



Investigation of disease hazards in cattle in South of Italy (Sicily)

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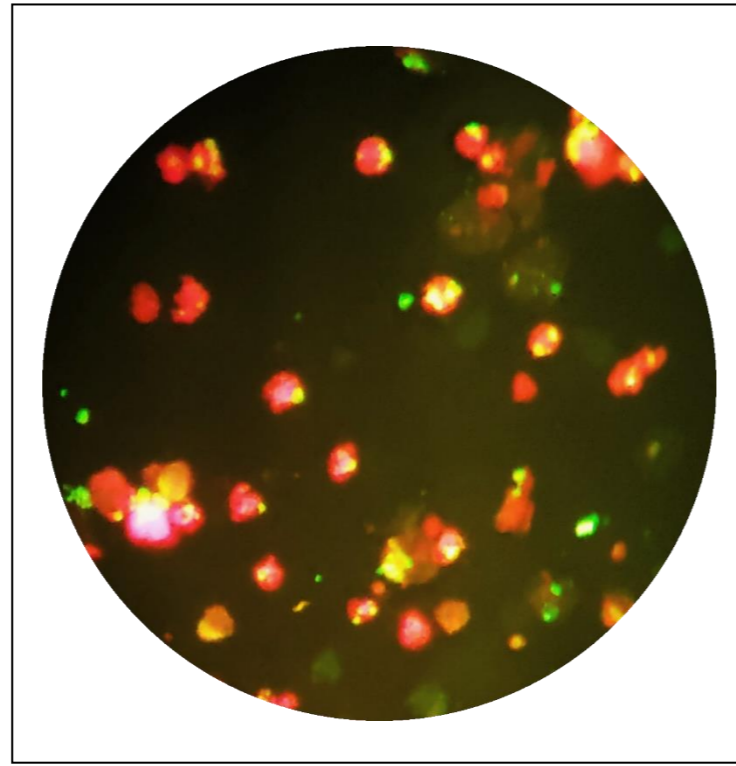


Fig. 1 Positive sample of *Theileria annulata* to fluorescence microscope

This is a cross-sectional study

- ✓ assessing the presence of antibodies in ruminants against *Coxiella burnetii*, *Toxoplasma gondii*, *Neospora caninum*, *Chlamydophila abortus* and *Theileria annulata*

- ✓ determining the molecular status for *T. gondii*

in cattle in Sicily

Seroreactive animals were found for all diseases studied except for *C. abortus*

	Seroprevalence	CI95%
<i>T. annulata</i>	93.7%	0.91-0.95
<i>N. caninum</i>	13.0%	0.09-0.16
<i>C. burnetii</i>	4,2%	0.02-0.06
<i>T. gondii</i>	6,1%	0.04-0.8

COINFECTIONS

- N. 56 *N. caninum*-*T. annulata* by serology
- N. 19 *C. burnetii*-*T. annulata* by serology
- N. 4 *N. caninum*- *T. gondii*- *T. annulata* by serology
- N. 1 *N. caninum*-*T. annulata* by serology
- N. 1 *T. gondii* by PCR- *T. annulata* by serology

Since all co-infections are associated with *T. annulata* it can be hypothesized that this is a highly prevalent disease and that it can determine a carrier status among animals with a high risk of cross-associated infections.

The prevalence rates of the diseases analyzed in Sicily among the bovine population fluctuate from medium to high, with the highest percentages in the province of Palermo, for all the infections detected 87%, followed by Agrigento (12,7%) and thus require official control measures.

This is the first study that has identified *C. burnetii* in cattle associated with *N. caninum* and *T. gondii*. Future studies should be conducted to investigate how widespread this pathogen is in sicilian cattle herds.

478 bovine blood samples were collected from 208 sicilian farms in the province of Palermo (402 from 171 farms), Agrigento (52 from 25 farms) e Trapani (24 from 12 farms), between September 2020 and September 2021

A simple random sample was determined with proportional stratification and an expected prevalence of 5%. The simple random sample was stratified by province with weights proportional to the total number of cattle within the province. An extraction step of bovine serum from herds in each province was calculated. The extraction step is different for each province.

DIFFERENT APPROACHES OF LABORATORY DIAGNOSIS

Serology

Commercial *ELISA*
(spectrophotometer Multiskan SkyHigh (Thermo Scientific at 450nm)
IFA test
(microscope Leica with 10x objective) (Fig. 1)

Molecular assays

REAL TIME PCR SEQUENCING for *T. gondii* [3]

Infectious Agent	Test Kit	Manufacturer	Sensitivity	Specificity
<i>N. caninum</i>	ID Screen® Neospora caninum Indirect Multi-species	ID.vet Innovative Diagnostics, Grabels	100% (CI95%: 98.8–100%)	100% (CI95%: 99.41–100%)
<i>C. burnetii</i>	ID Screen® Q fever indirect Multi-species	ID.vet Innovative Diagnostics, Grabels	100% (CI95%: 89.28–100%)	100% (CI95%: 97.75–100%)
<i>C. abortus</i>	ID Screen® Chlamydophila abortus indirect Multi-species	ID.vet Innovative Diagnostics, Grabels	100% (CI95%: 98.8–100%)	100% (CI95%: 99.41–100%)
<i>T. gondii</i>	ID Screen® Toxoplasmosis indirect Multi-species	ID.vet Innovative Diagnostics, Grabels	100% (CI95%: 98.8–100%)	100% (CI95%: 99.41–100%)
<i>T. annulata</i>	Home-made	/	100% (CI95%: 98.8–100%)	100% (CI95%: 99.41–100%)

MOLECULAR METHOD	PRIMERS, PROBES	TARGET	PCR PRODUCT LENGTH
<i>T. gondii</i> Real Time PCR	AF1 AF2 Toxo P	529-bp repeat element	97 bp
<i>T. gondii</i> Nested PCR	Toxo P1 Toxo P4 Toxo P2 Toxo P3	BI gene	98 bp

T. Annulata

Palermo → 84.15% (90.05% farm)

Agrigento → 10.71% (88.0% farm)

Trapani → 5.14% (91.6% farm)

C. burnetii

Palermo → 90.00% (7.6% farm)

Agrigento → 10.00% (8.0% farm)

Trapani → 8.33% (16.7% farm)

N. caninum

Palermo → 79.03% (18.1% farm)

Agrigento → 11.29% (12.0% farm)

Trapani → 9.68% (50.0% farm)

Seroprevalence

PA → 72.41% (12.2% farm)

AG → 20.68% (12,0% farm)

TP → 6.91% (16.66% farm)

Molecular prevalence

PA → 0.24% (0.03% farm)

AG → 0.00% (0.0% farm)

TP → 0.00% (0.0% farm)

AIM

MATERIALS AND METHODS

RESULTS

CONCLUSIONS

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