



IZSAM G.CAPORALE  
TERAMO

parte II  
**Risultati**

**Marco Di Domenico**

Teramo, 3 novembre 2016

Centro Internazionale per la Formazione e l'Informazione Veterinaria "F. Gramenzi" (CIFIV)



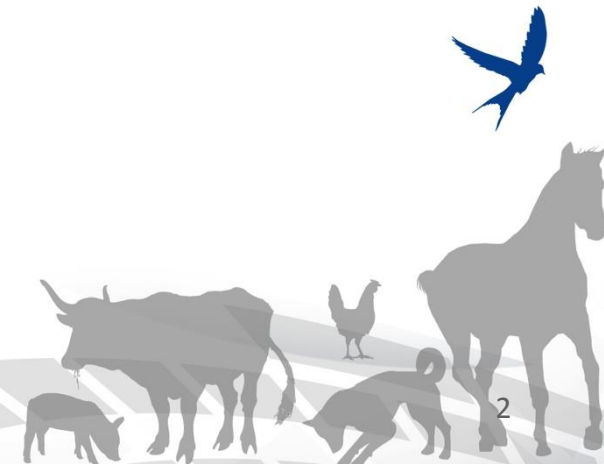
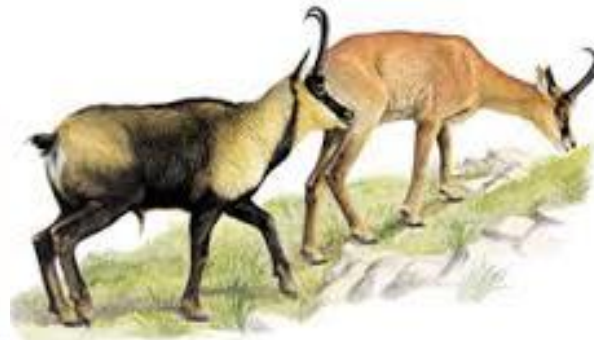
# *Anaplasma phagocytophilum* nei selvatici

Species	Spleen	
	tested	positive (%)
red deer	12	9 (75%)
Apennine chamois	9	6 (66.7%)
roe deer	59	32 (54.2%)
fallow deer	1	0
mouflon	6	0
<b>Total</b>	<b>87</b>	<b>47 (54%)</b>



## *Ixodes ricinus*

tested	positive (%)
18	2 (11.1%)
0	0
59	22 (37.3%)
0	0
0	0
<b>77</b>	<b>24 (31.2%)</b>





### Detection of *Anaplasma phagocytophilum* genotypes that are potentially virulent for human in wild ruminants and *Ixodes ricinus* in Central Italy



M. Di Domenico, I. Pascucci, V. Curini, A. Cocco, F. Dall'Acqua, C. Pompili, C. Cammà\*

Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise, Teramo, Italy

#### ARTICLE INFO

##### Article history:

Received 3 June 2015  
Received in revised form 16 March 2016  
Accepted 17 March 2016  
Available online 10 March 2016



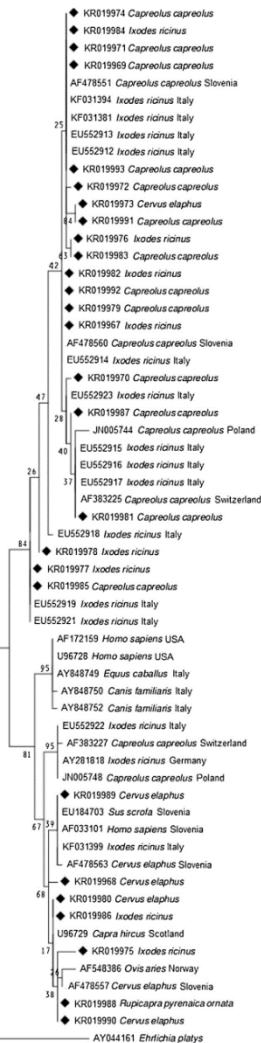
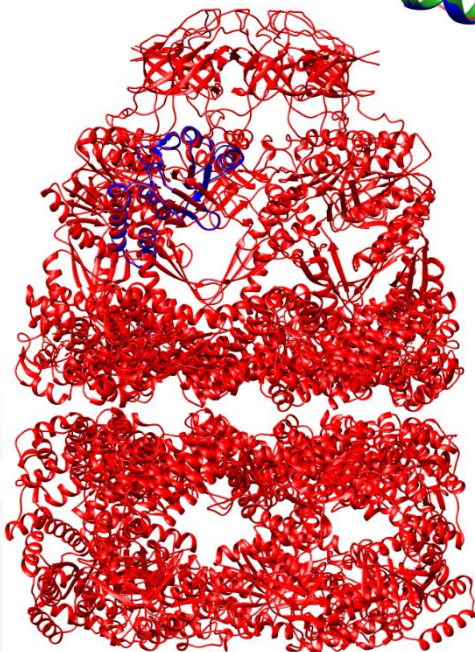
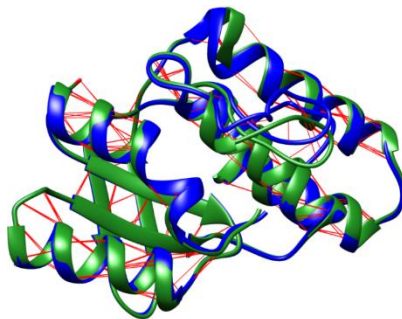
...one zoonosis worldwide. As is the case related to the environmental conditions, such as, climate and vegetation. In this study we analyzed samples from Central Italy, and 77 *Ixodes ricinus*



analisi della sequenza parziale del gene *GroEL*



# Complesso GroEL-GroES



lineage II

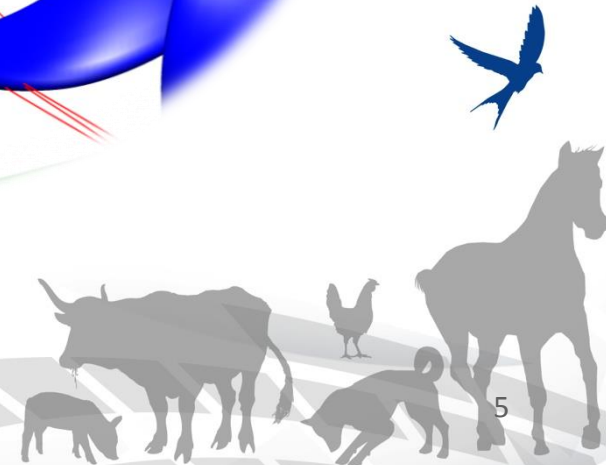
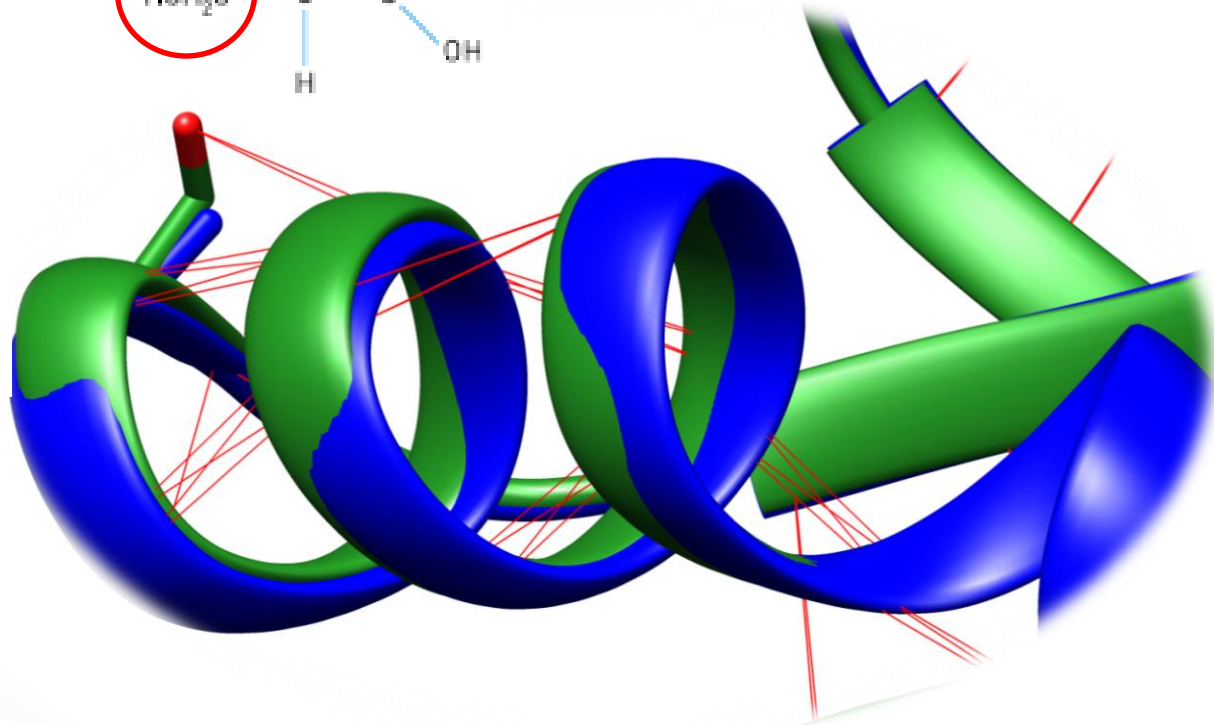
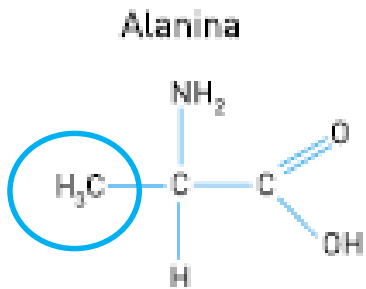
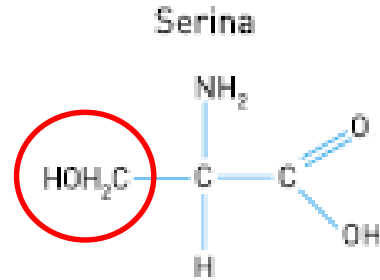
A-Var

lineage I

S-Var



# Varianti Ala - Ser



# Considerazioni



✓ Diagnosi nell'uomo

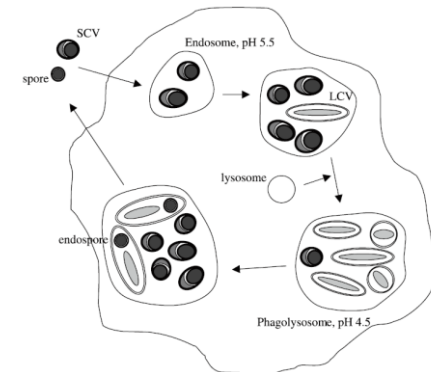
✓ Approfondire le conoscenze sui meccanismi di patogenicità di *Anaplasma phagocytophilum*

✓ Capire il ruolo della malattia nei selvatici



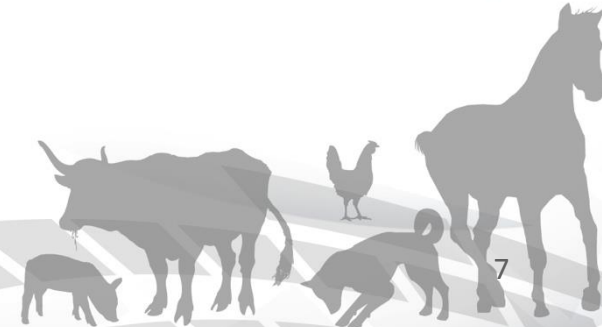
# *Coxiella burnetii*

- ✓ coccobacillo Gram –
- ✓ intracellulare obbligato
- ✓ diffusione mondiale
- ✓ pleomorfo



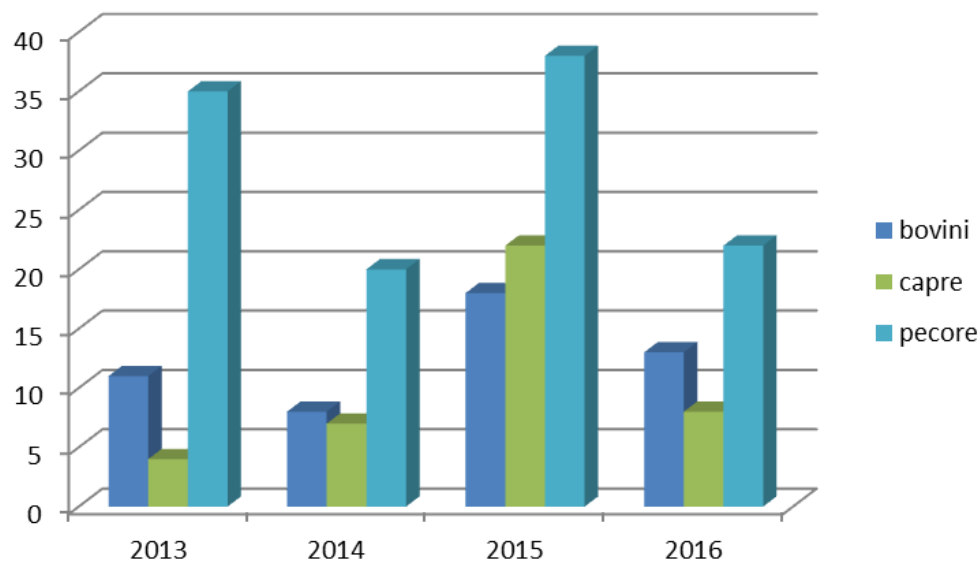
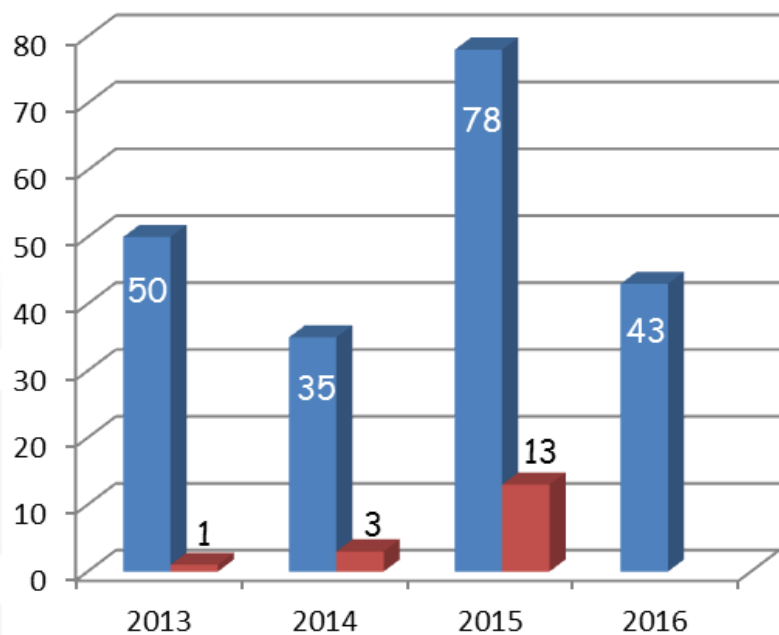
Arricau-Bouvery et al. 2005, *BM Microbiology*

The *Coxiella burnetii* Parasitophorous Vacuole. Springer Eds. 2012



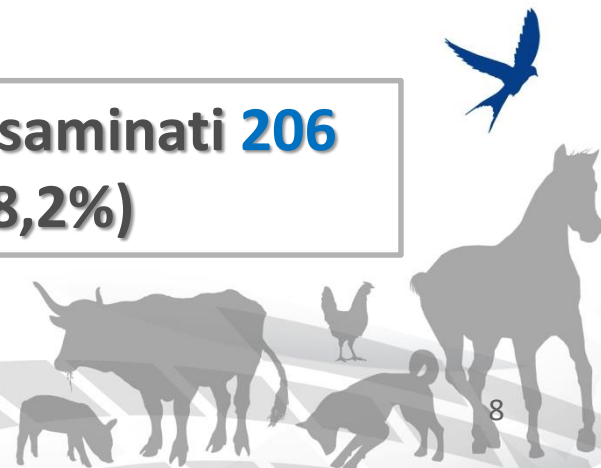
# Coxiella burnetii diagnostica

## casi di aborto nei ruminanti domestici



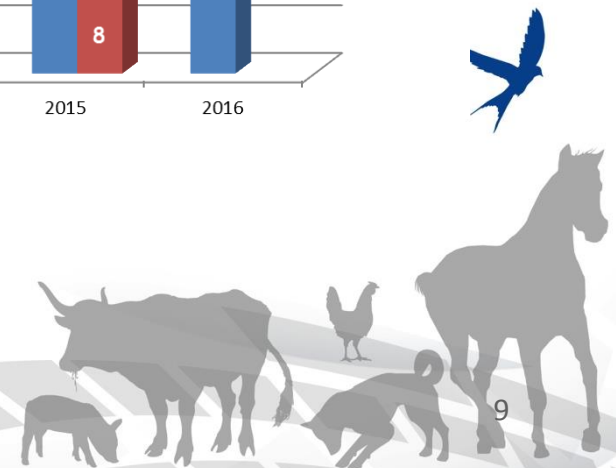
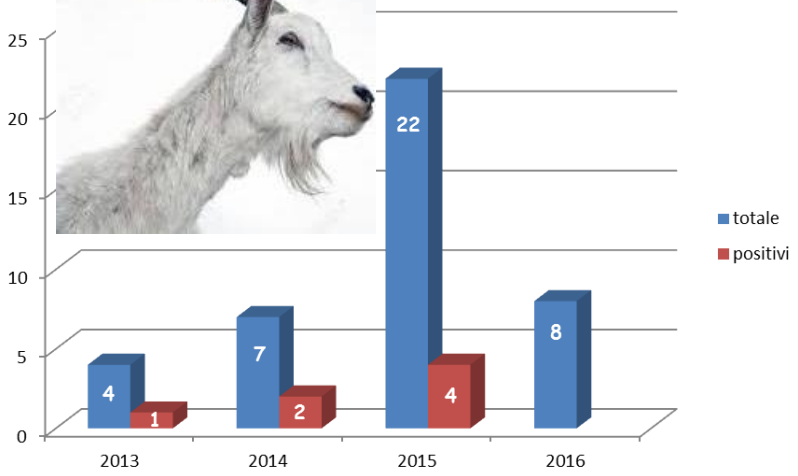
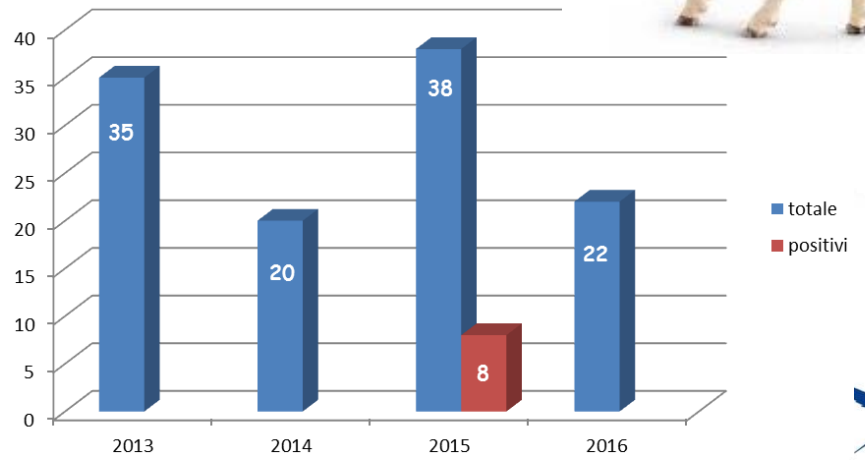
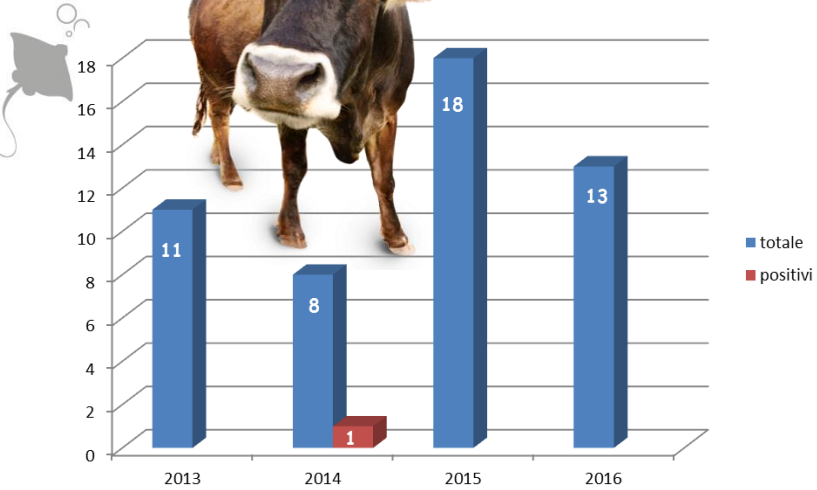
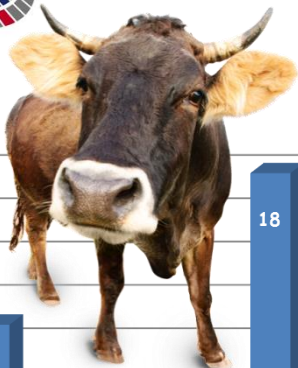
■ campioni analizzati  
■ positivi

**totale casi esaminati 206**  
**positivi 17 (8,2%)**





# Coxiella burnetii diagnostica



# *Coxiella burnetii* typing

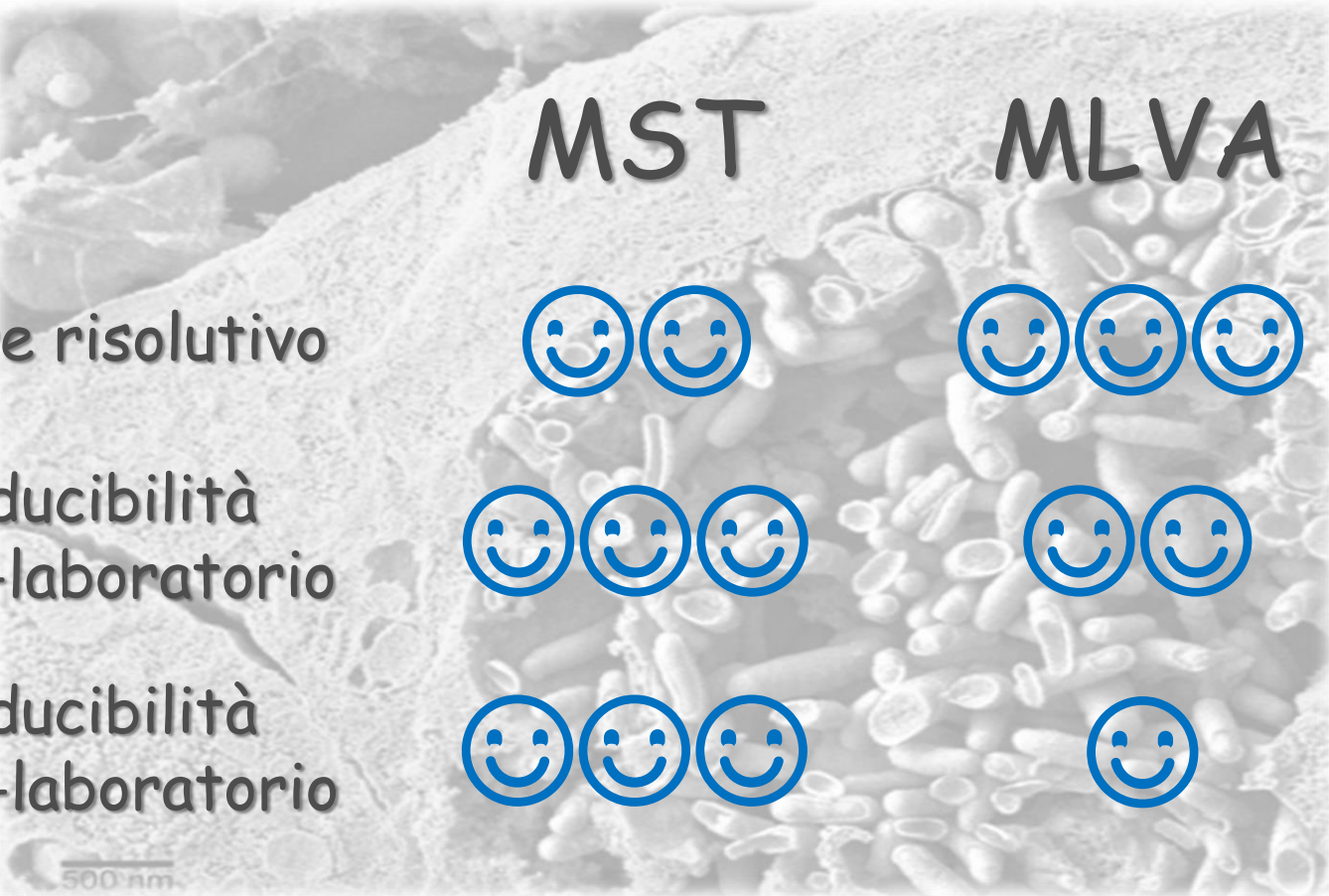
potere risolutivo




riproducibilità  
intra-laboratorio



riproducibilità  
inter-laboratorio



# *Coxiella burnetii* typing MST



VECTOR-BORNE AND ZOO NOTIC DISEASES  
Volume 14, Number 10, 2014  
© Mary Ann Liebert, Inc.  
DOI: 10.1089/vbz.2014.1587

## *Coxiella burnetii* in Central Italy: Novel Genotypes Are Circulating in Cattle and Goats

Marco Di Domenico, Valentina Curini, Fabrizio De Massis, Andrea Di Provvido,  
Massimo Scacchia, and Cesare Cammà

### Abstract

Genotyping of bacteria is critical for diagnosis, treatment, and epidemiological surveillance. *Coxiella burnetii*, the etiological agent of Q fever, has been recognized to have a potential for bioterrorism purposes. Because few serosurveys have been conducted in Italy, there is still limited information about the distribution of this pathogen in natural conditions. In this paper, we describe the genotyping of *C. burnetii* strains by multispacer

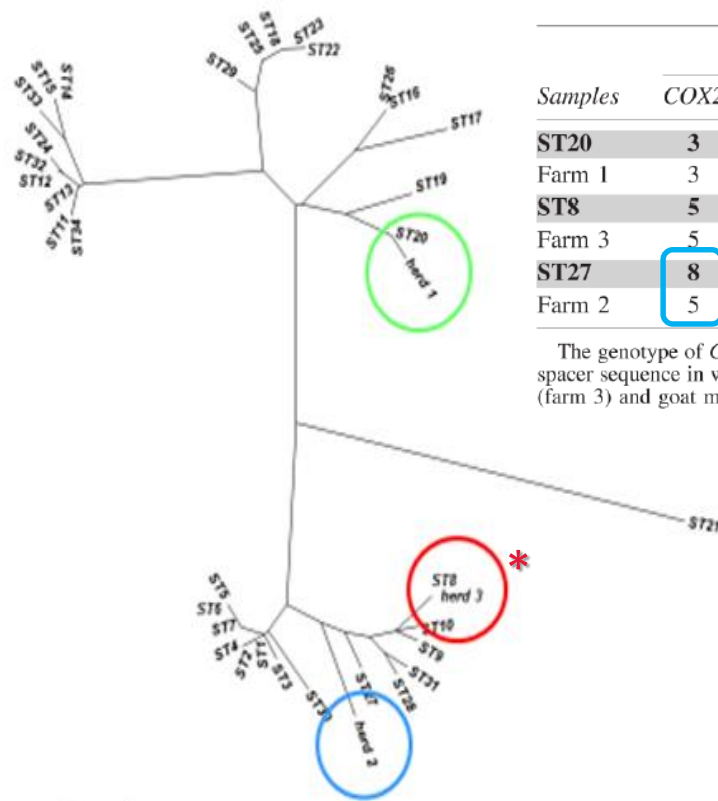


# Coxiella burnetii typing MST

TABLE 2. COMPARISON BETWEEN THE REFERENCE SEQUENCE TYPE AND THE NOVEL GENOTYPES DESCRIBED IN THE PRESENT STUDY

Samples	Spacers									
	COX2	COX5	COX18	COX20	COX22	COX37	COX51	COX56	COX57	COX61
ST20	3	2	6	1	5	4	4	10	6	5
Farm 1	3	2	6	1	5	4	4	10	Novel	5
ST8	5	4	2	5	1	5	3	3	4	4
Farm 3	5	4	2	5	1	5	3	2	4	4
ST27	8	4	8	5	7	5	2	3	4	6
Farm 2	5	4	8	5	8	5	2	3	4	6

The genotype of *C. burnetii* from cattle milk samples (farm 1) was similar to sequence type 20 (ST20), with the exception of the Cox57 spacer sequence in which a novel allele was described in the present study. Different alleles were not found in *C. burnetii* from goat fetus (farm 3) and goat milk (farm 2); however, in both cases the allelic combination generated unique STs.



3\* nuovi Sequence Type

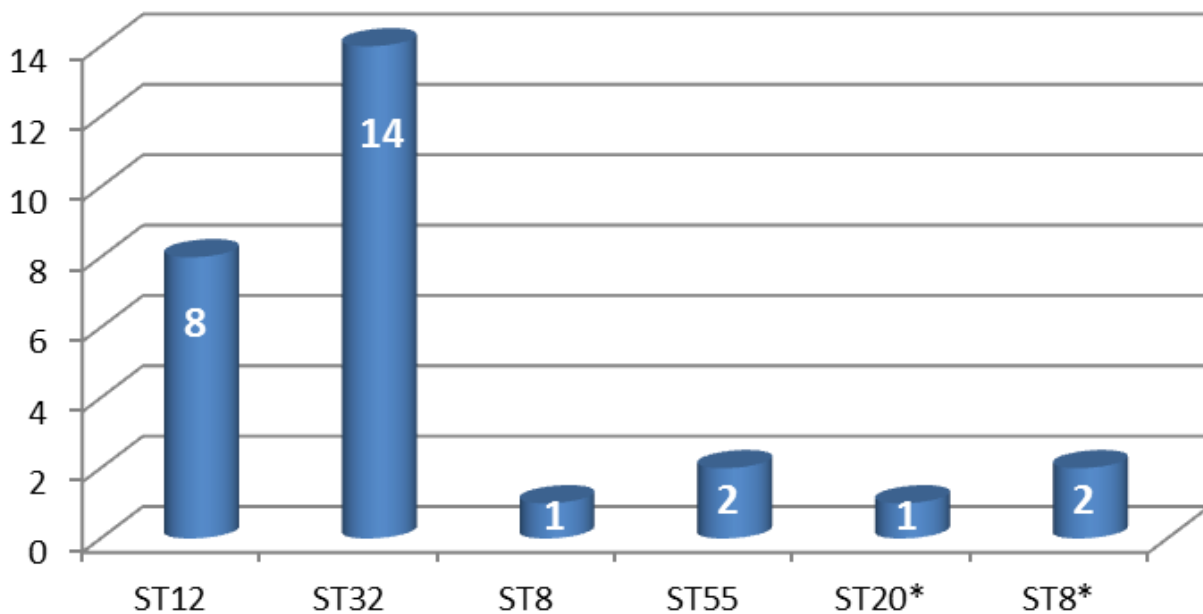
[http://ifr48.timone.univ-mrs.fr/mst/coxiella\\_burnetii/](http://ifr48.timone.univ-mrs.fr/mst/coxiella_burnetii/)



# *Coxiella burnetii* typing MST



IZSAM 9  
IZSLT 14  
UniPi 5  
28 ceppi




22/28 (78%) appartengono a **ST12** e **ST32**

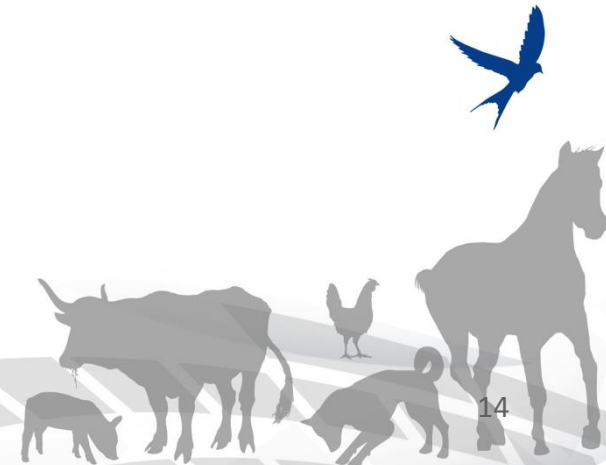
**ST12**




**ST32**



- 
- ✓ Isolamento dei ceppi
  - ✓ Diagnosi nell'uomo
  - ✓ Conoscere i ceppi circolanti nel territorio e avere a disposizione database per la rapida identificazione e rintracciabilità dei focolai (epidemiologia molecolare)
  - ✓ Genoma



# Rickettsia nelle zecche



uomo  
cinghiale  
cervo



*Dermacentor marginatus*

*Rickettsia slovaca*

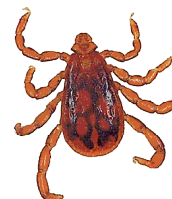
uomo  
capriolo  
cervo  
lupo  
gatto  
capra  
volpe



*Ixodes ricinus*

*Rickettsia monacensis*  
*Rickettsia helvetica*

uomo  
capriolo  
tasso  
lepre



*Rhipicephalus turanicus*

*Rickettsia massiliae*  
*Rickettsia conorii*

lupo  
volpe  
tasso  
riccio



*Ixodes hexagonus*

*Rickettsia slovaca*  
*Rickettsia felis*

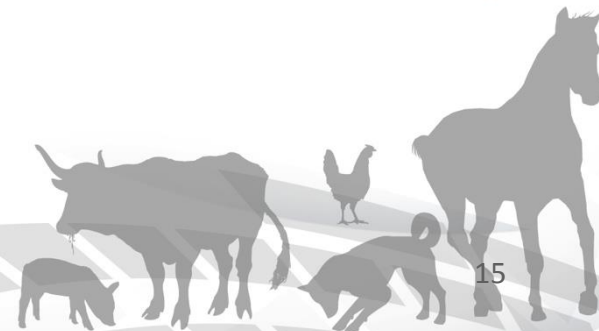


topo  
cane  
volpe  
lupo



*Rhipicephalus sanguineus*

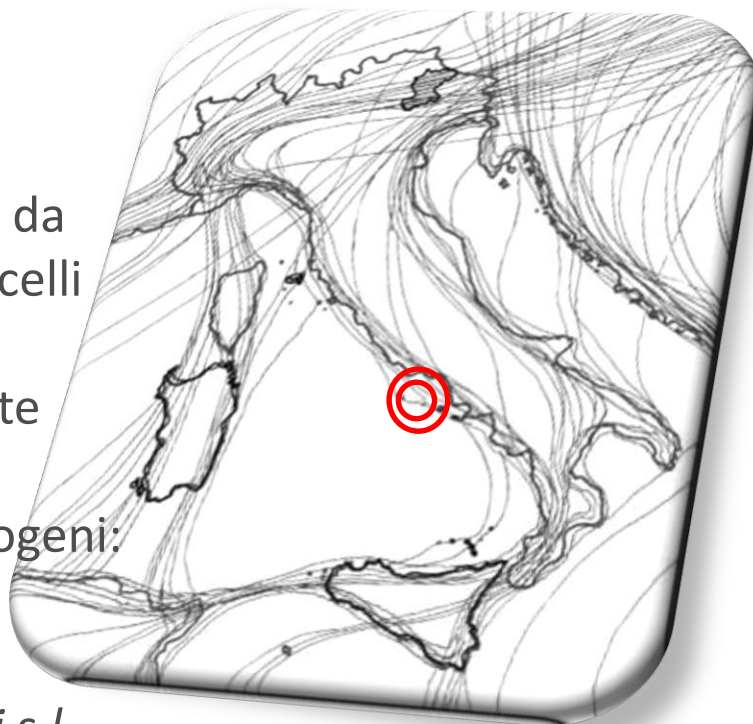
*Rickettsia massiliae*



# Patogeni nelle zecche degli uccelli migratori

- 450 zecche raccolte da diverse specie di uccelli migratori
- Identificate mediante sequenza 12S
- Analizzate per i patogeni:

- *Rickettsia sp.*
- *Borrelia burgdorferi s.l.*
- *Coxiella burnetii*



- Identificazione di 3 generi *Hyalomma*, *Amblyomma* e *Ixodes*
- 160 zecche positive solo per *Rickettsia sp.*

- 1 *R. africae*
- 1 *Rickettsia sp.*
- 158 *R. aeschlimanii*



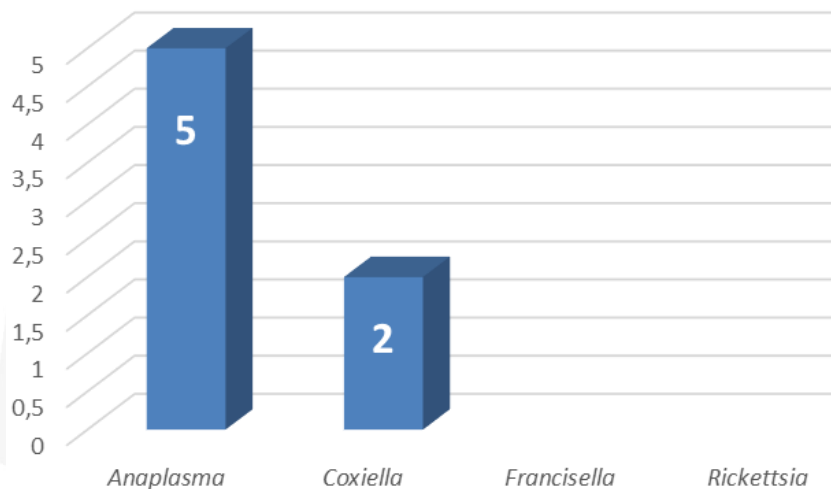


# Sorveglianza sanitaria cinghiali



231

campioni positivi



*Dermacentor marginatus*



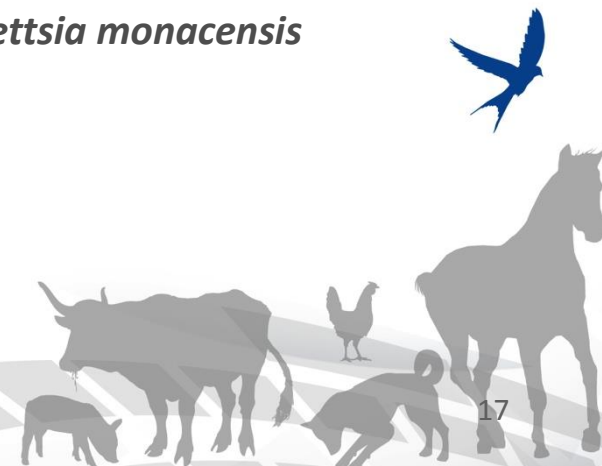
118 zecche  
40 pool

## PCR Real Time

- 31 positivi per *Rickettsia* sp 77,5%
- 1 *Coxiella burnetii* 0,7%

## sequenza parziale OmpA

- 29 *Rickettsia slovacae*
- 1 *Rickettsia raoultii*
- 1 *Rickettsia monacensis*



# Micromammiferi e zecche part II

Real Time PCR specifica per *A. flavicollis* e *A. sylvaticus*


- ✓ Coxiella 0/132 topi – 0/287 zecche
- ✓ *Borrelia afzelii* 5/132 (3,8%) topi – 30/287 (10,5%) zecche
- ✓ *Rickettsia sp.* 0/132 topi – 12/287 (4,2%) zecche

Sono stati catturati e prelevati 143 roditori delle specie *M. glareolus* (29%) e *Apodemus sp.* (71%). Sui campioni di sangue conservati mediante FTA sono stati eseguiti i test RealTime per i patogeni selezionati

	<i>Borrelia burgdorferi s.l.</i>		<i>Coxiella burnetii</i>	
Specie	positivi / esaminati	Prevalenza stimata (95%CI)	positivi / esaminati	Prevalenza stimata (95%CI)
<i>Apodemus sp.</i>	7/101	6.93% (3.45% - 13.63%)	2/101	1.98% (0.61% - 6.90%)
<i>Myodes glareolus</i>	5/42	11.90% (5.30% - 25.08%)	0/42	0% (0% - 6.73%)
<b>Totali</b>	<b>12/143</b>	<b>8.39%</b> (4.89% - 14.10%)	<b>2/143</b>	<b>1.40%</b> (0.43% - 4.93%)



# Alcune considerazioni generali



Potenziare le  
capacità  
diagnostiche

Pervenire ad una  
diagnosi eziologica  
sia nell'uomo sia  
negli animali

Definire la  
circolazione dei  
patogeni nelle  
popolazioni


Identificare i  
genotipi e/o  
specie e  
genospecie

Conoscere  
ecologia dei  
diversi genotipi,  
specie e/o  
genospecie

Valutare il rischio  
potenziale per gli  
animali e per  
l'uomo

Incrementare la  
collaborazione  
con la medicina  
umana

Conferma  
diagnostica su  
vettori e su  
campioni  
biologici



Incrementare la  
notifica. Disporre di  
ceppi o DNA di  
patogeni implicati in  
casi umani



**grazie per l'attenzione!**

