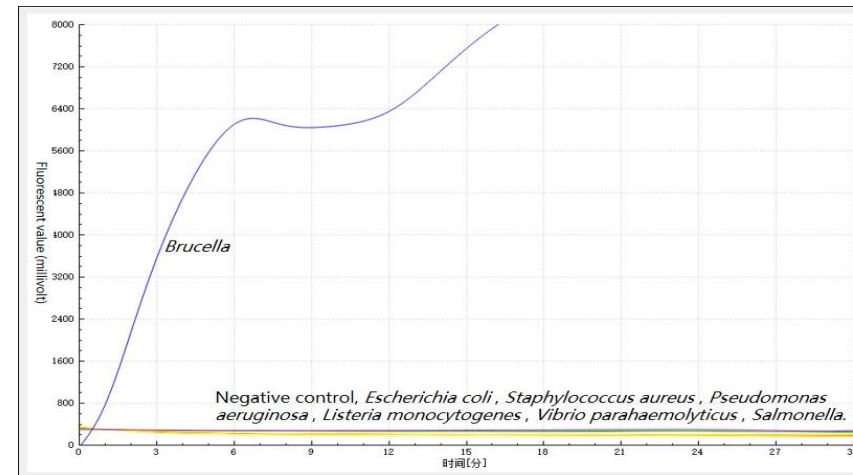
2.2.2 RAA

17

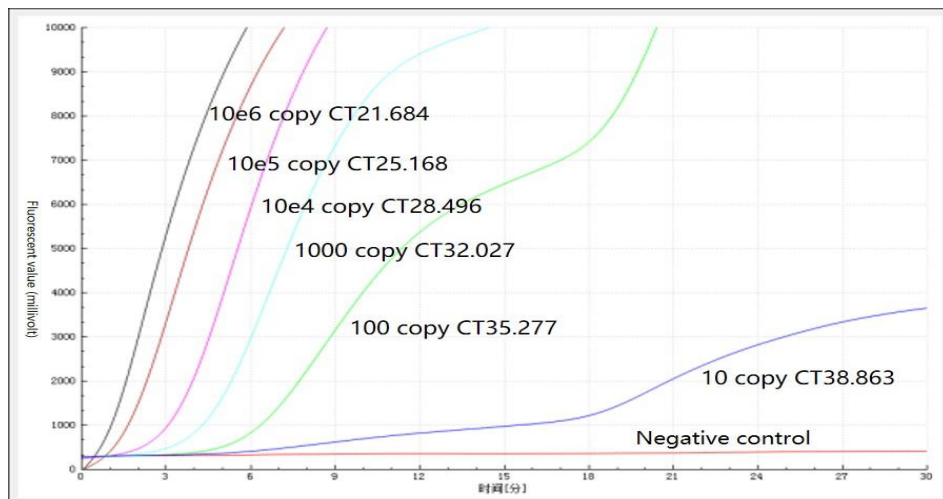
□ Principle



□ specific



□ sensitive: comparable to qPCR



□ practical: fast test on site

- ✓ short detection time
(35 ' extraction+20 ' amplification = 55 min)
- ✓ Easy to operate
- ✓ Suitable in resource-limited settings





2.2.2 RAA

- RAA, suitable for rapid on-site detection, has become a routine detection method and is easy to popularize

RAA primers and probe

BRU-F1	TCAATGCGATCAAGTCGGGCGCTCTGGAGTC
BRU-R1	TCCTTACGCGCAACGATATGGATCGTTTCCG
BRU-P1	CTTTATGATGGCAAGGGCAAGGTGGAAGA (i6FAMdT) (idSp) (iBHQ1dT) GCGCCTTCTGGCGAC-3'C3spacer

Clinical sample test results					
Order number	Sample name	Fluorescent PCR results		Fluorescent RAA results	
		CT	Results	TT	Results
1	17#-1A	--	Negative	--	Negative
2	17#-1B	--	Negative	--	Negative
3	17#-1C	--	Negative	--	Negative
4	17#-1D	35.61	Positive	2.67	Positive
5	17#-1E	--	Negative	--	Negative
6	17#-1F	--	Negative	--	Negative
7	17#-1G	--	Negative	--	Negative
8	17#-1H	--	Negative	--	Negative
9	17#-2A	33.44	Positive	2.33	Positive
10	17#-2B	--	Negative	--	Negative
11	17#-2C	--	Negative	--	Negative
12	17#-2D	--	Negative	--	Negative
13	17#-2E	--	Negative	--	Negative
14	17#-2F	--	Negative	--	Negative
15	11#-4C	--	Negative	--	Negative
16	11#-4D	--	Negative	--	Negative
17	11#-4E	--	Negative	--	Negative
18	P34	24.12	Positive	0.33	Positive
19	P35	-	Negative	-	Negative
20	11#-4F	--	Negative	--	Negative
21	11#-4G	--	Negative	--	Negative
22	11#-4H	--	Negative	--	Negative
23	P33	29.82	Positive	0.33	Positive
24	P36	30.64	Positive	2	Positive
25	P37	-	Negative	-	Negative
26	P38	37.37	Positive	2.67	Positive
27	P39	-	Negative	-	Negative
28	P40	34.88	Positive	0.33	Positive
29	P57	19.6	Positive	0	Positive
30	P58	21.9	Positive	0	Positive

3

Control Policies and Challenges



3.1 National plan for animal brucellosis control

20

Ministry of Agriculture and Rural Affairs (MARA)

- Brucellosis Prevention and Control Five-Year Action Plan for Livestock (2022-2026), issued in March 2022
- Five-year Action Plan for the Prevention and Control of Zoonotic Diseases in Livestock (2022-2030), issued in September 2022

农业农村部文件

农牧发〔2022〕31号

农业农村部关于印发《全国畜间人兽共患病防治规划（2022—2030年）》的通知

各省、自治区、直辖市及计划单列市农业农村（农牧）、畜牧兽医厅（局、委），新疆生产建设兵团农业农村局，部有关直属事业单位：

为贯彻落实2022年中央一号文件要求，做好人兽共患病源头防控，保障畜牧业生产安全、公共卫生安全和国家生物安全，按照《中华人民共和国动物防疫法》等有关法律法规规定，我部制定了《全国畜间人兽共患病防治规划（2022—2030年）》。现印发你们，请结合本地实际，认真组织实施。

农业农村部

2022年9月14日

— 1 —

农业农村部文件

农牧发〔2022〕13号

农业农村部关于印发《畜间布鲁氏菌病防控五年行动方案（2022—2026年）》的通知

各省、自治区、直辖市及计划单列市农业农村（农牧）、畜牧兽医厅（局、委），新疆生产建设兵团农业农村局，部属有关事业单位：

为贯彻落实2022年中央一号文件有关要求，强化畜间布鲁氏菌病防控，保障畜牧业生产安全、公共卫生安全和生物安全，我部制定了《畜间布鲁氏菌病防控五年行动方案（2022—2026年）》。现印发你们，请结合本地实际，认真组织实施。

农业农村部

2022年3月28日

— 1 —



3.2 Five-year Action Plan

- Implementing classified policies
- Promote prevention and control in contiguous areas at the county level
- The immunity zone mainly focuses on implementing vaccination
- Non-immune area primarily focus on test-cull till eradication

Each county should adopt at least one of these control strategies

—Control at the source, emphasizing key points

- Pay special attention to the prevention and control of brucellosis in breeding cattle, sheep, dairy cows, dairy goats, and meat sheep
- Efforts should be made in susceptible animals such as cattle, pigs, deer, camels, and dogs, in order to effectively reduce the prevalence



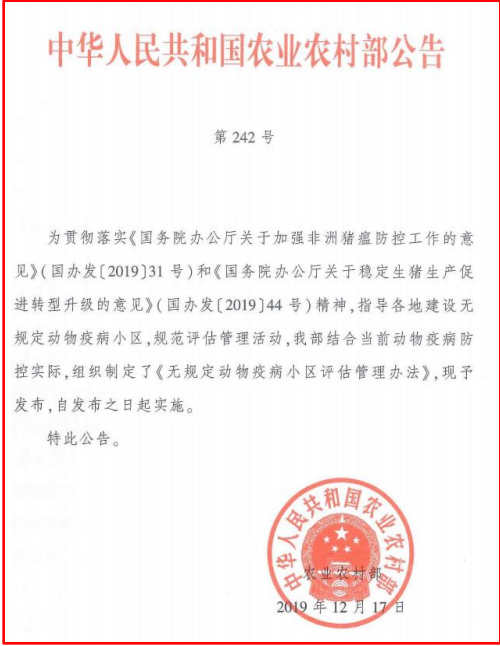
3.2 Five-year Action Plan

- Blue: northern, immunization area
- Yellow: southern, non-immunization area

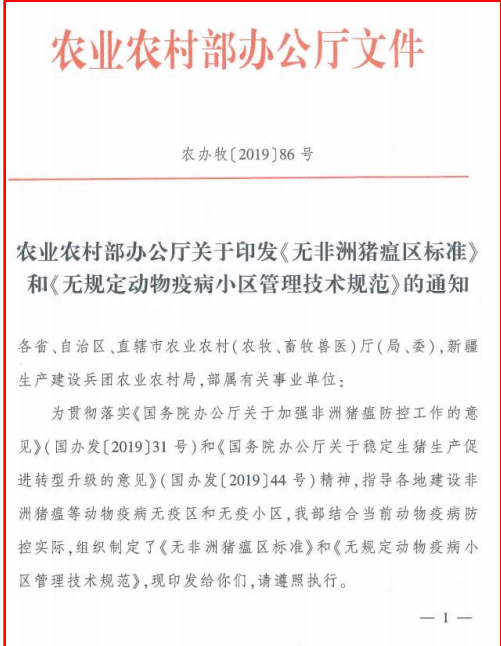




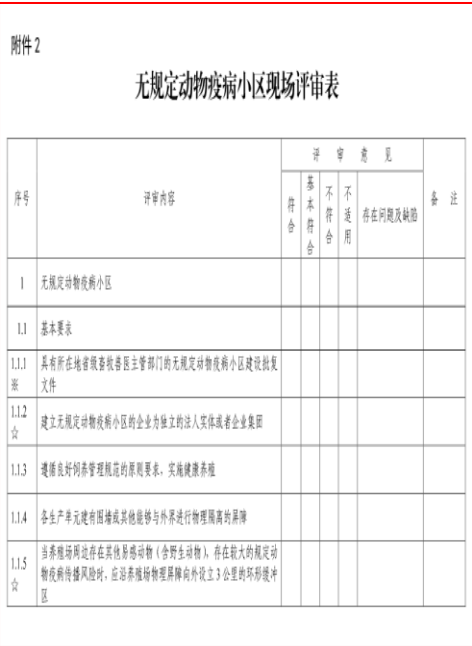
《The Animal Epidemic Prevention Law of P.R.China》



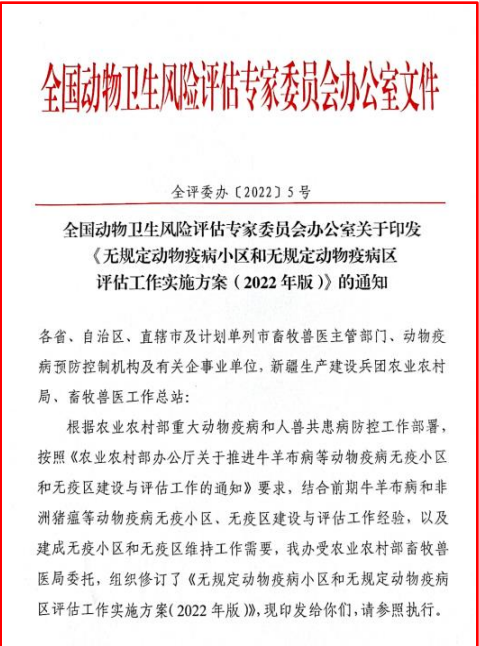
《Regulation on evaluation and management for brucellosis-free zone and sub-zone》



《Technical specification for management of brucellosis-free zone and sub-zone》



《On-site review form for brucellosis-free zone and sub-zone》



《Implementation plan for evaluation of brucellosis-free zone and sub-zone》



3.3.1 Cattle/small ruminant brucellosis-free sub-zone

24

Type	Rules
Brucellosis-free sub-zone with immunization	Use vaccines authorized by government, immunize cattle within 6 months of age and sheep within 3 months of age, and do not immunize adult cattle/sheep
	New-born cattle/sheep should be given identification during immunization
	Do not import immunized cattle/sheep from other region
	Perform two serological tests on all sexually mature cattle/sheep, with negative results (Sheep after 6 months old, cattle after 12 months old)
Brucellosis-free sub-zone with none-immunization	No vaccination in the past 36 months



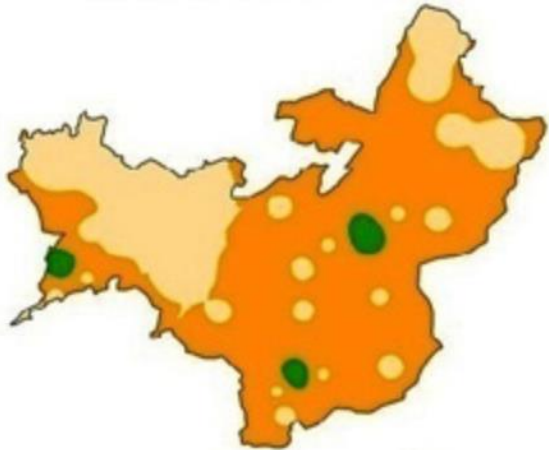
3.3.2 Progress of disease-free zone and sub-zone

25



Under construction or planned brucellosis-free area

Inner Mongolia Autonomous Region-Bayannaoer, Alshan, Alxa
Shaanxi-Shangluo, Hubei-Yichang, Chongqing-Zhongxian,
Zhejiang-Huzhou, Shandong-Weihai, Gansu-Minqin



The brucellosis-free sub-zones have been established (46 farms in 16 provinces)

Shandong, Inner Mongolia, Zhejiang, Chongqing, etc.



3.4 Challenges

- Implementation of eradication plans require several decades, continuous financial support
- There is need to improve frontline worker teams, including personnel for epidemic prevention, sampling, quarantine, laboratory testing
- There is need for cooperation between livestock breeders and businesses
- The movement control of test-positive herds is necessary but still difficult





3.5 Explore the risk classification of test-positive animals

27



- Differentiate animals between sera test-positive and pathogen test-positive
- Explore classified slaughter strategy for healthy, sera test-positive, pathogen test-positive and sick animals based on risk assessment
- Coordinate slaughter testing, labeling traceability, transport supervision, personnel protection, and other measures to reduce the risk of transmission



World Organisation
for Animal Health
Founded in 1924

Thank you!

China Animal Health and Epidemiology Center

369 Nanjing Road, Shibei District, Qingdao
Shandong Province
China

fanxiaoxu@cahec.cn

