

BRUCELLA MELITENSIS OCCURENCE IN CYPRUS 2022





BACKGROUND

BRUCELLA MELITENSIS:

Cyprus has been recognized as Brucellosis (*B. melitensis*) officially free member state since 2015,

(Commission Implementing Decision (EU) 2015/129 of 26 January 2015).

- Last case of *Brucella melitensis* in bovine population occurred in 2008 - serologically and in sheep and goat population in 2010 - serologically (last culture confirmed case 2009).

BRUCELLA ABORTUS:

Cyprus has been recognized as Brucellosis (*B. abortus*) officially free member state (Commission Implementing Decision (EU) 2017/1910 of 17 October 2017).

• Brucella abortus was isolated in the period 1921-32 from imported cattle. Since then no outbreak of Brucella abortus in cattle occurred since 2017.

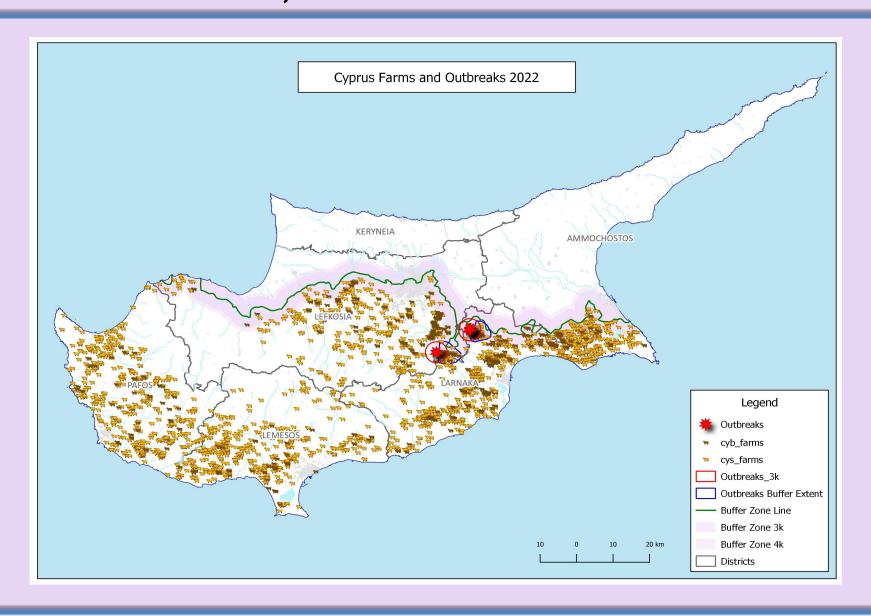
CATTLE POPULATION (IRSC 31/12/2021)

BOVINE ANIMALS	FARMS	TOTAL NUMBER OF ANIMALS	ANIMALS >12 MONTHS	ANIMALS >24 MONTHS (milking cows)
Breeding, double direction-milk and meat production farms with more than 10 animals >24 months old (milking cows)	259	81427	58684	42187
Breeding, double direction-milk and meat production farms with less than 10 animals >24 months old (milking cows)	30	429	331	98
Local breed (no milk) kept for the maintenance of the breed	58	1050	826	
Fattening (only males, only for slaughter)	25	551	247	
TOTAL	372	83457	60088	42285

SHEEP AND GOAT POPULATION IRSC 31/12/2021

SHEEP and GOATS	FARM S	TOTAL NUMBER OF ANIMALS	ANIMALS >6 MONTHS
Breeding, dual	2569	475944	471870
production, milk and meat production farms			
Animal Collection only	62	5772	5772
for slaughter	<u> </u>	- · · -	
(for up-to 29 days)			
Fattening only for	13	385	385
slaughter (<11 moths of			
age)			
TOTAL	2644	482101	478027

MAP 1: CATTLE HERD, SHEEP AND GOAT FLOCKS DISTRIBUTION



SURVEILLANCE PROGRAM

Cattle

Bulk milk sampling is carried out in all female animals over 24 months of age in holdings with more than 10 animals Sampling is performed twice a year

Samples are tested using ELISA method. In case of a positive sample, blood samples from all animals over 12 months are collected

Pre-movement tests with blood samples are performed prior to the movement of animals

Ovine and caprine

Two stage sampling stratified according to district level (farms to be tested) and herd size (animals to be tested) to detect prevalence of 0,2% and 95% confidence level

Pre-movement tests in blood samples are performed prior to the movement of animals in a 3km zone from the green-line

- ✓ Abortions are notifiable to the Veterinary Services
- ✓ All samples (embryo and blood) submitted due to abortions in the Veterinary Services are examined also for brucellosis

CASE 1- CATTLE FARM- OUTBREAK CY-B.MELIT-2022-00001

22.03.2022: The ELISA bulk milk test from one dairy cattle farm showed positive reaction **23.03.2022 & 24.03.2022:** Blood samples were collected from dairy cattle > 12 months for further laboratory examination with Rose Bengal and CFT tests and in 25.03.2022, out of 372 animals > 12 months,

54 cattle had been found positive for Brucella (Rose Bengal and CFT tests in parallel).

- 24.03.2022: Instructions for increased biosecurity measures and restrictions were imposed on the farm
- The officially free status of the holding was revoked and a scheduled for blood sampling was set within the control measures implemented for the specific positive holding
- Movement restrictions
- Isolation of positive from the negative bovine animals
- Separation, seizure and destruction of raw milk deriving from positive animals
- Epidemiological Investigation started
- **06.04.2022:** Brucella melitensis was isolated and identified from individual milk samples from the positive animals
- **15.04.2022:** An awareness meeting with the farmers was organized in order to update them as regards implementing better management of their farms in relation to brucellosis.
- **19.04.2022-19.5.2022:** 32 additional cattle were found positive.

All animals directed to slaughter.

CASE 1 – EPIDEMIOLOGICAL INVESTIGATION

- . Location of farm in the buffer zone (Louroujina area)
- . No incoming and outcoming animal movements were recorded
- . Lack of an established biosecurity plan (protocol)
- . No records of visitors were present
- . No fencing was available for setting the boundaries of the holding and preventing wildlife, stray animals and unwanted people and vehicles from approaching the animals and the feeding resources
- . No controlled entry for vehicles
- . Multiple agricultural roads leading to the holding
- . No disinfection station for incoming/outcoming vehicles.
- . Roughage were sourced from fields within the Green Line
- . Wildlife Diversity: The infected holding is located in the Buffer Zone. In this area hunting is forbidden and no pesticides are used, due to the absence of intensive agricultural cultivation.
- . These factors favor the presence of wild animals (hares, foxes, hedgehogs, birds, bats, snakes, wild dogs).

Most probable source of infection is contact with animals/use of forages related to the occupied

CASE 2 OUTBREAK CY-B.MELIT-2022-00003 IN CATTLE LINKED WITH CASE 1

24.05.2022: In terms of the blood sampling from all bovine animals > 12 months old, in Ahienou area after detecting Case 1 in cattle, one cow was found positive in RB and CFT (parallel testing) out of 506 animals.

25.05.2022: instructions for increased biosecurity measures and restrictions were imposed to the farm as in case 1 and epidemiological investigation started.

27.5.2022: Slaughter of the 1 positive animal, samples taken for culture.

7/6/2022: Brucella melitensis was isolated and identified

Same location with case 1 in cattle (160 m distance) in the buffer zone (Louroujina area)

The positive animal was imported from Germany and gave birth in 12/5/2020. Since then it was not fertilized due to health issues and did not give milk. The animal was kept in the dry period area.

Same situation on biosecurity as case 1 in cattle

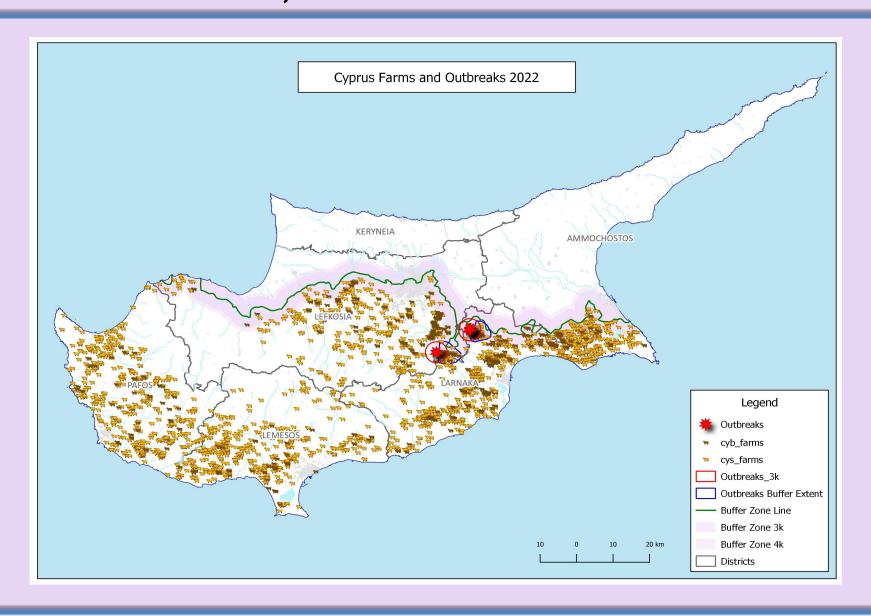
Roughage sourced from fields within the Green Line

The road leading to the farm passes next to case 1 farm

All animals bought and sold were examined for Brucellosis (pre-movement test)

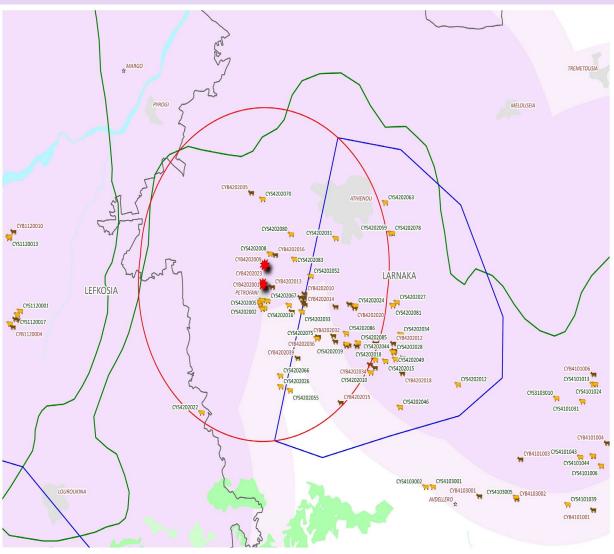
22 registered animals are missing from the farm and investigation is on-going.

MAP 1: CATTLE HERD, SHEEP AND GOAT FLOCKS DISTRIBUTION

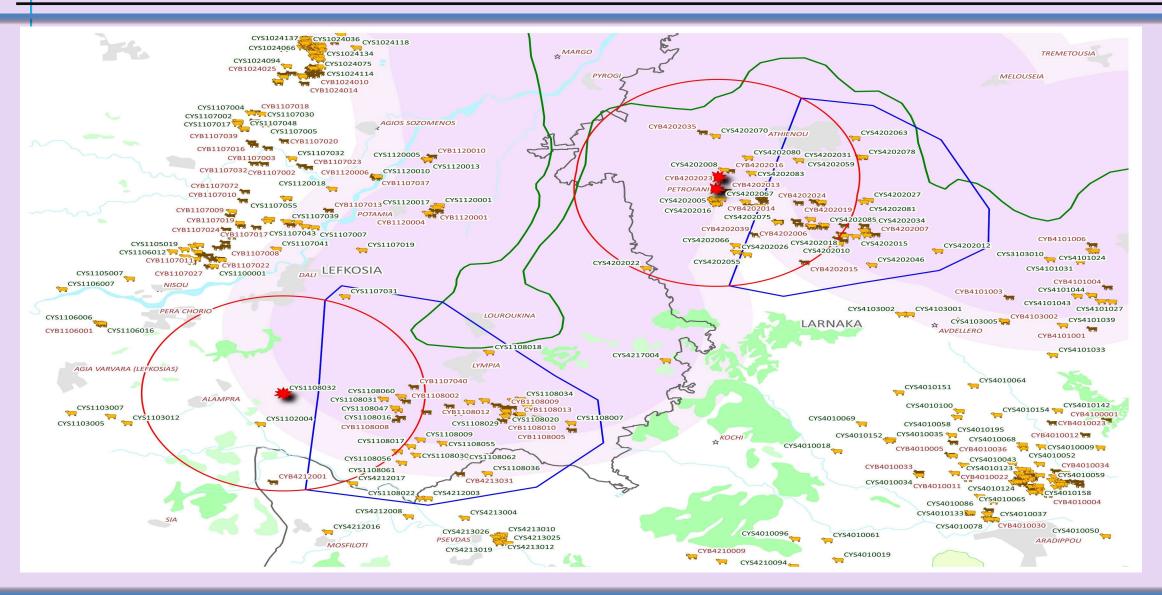


GEOLOCATION OF THE OCCURRED INCIDENCES BRUCELLA MELITENSIS 2022





GEOLOCATION OF THE OCCURRED INCIDENCES AND AREA SURVEYED



CASE 1 IN SHEEP AND GOATS OUTBREAK CY-B.MELIT-2022-00002

17.05.2022: In terms of the surveillance programme in sheep and goats in Nicosia district 6 animals showed positive reactions in RB and CFT in a flock in Lympia Area (Nicosia District).

17 and 18.05.2022: instructions for increased biosecurity measures and restrictions were imposed to the farm as in case 1 and epidemiological investigation started.

19.05.2022: seizure and killing of the 6 positive animals and sampling for cultures.

23-24.5.2022: blood sampling from all animals > 6 months (584 animals) and confiscation. 40 animals were positive in RB and CFT (parallel tested)

24-26.05.2022: slaughter/killing of all animals

25.05.2022: *Brucella melitensis* was isolated and identified

Epidemiological investigation still on going Location of farm in the buffer zone (Louroujina area) as case

1 and 2 in cattle

Lack of an established biosecurity plan (protocol)

Lack of disinfections

No records of visitors

No fencing was available for setting the boundaries of the holding and preventing wildlife, stray animals and unwanted

people and vehicles from approaching the animals and the feeding resources

No controlled entry for vehicles

No disinfection station for incoming/outcoming vehicles Roughage were sourced from own fields which are located around his farm

9 animals without ear-tags and investigation is on-going. High risk owner for illegal acts

FURTHER MEASURES TAKEN AFTER CASE 1, 2 AND 3

Bovines

27/03 -4/4/2022 and 2-6/5/2022: bulk milk was tested with negative results from all dairy cattle farms in Cyprus including the affected area (Athienou).

Already started:

- Blood sampling from all bovine animals > 12 months old in Ahienou area. Case 2 <u>From this</u> <u>sampling case 2 in cattle was detected</u> (serology 24/5/2022).
- Bulk milk sampling (one sample per 100 cows) in the rest of Larnaca and Nicosia District. To be repeated in October 2022.
- Bulk Milk sampling from all dairy farms in Cyprus in September 2022 (except Larnaca and Nicosia District) and in November 2022 (from all districts).

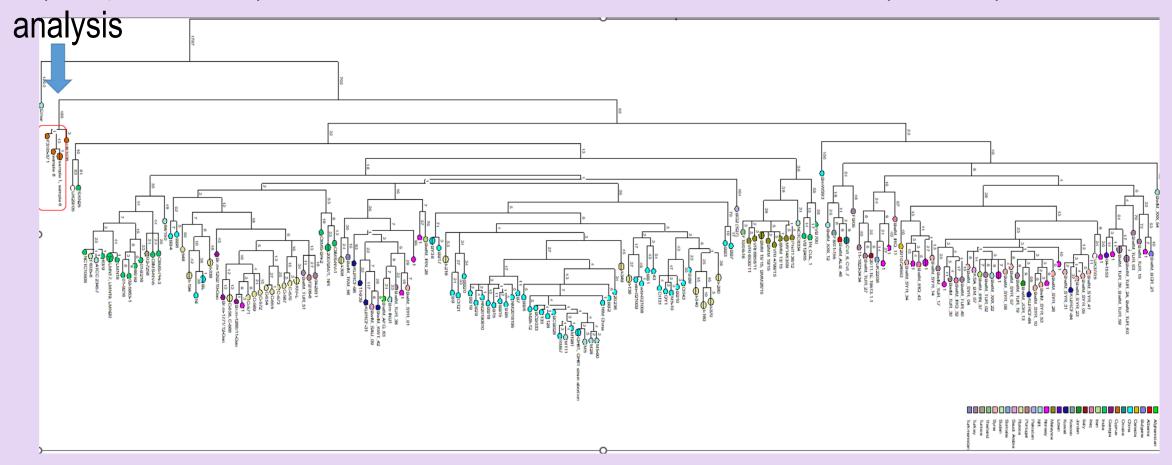
Ovine and caprine animals

28.03.2022: individual blood sampling program from all small ruminants >6 months within Athienou area was performed with negative results.

- Already started: Second blood sampling from all small ruminants > 6 months within Athienou area
- Same measures as above are also taken for Nicosia District
- Until the end of the year: surveillance programme covering 30% of small ruminants >6 months in the rest Larnaca and Nicosia District surveillance program covering 10% of small ruminants >6 months in the rest Districts (except Larnaca and Nicosia District). From this sampling case 1 in sheep and goats was detected (serology 17/5/2022).

SNP ANALYSIS

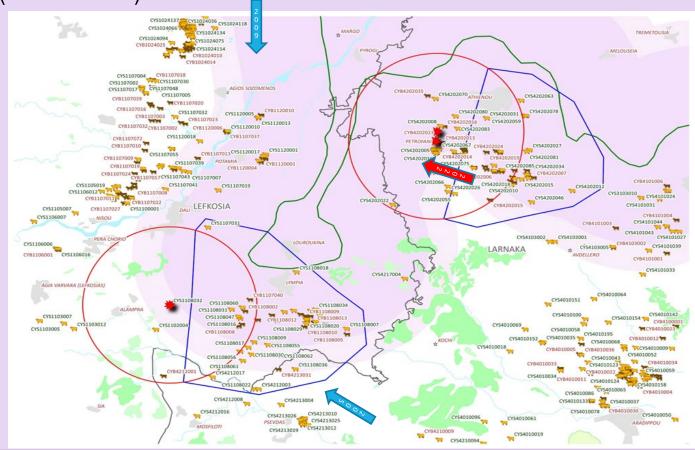
Whole Genome Sequencing (WGS) was performed for case 1 in cattle strains (samples 1, 6, 8) and raw data were sent to Brucellosis EURL (ANSES) for SNP



SNP analysis- the Cypriot Brucella melitensis cluster

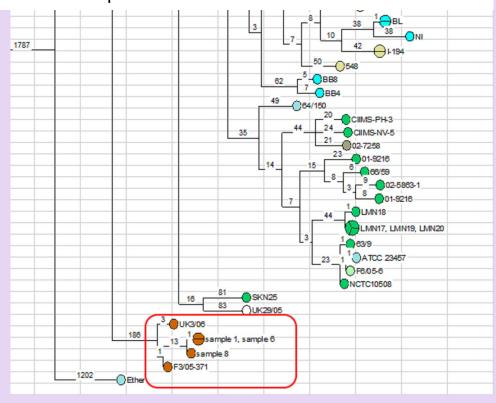
The strain isolated from case 1 in cattle is the strain which is circulating in Cyprus.

Further SNP analysis will be performed including also the strains from case 2 in cattle and case 1 in sheep and goats and an old strain of 2009 (CYS1108047).



UK3/06: Human strain from a traveler to Cyprus **_2006**-see map

F3_05_371: Strain from an old Brucella outbreak of **2005** in sheep and goats (CYS4217001)-see map Sample 1, 6, 8 strains isolated from case 1 in cattle in 2022 see map



CONCLUSIONS

Brucellosis is present in the occupied area of Cyprus:

«...The most important disease affecting mainly animal production is brucellosis in the northern part of Cyprus. Brucellosis is currently only

endemic throughout the northern part of Cyprus...». Technical assistance to improve implementation of food safety standards and disease crisis preparedness Service Contract No: 2021/423-933 Publication Reference: EuropeAid/140337/DH/SER/CY Implemented by NSF EC Consortium, Annex 3 to inception report

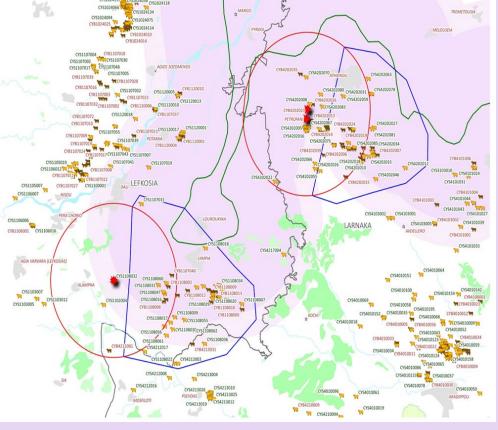
programmes. The EU Aid Programme also contributed clarification of animal health situation as well as initiation of the elimination of priority animal diseases in the northern part of Cyprus (such as brucellosis). Thanks to the EU support, from 2016 to 2019, prevalence of brucellosis among sheep and goats herds declined by 40% (from 10% herd prevalence in 2017 to 6% in 2019). In the same period the prevalence of brucellosis among cattle herds declined by 20% (from 10% herd prevalence in 2017 to 8% in 2019). In addition, the EU support programme actions implemented during 2020 and 2021

2 EU Aid Programme for the Turkisi-b Cypriot community (Regulation (EC) No 389/2006.



have achieved reaching the (preliminary) result of herd prevalence around 4% in cattle and sheep and goat population.

SPECIFIC TERMS OF REFERENCE Support in the area of animal health in the northern part of Cyprus FWC SIEA 2018-9847 LOT 1: Sustainable management of Natural Resources and resilience



The most probable source of infection in the buffer zone (Louroujina area) is contact with animals/use of forages related to the occupied areas.

¹https://ec.europa.eu/info/funding-tenders/funding-opportunities/funding-programmes/overview-funding-programmes/aid-programme-turkish-cypriot-community en-.

THANK YOU

I would like to express my thanks and my gratitude and to DG SANTE for their quick response to offer support through the EUVET formation in clarifying the source of the current case as well as to Jose Luis Saez Llorente and Fabrizio De Massis of the EUVET team configuration in investigating the incidences.

My whole-hearted thanks to my CyVS's collaborators and personnel for devoting their selves in proceeding with diligently performing the proper epidemiological investigation, follow up and management of the incidences as well as to our laboratory personnel, our personnel in the field as well as the administrative personnel who have offered and keep offering their services and devotion in combating and clearing these cases targeting the continuation of our BOFS Status.