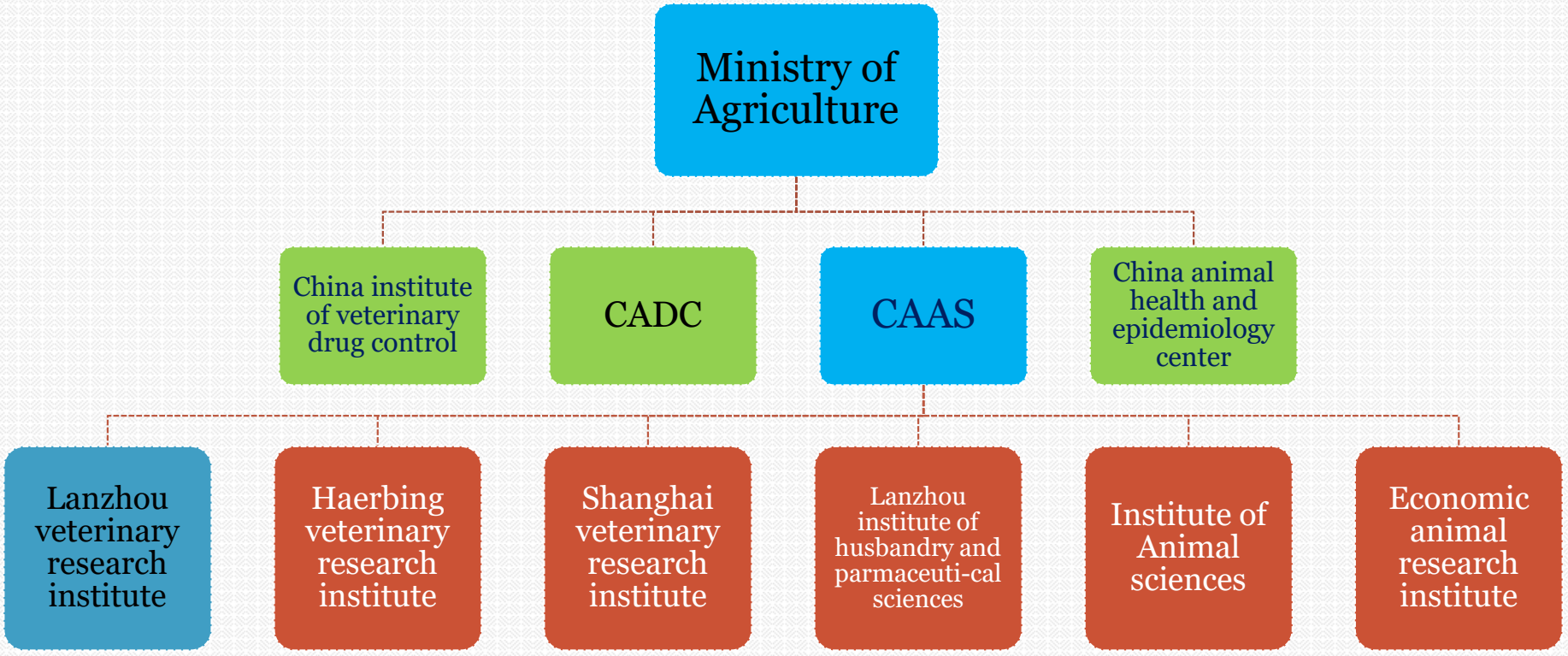
Activities **on tick borne diseases in China**

Zhijie Liu

Contents

- Brief introduction of the institute
- Activities on Piroplasm and *Anaplasma*
- Current situation of African swine fever

National Veterinary Research Institutes



Lanzhou Veterinary Research Institute

Total staff: 269

Prof: 41 (Ph D supervisor: 24)

Associate prof: 64

Centers of Excellence in Animal Diseases

National Designations

- State Key Laboratory of Veterinary Etiological Biology
- National Foot and Mouth Diseases Reference Laboratory

Ministry of Agriculture Designations

- Key Laboratory of Animal Virology
- Key Laboratory of Eipzootic Diseases
- Key Laboratory of Zoonosis

Gansu Province Designations

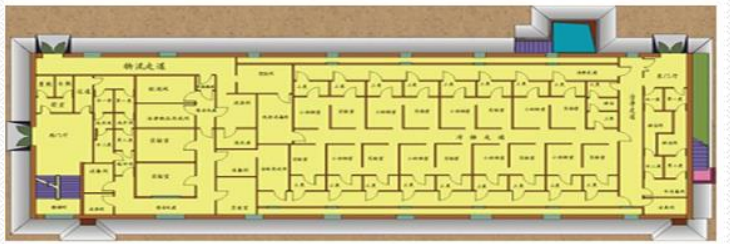
- Key Laboratory of Veterinary Parasitology
- Gansu Biological Detection Research Centre

International Reference Laboratory Designations

- OIE Foot and Mouth Diseases Reference Laboratory
- OIE Ovine Theileriosis Reference Laboratory

Facilities of National Capacity:
A national high-containment animal health center

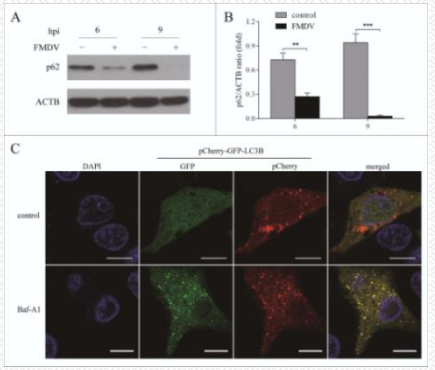
P3 bio-safety animal experimental facility



ABSL-3 Laboratory

Our Impact – What We Deliver

- **Research**
  - Epidemiology
  - Pathogenesis and immunity
  - Novel vaccine and diagnostic tools
- **Diagnostic Service**
  - Rapid response service (FMD)
  - Pathogen characterisation and emerging diseases
  - Clinical investigation on site and surveillance
- **Technical Advice and Training**
  - Private practice/government veterinarians
  - Laboratory capacity building
  - International partners: FAO, OIE



Institutions working with Piroplasm or *Anaplasma*

- Lanzhou Veterinary Research Institute, CAAS
- Huazhong Agricultural University
- Xinjiang Agricultural University
- Henan Agricultural University
- Northwest University
- Beijing Institute of Microbiology and Epidemiology
- National Institute for Communicable Disease Control and Prevention

Ectoparasites and Vector-Borne Diseases Team

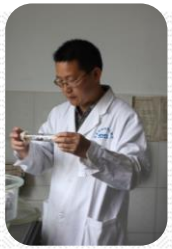
- **Main focus:**
  - *Theileria*, *Babesia*
  - *Anaplasma*, lyme disease
  - Ticks
  - Bluetongue, BEF, BVD, Africa swine fever
- **Piroplasm collection and identification:**
  - More than 100 species, strains or isolates of piroplasm
  - Newly designated 3 Theilerias, *T. sinensis*, *T. luwenshuni*, *T. uilenbergi*
  - Newly found but undesignated 2 Babesias, one from sheep and another from cattle
- **Main studies:**
  - Genomics of *Babesia*, *Theileria*, *Anaplasm*
  - Attenuated vaccine for *T. annulata*
  - Biology and biocontrol of ticks
  - Passive surveillance of African swine fever

Prof. Dr. Hong Yin
Email: yinhong@caas.cn

Piroplasm collection

Working groups

- **Piroplasm working group,**
  Leading by Prof. Dr. Jianxun Luo
  Mainly focus on *Babesia* and *Theileria*



- **Tick working group,**
  Leading by Prof. Dr. Guangyuan Liu
  Mainly focus on ticks

- **Tick- borne bacteria working group,**
  Leading by Dr. Zhijie Liu
  Mainly focus on *Anaplasm*, *Borrelia* and ASF

- **Vector-borne virus working group,**
  Leading by Dr. Junzheng Du
  Mainly focus on BTV, BEFV, BVDV and Japanese B encephalitis

Known *Babesia* species in China

	***Babesia* species**	**Host**	**Tick vector**
Isolated (10)	*B. bovis*	Cattle, Buffalo	*R. (Boophilus) microplus*
	B. bigemina	Cattle, Buffalo	*R. (Boophilus) microplus*
	B. major	Cattle	*H. punctate*
	B. ovata	Cattle	*H. longicornis, H. punctate*
	B. orientalis	Buffalo	*R. sanguineus*
	B. sp. Kashi	Cattle	*Hy. anatolicum, Hy. asiaticum, Hy. rufipes*
	B. motasi	Sheep, Goat	*H. longicornis, H. qinghaiensis*
	B. sp. Xinjiang	Sheep, Goat	*Hy. anatolicum*
	B. caballi	Equine	*D. nuttalli, D. silvarum, D. sinicus, D. niveus*
	B. gibsoni	Dog	*H. longicornis, R. sanguineus, R. haemaphysaloides*
Detected (6)	*B. divergens*	Bovine, Ovine	Not studied
	B. ovis	Sheep, Goat	Not studied
	B. trautmanni	Pig	Not studied
	B. perroncitoi	Pig	Not studied
	B. hongkongensis	Cat	Not studied
	B. canis	Dog	Not studied

Known *Theileria* species in China

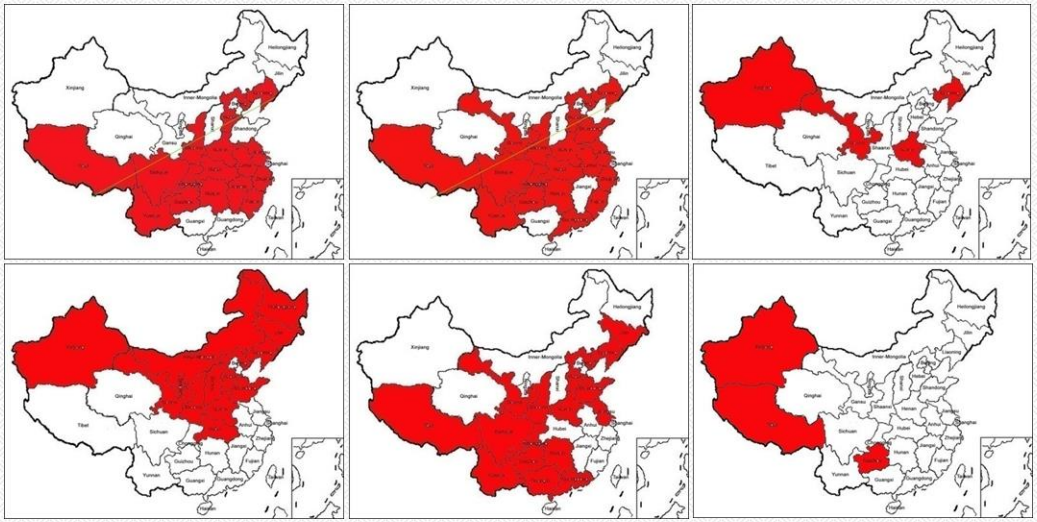
Theileria	**Host**	**Tick vector**
T. annulata	Cattle	*Hy. anatolicum, Hy. rufipes, Hy. dromedarii*
T. orientalis	Cattle	*H. longicornis*
T. sinensis	Cattle	*H. longicornis, H. japonica*
T. ovis	Sheep, Goat	*Hy. asiaticum*
T. uilenbergi	Sheep, Goat	*H. longicornis, H. qinghaiensis*
T. luwenshuni	Sheep, Goat	*H. longicornis, H. qinghaiensis*
T. equi	Equine	*D. nuttalli, D. silvarum, D. niveus, R. sanguineus*

Diagnostic and detection methods available in laboratory

Method	Targeting pathogen
Blood smear examination	All of the *Babesia* and *Theileria* sepcies
Lymph node centesis	*Theileria*
Inoculation to animals	All of the *Babesia* and *Theileria* sepcies
In vitro cultivation	*Babesia* and *T. annulata*
ELISA	*T. annulata, T. luwenshuni, T. uilenbergi; B. bovis, B. bigemina, B. motasi, B. orientalis, B.* sp. Xinjiang;
PCR	All of the *Babesia* and *Theileria* sepcies
LAMP	*B. motasi, B.* sp. Xinjiang, *B. bovis, B. bigemina, T. annulata, T. orientalis, T. luwenshuni, T. uilenbergi*
Reverse line blot	All of the *Babesia* and *Theileria* sepcies for ruminants

The first large-scale survey of piroplasm

Method: Examination of blood smears + literature review
Period: 1991.4-1993.10
Range: 26 provinces, 3184 cattle herds
Target: Bovine piroplasm
Achievements: Identified 6 species, *B. bigemina* in 17 provinces, *B. bovis* in 15 provinces, *B. major* +*B. ovata* in 4 provinces, *T. annulata* in 13 provinces, *T. orientalis* in 15 provinces and *T. mutant* in 3 provinces



The second large-scale survey of piroplasm

Method：ELISA

Period：2005 - 2014

Range：17 - 22 provinces, more than 2000 herds for each pathogen

Target：*B. bovis*, *B. bigemina*, *T. annulata*, *T. senensis*, *T. sergenti*, *B. motasi*, *B*. species Xinjiang, *T. luwenshuni* and *T. uilenbergi*

Achievements：Obtained the basic data about the situation of these pathogen infection in China.

Babesia bovis

Method: rRAP-1 ELISA

Period: 2010 - 2014

Range: 17 provinces

Sampling: 2364 herds

Results: Positive rates, 6.40 - 47.27%

Province	Prefecture (No. of positive sera/ No. of sera)	No. of positive	No. of sera	Positive ratio (%)
Gansu	Zhangye (4/74) Gannan (61/89) Qingyang (39/57)	104	220	47.27
Qinghai	Haibei	95	207	45.89
Xinjiang	Hetian (4/14) Yili (42/108)	46	122	37.70
Henan	Zhengzhou	26	73	35.62
Shanxi	Yuncheng	16	54	29.63
Yunnan	Xishuangbanna (10/40) Wenshan (3/32) Honghe (7/31) Dehong (16/32) Qujing (4/15)	40	150	26.67
Hebei	Baoding	8	32	25.00
Jilin	Jilin (5/29) Changchun (8/24)	13	53	24.53
Liaoning	Dalian (8/29) Benxi (9/29) Anshan (3/24)	20	82	24.39
Guangdong	Maoming (16/60) Qingyuan (1/25) Zhaoqing (8/25)	25	110	22.73
Tibet	Lasa	106	555	19.10
Inner Mongolia	Hulunbeier (7/46) Baotou (4/27) Xilinguoluomeng (9/35) Xinganmeng (7/39) Wulanchabu (6/19) Alashan (5/15) Bayanzhuoer (1/39)	39	220	17.73
Chongqing	Wanzhou (0/26) Jiangjin (10/31)	10	57	17.54
Guizhou	Anshun (1/24) Qiandongnan (6/29) Qianxinan (5/50) Qiannan (5/31) Tongren (3/31)	20	165	12.12
Hunan	Huaihua (4/28) Changde (4/28) Yongzhou (0/23)	8	79	10.13
Sichuan	Panzhihua (5/30) Luzhou (0/30)	5	60	8.33
Guangxi	Guilin (6/35) Baise (2/60) Chongzuo (0/30)	8	125	6.40
Total		589	2364	24.92

Babesia bigemina

Method: rRAP-1 ELISA

Period: 2010 - 2013

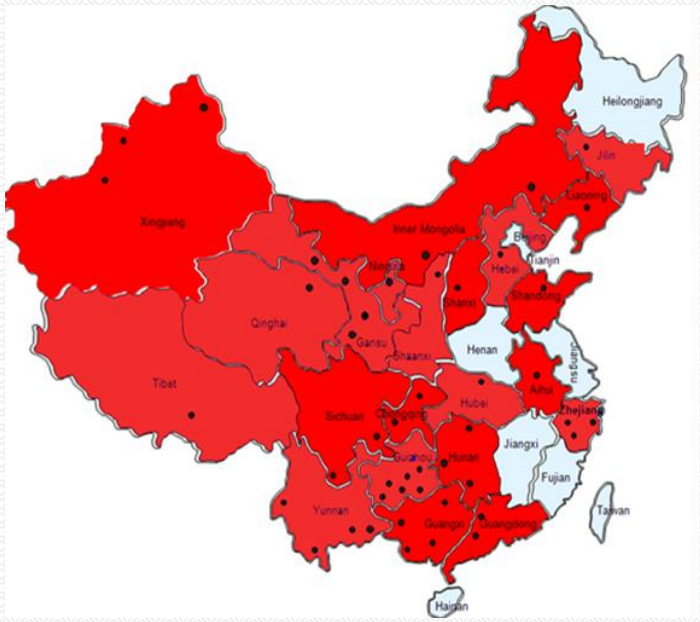
Range: 6 provinces

Sampling: 516 herds

Results: Average positive rates, 18.4%

Babesia motasi

Method: Crude antigen ELISA
Period: 2005 - 2010
Range: All over the country
Sampling: 3204 herds
Results: Average positive rates, 43.5%



Province	Prefecture (no. of positive sera/no. of sera)	No. of sera	No. of positive sera	Positive ratio (%)
Inner Mongolia	Chifeng	134	122	91.0
Guangdong	Qingyuan (30/86) Zhaoqing (31/38)	74	61	82.4
Hunan	Yongzhou (25/27) Huaihua (23/29) Changde (19/26)	82	67	81.7
Shandong	Dongying	91	70	76.9
Liaoning	Liaoyang	195	143	73.3
Chongqing	Jiangjin (20/30) Wanzhou (22/28)	58	42	72.4
Sichuan	Panzhihua (20/32) Luzhou (22/31)	63	42	66.7
Shanxi	Lvliang	50	31	62.0
Xinjiang	Aksu (38/90) Aksu (86/187) Ili (143/187)	464	267	57.5
Guangxi	Chongzuo (13/18) Baise (30/60) Nanning(9/31) Guilin (26/39)	148	78	52.7
Anhui	Hefei	144	74	51.4
Yunnan	Wenshan (9/29) Xishuangbanna (13/32) Qujing (12/35) Dehong (20/32) Honghe (16/32)	160	70	43.8
Gansu	Lanzhou (4/91) Wuwei (15/62) Gannan (97/224)	337	116	34.4
Qinghai	Haibei	96	33	34.4
Guizhou	Qiandongnan (9/29) Qiannan (11/30) Qianxinan (7/34) Anshun (15/32) Guiyang (11/32)	157	53	33.8
Ningxia	Wuzhong	80	22	27.5
Zhejiang	Jinhua (3/17) Taizhou (7/29) Lishui (3/19)	65	13	20.0
Hebei	Baoding	396	63	15.9
Tibet	Lhasa	113	13	11.5
Shaanxi	Yulin	74	7	9.5
Hubei	Suizhou	37	3	8.1
Jilin	Songyuan	186	3	1.6
Total		3,204	1,393	43.5

Babesia sp. Xinjiang

Method: Crude antigen ELISA

Period: 2005 - 2010

Range: 22 provinces

Sampling: 3857 herds

Results: Positive in 20 province, average positive rates, 31.7%

Bovine theileriosis

Method: rMPSP ELISA

Period: 2010 - 2013

Range: 17 provinces

Sampling: 2473 herds

Results: Average positive rates, 43.6%

Ovine theileriosis

Method: rTISP ELISA

Period: 2010 - 2013

Range: 21 provinces

Sampling: 2351 herds

Results: Average positive rates 41.0%

Investigation by DNA based tests

Method: PCR, Revers line blot, LAMP

Sampling: Less than 1000 for each test

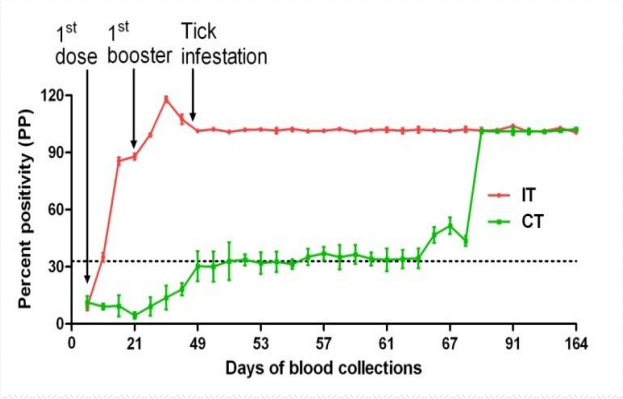
Results: Obtained regional epidemiological data, detected some new species such as *T. capreoli*, *Theileria* BO302-SE, *Theileria* sp. OT3, *Theileria* sp. RSR, ect.

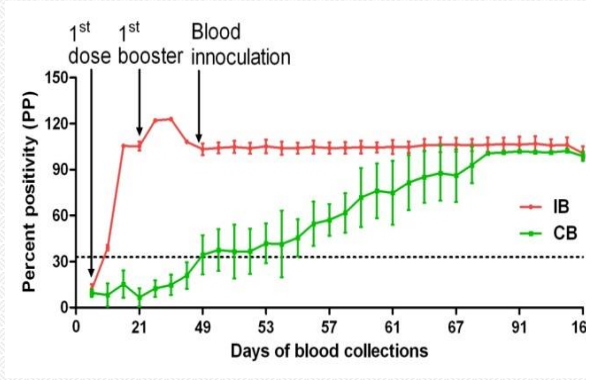
Attenuated Vaccine against *T. annulata*

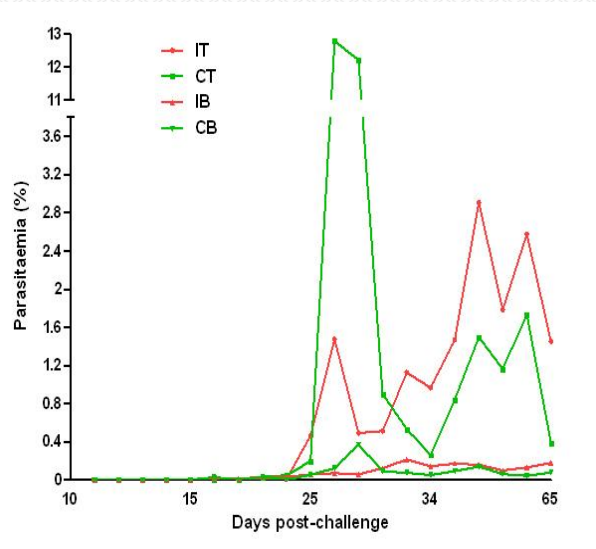
- Schizonts infected lymph cell passaged *in vitro* to make the attenuated vaccine. When injected to animal, the merozoites did not appear in red cell.
- This vaccine was used in 1970s, its protection efficacy was 85.8-99.1%.

Vaccine using recombinant protein

- The rTuIP was used to produce recombinant antigen vaccine against *T. luwenshuni* infection. Only partial protection was observed.





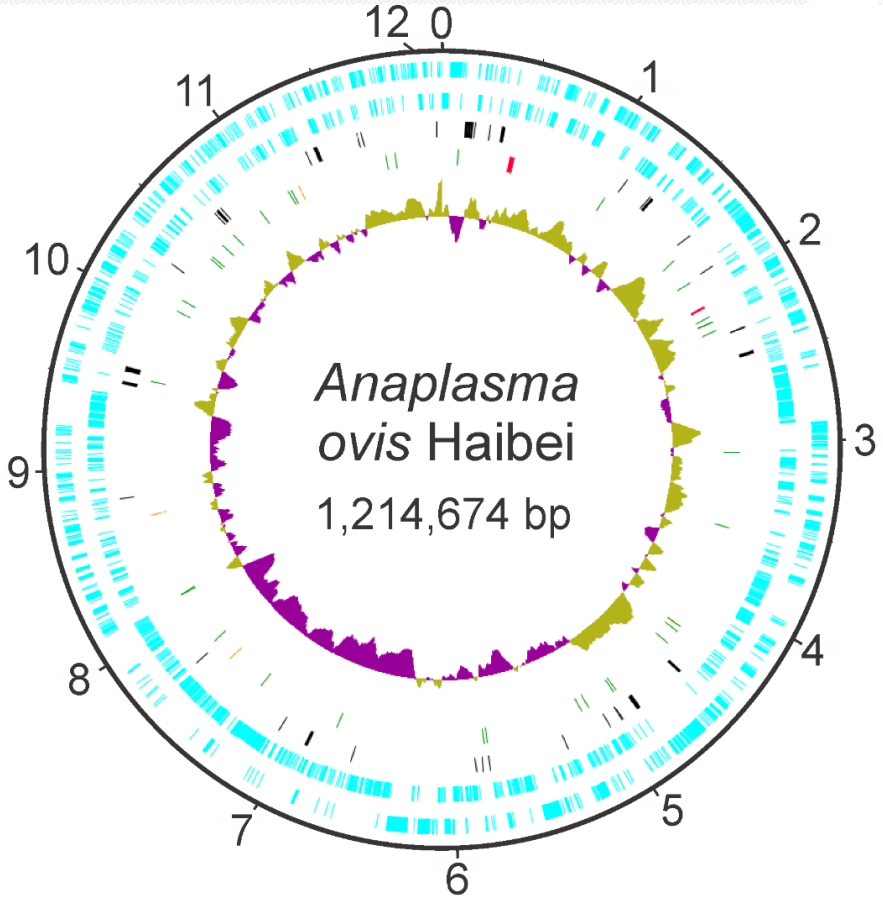


Anaplasma

Anaplasma	**Host**	**Tick vector**
A. marginale	Cattle	*R. (Boophilus) microplus, Hy. asiaticum, I. ricinus, D. pictus*
A. ovis	Sheep, Goat	*D. nuttalli, Rh. pumilio, Hy. asiaticum*
A. bovis	Cattle	*Not confirmed*
A. phagocytophilum	Mammals	*I. persulcatus*
A. platys	Dog	*Not studied*
A. capra	Human, ruminants	*Unknown*

PCR available for all of the species, ELISA available for *A. marginale* and *A. ovis*.

Genome project for *A. ovis*

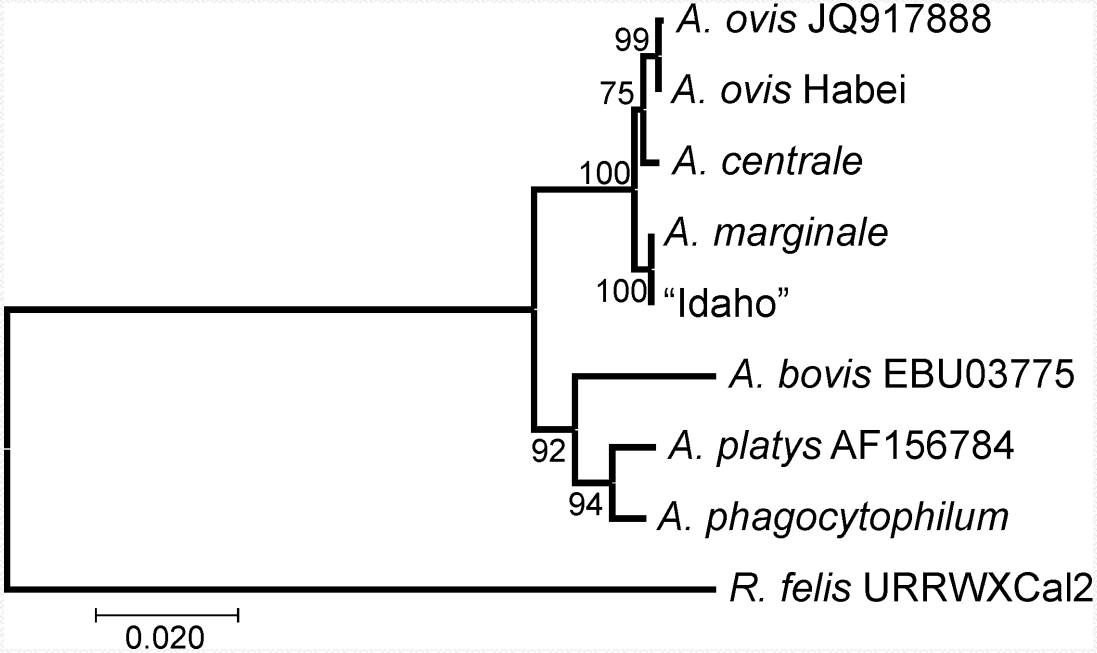


Circular map of the *Anaplasma ovis* genome. The outermost ring shows the genome size in 100 kb increments. Moving inwards, the light blue marks indicate the coding sequences on the forward and reverse strands, the black marks indicate pseudogenes, the next ring contains the noncoding RNAs with tRNAs in green, rRNAs in red and other ncRNAs in orange. The innermost ring shows the GC skew, with olive green being positive and purple being negative.

General Features of *Anaplasma* Genomes

	A. ovis Str Haibei	*A. marginale* Str St. Maries	*A. centrale* Str Israel	*A. phagocytophilum* Str HZ
Total Bases	1,214,674	1,197,687	1,206,810	1,471,282
CDS Count	938	949	925	1066*
tRNAs	37	37	37	37
nc RNAs	3	3	3	3
rRNAs	3	3	3	3
tmRNA	1	1	1	1
Pseudogenes	44	20	24	111
Functional pseudogenes	15	14	16	75
Coding %	83.0	85.4	84.4	68.2
GC %	49.0	49.9	50.0	42.6

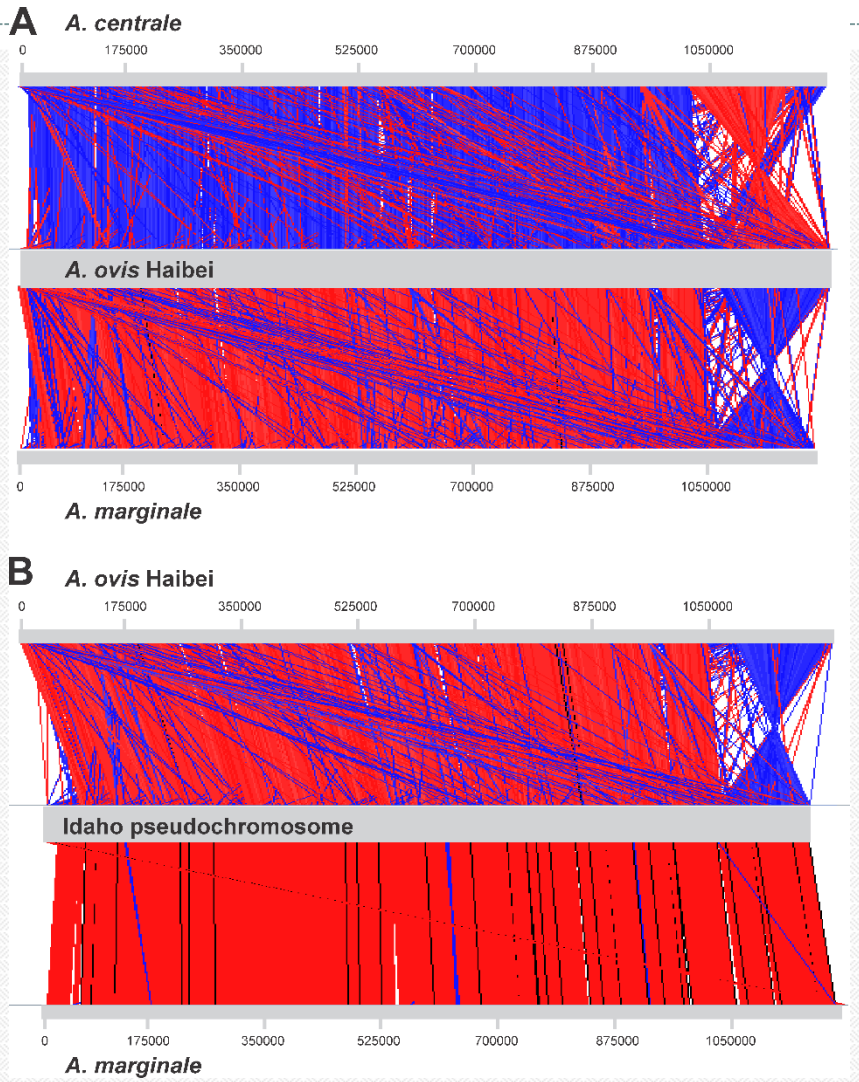
Genome project for *A. ovis*



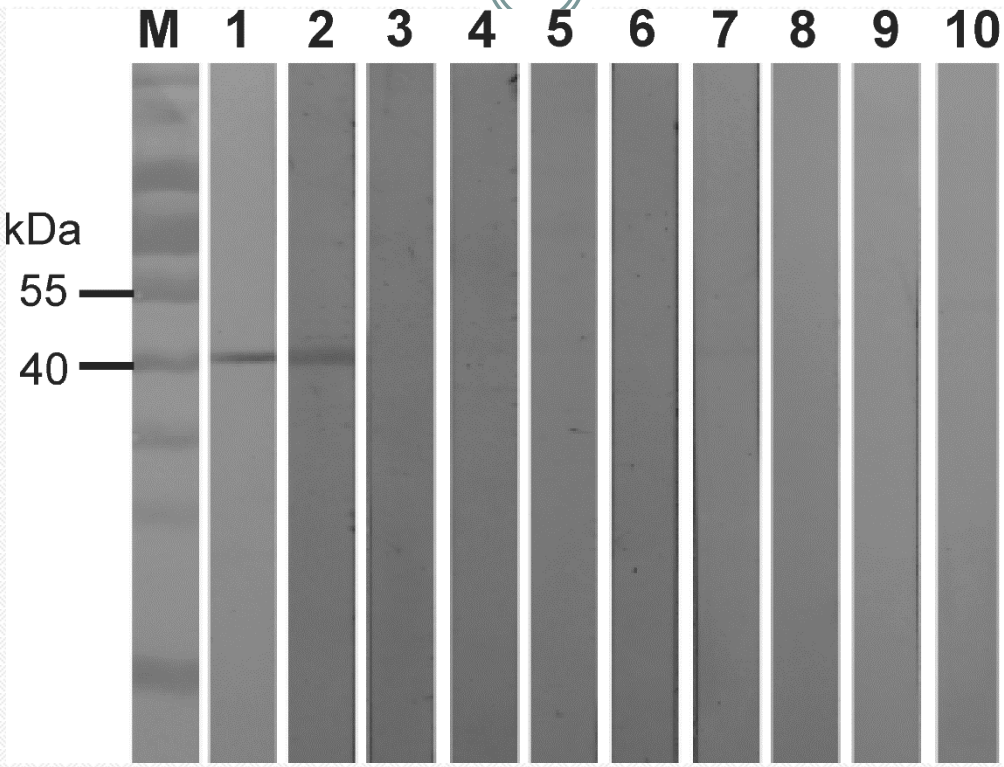
Phylogenetic tree based on 16S sequences. Other 16S sequences were taken from whole genomes with accession numbers as follows: *A. centrale*: CP001759; *A. ovis* Haibei: CP015994; *A. marginale*: CP00030; *A. phagocytophilum*: CP000235.

Whole genome alignment of *Anaplasma* species

Panel A shows the comparison of *A. centrale* (CP001759) and *A. marginale* (CP000030) with *A. ovis* Haibei (CP015994). The *A. centrale* genome was flipped for the alignment. Panel B shows the alignment of *A. ovis* Haibei and *A. marginale* with the Idaho pseudochromosome . Red indicates regions of identity in the same orientation while blue indicates regions of identity with the opposite orientation.

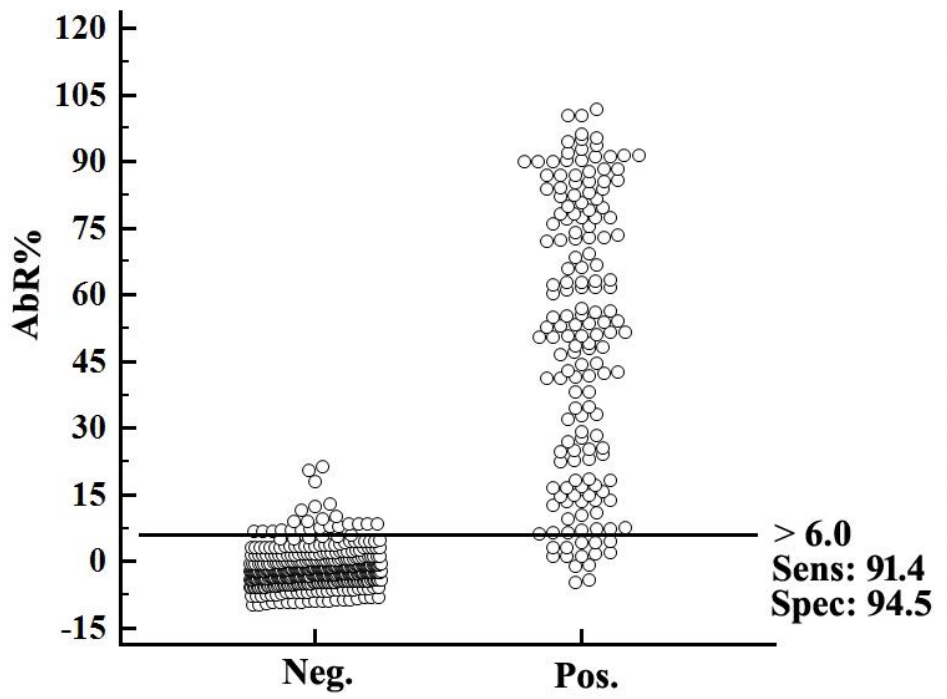


Antigenicity of recombinant protein AAAP of *A. ovis*



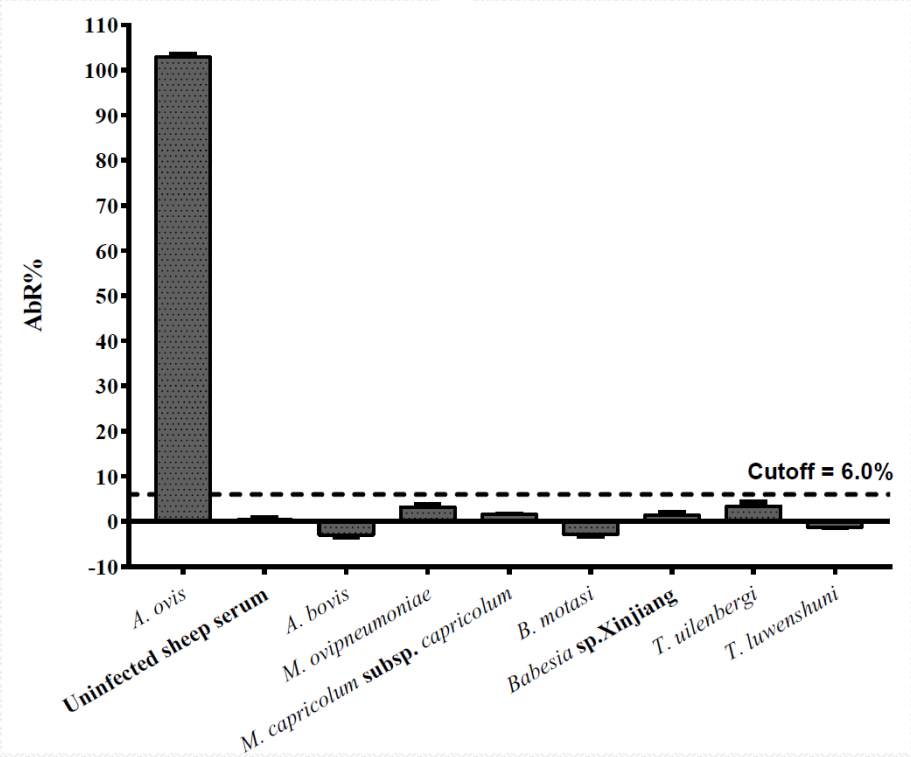
Lane 1: mouse anti-RGS-His antibody; Lane 2: *A. ovis*-positive sheep serum; Lane 3: serum from uninfected sheep; Lane 4–9, 11: positive sheep sera against *Mycoplasma ovipneumoniae*, *M. capricolum capricolum*, *Babesia motasi*, *Babesia* sp. Xinjiang, *Theileria uilenbergi*, *T. luwenshuni* and *A. bovis*, respectively; Lane 10: positive cattle serum against *A. marginale*

Establishment of rAAAP ELISA



The cut-off value of the rAAAP ELISA was determined to be 6.0% by testing 434 negative and 163 positive reference sera using interactive dot diagram. With this threshold, the calculated sensitivity and specificity were 95.1 and 95.4.

Specificity of the rAAAP ELISA



Reaction with positive sheep sera against *A. ovis*, *A. bovis*, *Mycoplasma ovipneumoniae*, *M. capricolum capricolum*, *Babesia motasi*, *Babesia* sp. Xinjiang, *Theileria uilenbergi*, *T. luwenshuni* and negative serum from uninfected sheep

Prevalence of *A. ovis* by the rAAAP ELISA

Method: rAAAP ELISA

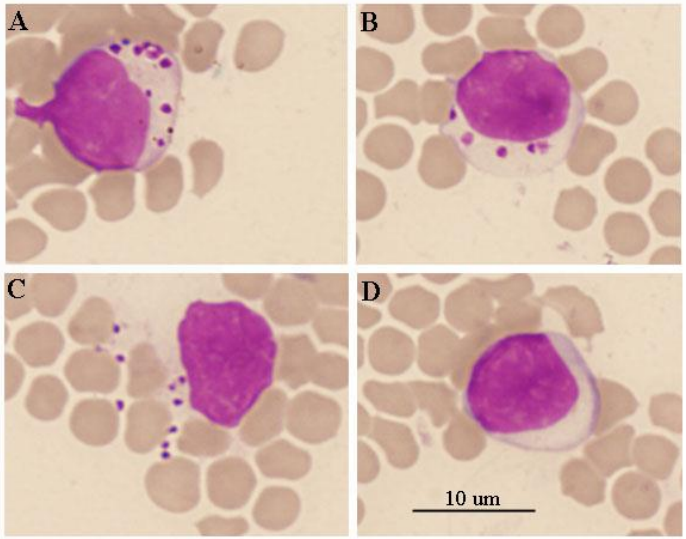
Period: 2010 - 2016

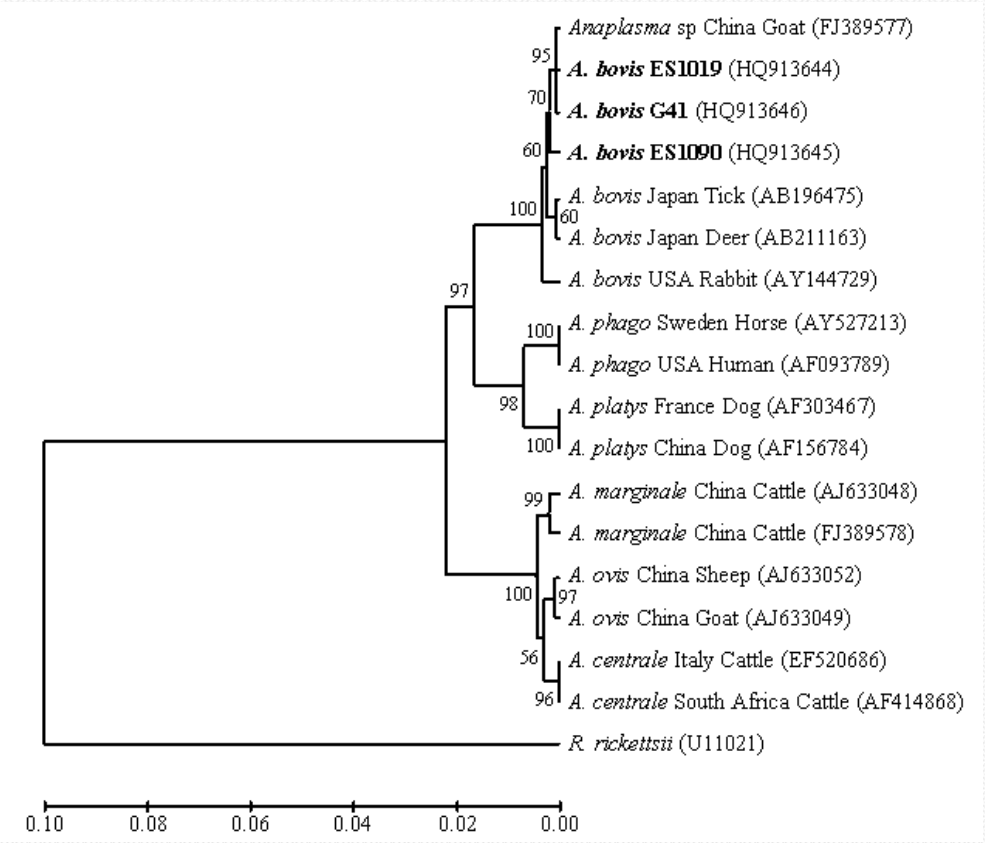
Range: 23 provinces

Sampling: 3138 herds

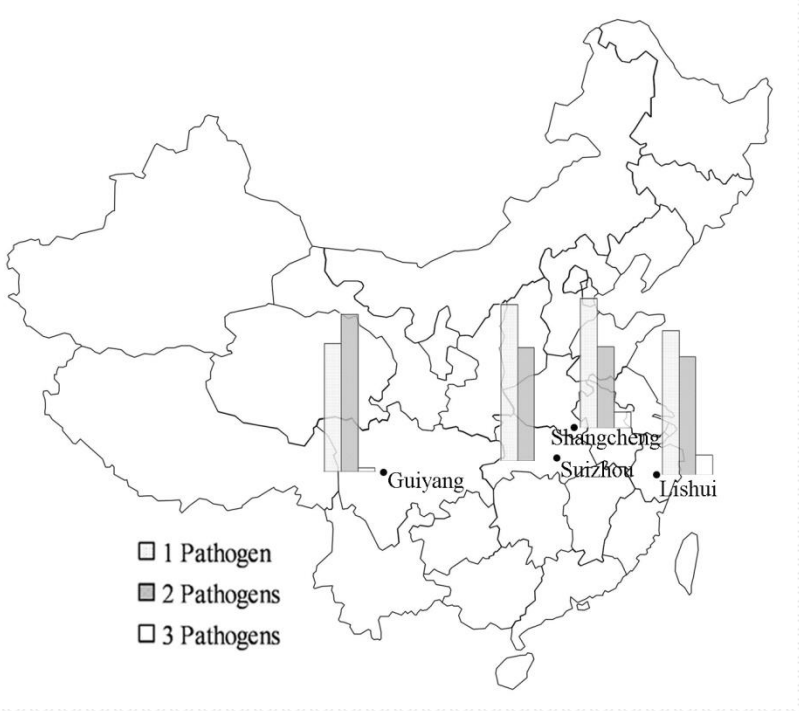
Results: Average positive rates 35.3%

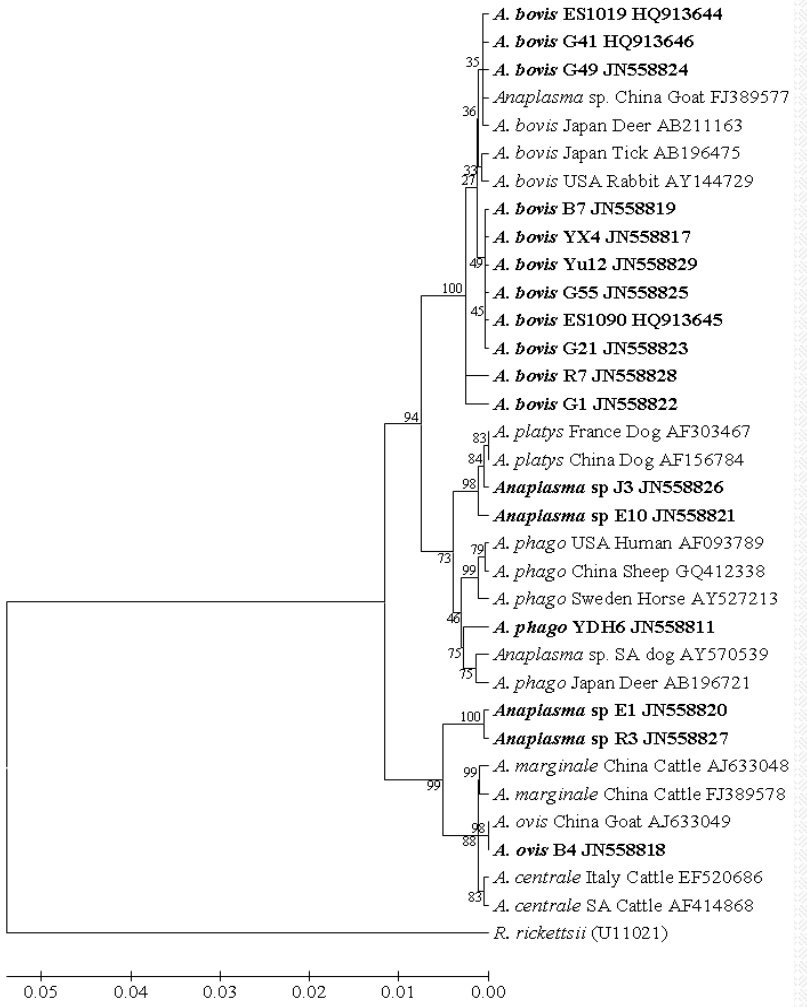
Isolation and identification of *A. bovis* from sheep





Epidemiological status of *Anaplasma* spp





Summary on piroplasm and *Anaplasma*

- Recent years, more and more piroplasm and *Anaplasm* have been identified and isolated.
- Many diagnostic tools have been developed, however, none of them has been commercialized and their use are limited in the laboratory.
- Diminazene Aceturate, Imidocarb and Primaquine phosphate are often used chemicals for the treatment of the infection.
- Vaccine against *T. annulate* did not use in the field because of its old fashion production procedures.
- The systematic studies on ecology and epidemiology need to be implement.

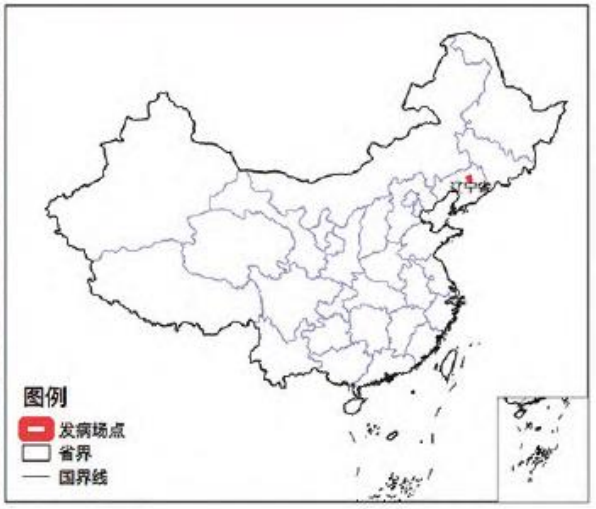
Activities on African swine fever

- Workshop on ASF epidemiology and diagnosis in September, 2010
- Regents transfer: genomic DNA of 17 ASFV isolates, positive and negative sera, virus antigens from CISA-INIA, Spain
- Techniques transfer: virus isolation, PCR (conventional PCR、Real-time PCR、multiplex PCR for differential diagnosis of ASF and CSF), genotyping for ASFV, Serological diagnostic methods (ELISA and IB)
- **We developed a PCR and LAMP methods using the materials**
- **We developed a recombinant NDV-vectored ASF vaccines (rNDV/P30, rNDV/P54 and rNDV/P72)**

Worried about ASF since last year

First discovery of ASF in Shenyang

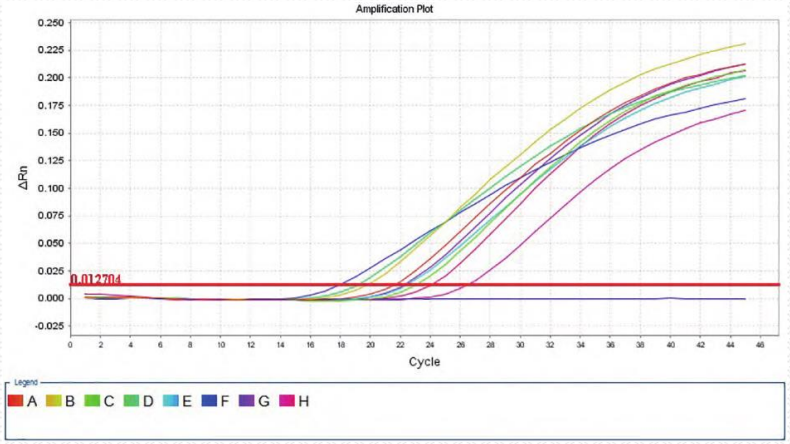
- From July 1 to August 1, a total of 47 of 383 pigs died on a farm in the Shenbei District of Shenyang, Liaoning Province, China.
- Postmortem showed extremely swollen and severely necrotic spleens.
- Pathologic changes included hemorrhages in tonsils and lungs, marbled lesions in mandibular and mesenteric lymph nodes, and diffuse hemorrhages in a large part of gastric serosa.



Wang Q, et al. Journal of China Animal Health Inspection. 2018;35:1–9

First discovery of ASF in Shenyang

- PCR and real time PCR were applied to detected the virus from two pig tissues.
- P72 gene, CD2v protein gene, TRS gene was successfully sequenced from the positive samples.
- P72 based ELISA from the INGENASA and P30 based ELISA from IDvet gave negative results.



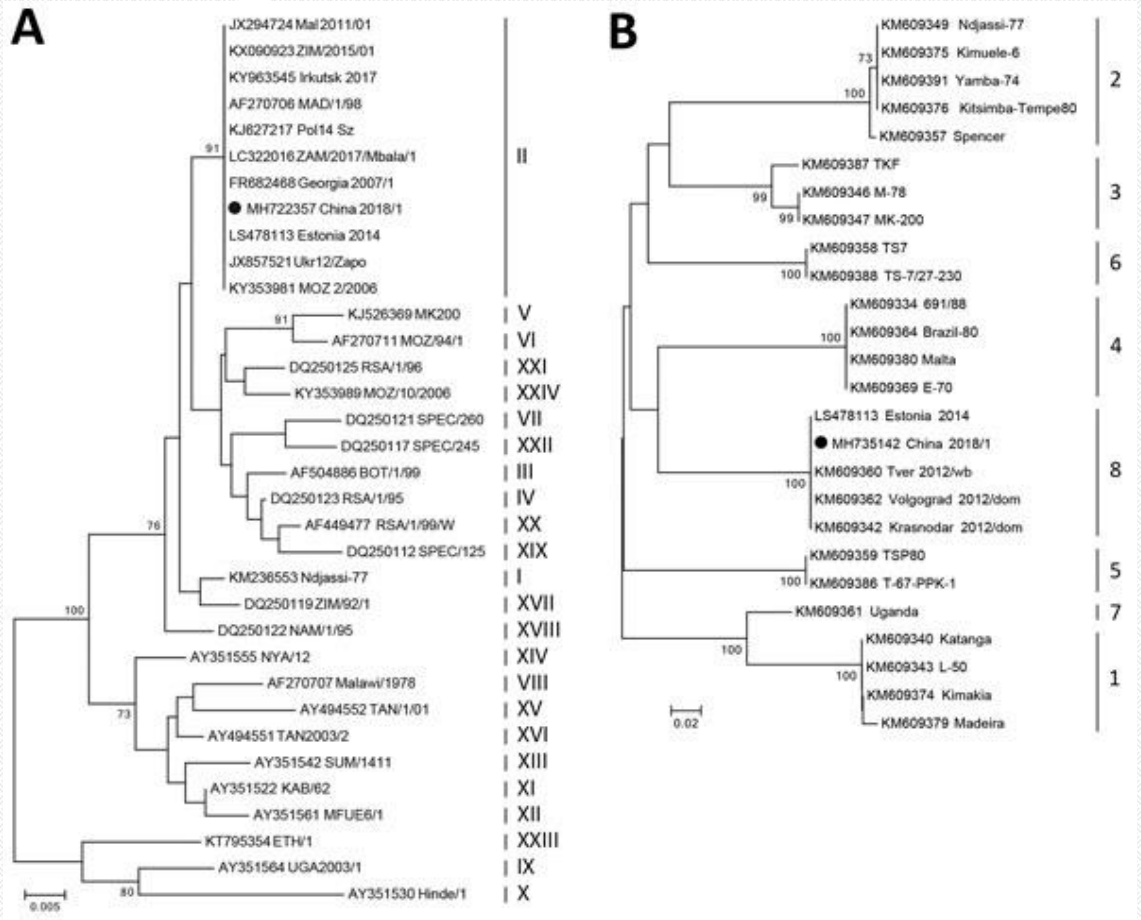

```
                10        20        30        40        50
ATGCAGCCCACTCACCACGCAGAGATAAGCTTTCAGGATAGAGATACAGC      50
TCTTCCAGACGCATGTTCATCTATATCTGATATTAGCCCCGTTACGTATC     100
CGATCACATTACCTATTATTAAAAACATTTCCGTAACTGCTCATGGTATC     150
AATCTTATCGATAAATTTCCATCAAAGTTCTGCAGCTCTTACATACCCTT     200
CCACTACGGAGGCAATGCGATTAAAACCCCCGATGATCCGGGTGCGATGA     250
TGATTACCTTTGCTTTGAAGCCACGGGAGGAATACCAACCCAGTGGTCAT     300
ATTAACGTATCCAGAGCAAGAGAATTTTATATTAGTTGGGACACGGATTA     350
CGTGGGGTCTATCACTACGGCTGATCTTGTGGTATCGGCATCTGCTATTA     400
ACTTTCTTCTTCTTCAG                                      417
```

Wang Q, et al. Journal of China Animal Health Inspection. 2018;35:1–9

First discovery of ASF in Shenyang

Phylogenetic analysis of a causative virus strain (China 2018/1) of an African swine fever outbreak, China, 2018.

A) p72 genotype; B) CD2v serogroup.



Ge S, et al. Emerg Infect Dis. 2018 Nov

Outbreaks of ASF

① Aug 3, Shenyang, Liaoning

② Aug 16, Zhengzhou, Henan

③ Aug 19, Lianyungang, Jiangsu

④ Aug 22, Wenzhou, Zhejiang

⑤ Aug 30, Wuhu, Anhui

⑥ Sept 2, Yicheng, Anhui

⑦ Sept 3, Yicheng, Anhui

⑧ Sept 3, Wuxi, Jiangsu

⑨ Sept 5, Jiamusi, Heilongjiang

Total of 14 outbreaks distributed in 6 provinces.

Outbreaks of ASF

Date of definitive diagnose	Site	No. of Pigs	No. of sickness	Morbidity	No. of death	Mortality
Aug. 3	Shenyang	383	47	12.3%	47	100%
Aug. 16	Zhengzhou	260	30	11.5%	30	100%
Aug. 19	Lianyungang	unknown	615	-	88	Culled
Aug. 22	Wenzhou	unknown	430	-	340	79.1%
Aug. 30	Wuhu	459	185	40.3%	80	Culled
Sept. 2	Yicheng	725	-	-	134	-
Sept. 3	Xuancheng	308	152	49.4%	83	54.6%
Sept. 3	Wuxi	97	12	12.4%	9	75%
Sept. 5	Jiamusi	87	39	44.9%	12	30.7%
Sept. 6	Jiamusi	203	26	12.8%	10	38.5%
	Wuhu	30	13	43.3%	4	30.8%
	Xuancheng	52	15	28.8%	15	100%

Main measures for control ASF

- Initiate emergency response plan;
- Block infected area and threating area;
- Culling and bio-safety disposal of the infected and contaminated animals;
- Disinfection of contaminated area;
- Forbidden all of the pigs and pig products move in or out of the blocking area.

Acknowledgements