

# Modulation of the One Health Approach to Tackle Brucellosis in Buffaloes and Cattle in Two Italian Territories with Different Characteristics

Alessandra Mazzeo<sup>1,\*</sup>, Patrizio Tremonte<sup>1</sup>, Nicola Rossi<sup>2</sup>, Carlo Ferrara<sup>3</sup>,  
Celestina Mascolo<sup>3</sup>, Silvia Jane Lombardi<sup>1</sup> and Elena Sorrentino<sup>1</sup>

<sup>1</sup>Department of Agricultural, Environmental and Food Sciences, University of Molise, Via Francesco de Sanctis s.n.c - 86100 Campobasso (IT), Italy

<sup>2</sup>Azienda Sanitaria Regionale del Molise, Department of Prevention - Complex Structure Animal Health, Piazza della Vittoria, 14 - 86100 Campobasso (IT), Italy

<sup>3</sup>Azienda Sanitaria Locale Caserta, Department of Prevention - Complex Structure Animal Health, Via Feudo San Martino - 81100 Caserta (IT), Italy

## Abstract:

*The new European Union animal health law and its rule concerning brucellosis in cattle and buffaloes*

The authors examine the latest European Union (EU) rules concerning eradication and surveillance of brucellosis and animal infectious diseases of EU concern.

*The Italian rules concerning brucellosis in cattle and buffaloes*

Italy is included in the EU co-financed compulsory eradication and surveillance programmes for brucellosis in cattle and in buffaloes in the frame of the EU and the related Italian laws, which allowed reaching the Brucellosis Free status without vaccination (former "Officially Brucellosis Free - OBF" status) in the majority of the northern and middle Italian Regions and in some middle and southern Provinces included in Regions where the infection persists.

*Epidemiology of brucellosis in the EU and in Italy*

In the Italian Province of Caserta, the highest prevalence of brucellosis in buffaloes is reported; in 2017-2021, a total of 314 outbreaks occurred, in which 39,163 heads tested positive. Here, brucellosis is threatening not only human health and the widespread buffaloes breeding but also the important satellite activities concerning the Protected Designation of Origin (P.O.D.) cheese "Mozzarella di Bufala Campana". The authors also discuss the reemerged brucellosis in cattle in the Molise Region, which despite bordering the Province of Caserta, shows different hydrographic, orographic, and breeding characteristics. In Molise, the reemerged brucellosis had a very different epidemiological course, which allowed it to limit the adoption of One Health measures.

*The One Health approach to tackle brucellosis in buffaloes*

In order to tackle brucellosis in Campania Region and in its Province of Caserta, the One Health approach has been predisposed through strict control of animal health, human health, and the environment. The adopted model could be exportable to territories having similar characteristics.

**Keywords:** One Health, *Brucella abortus*, Buffalo, Caserta, Campania, Molise, EU animal health law, Italian animal health rules, Epidemiology, Biosecurity, Environmental Control.

## INTRODUCTION

Italy is included in the European Union (EU) co-financed compulsory eradication and surveillance programmes for brucellosis in cattle and in buffaloes [1], which allowed reaching the Brucellosis-Free status (DF) without vaccination (former "Officially Brucellosis Free - OBF") in the majority of the northern and middle Italian Regions and in some middle and southern Provinces included in Regions where the infection

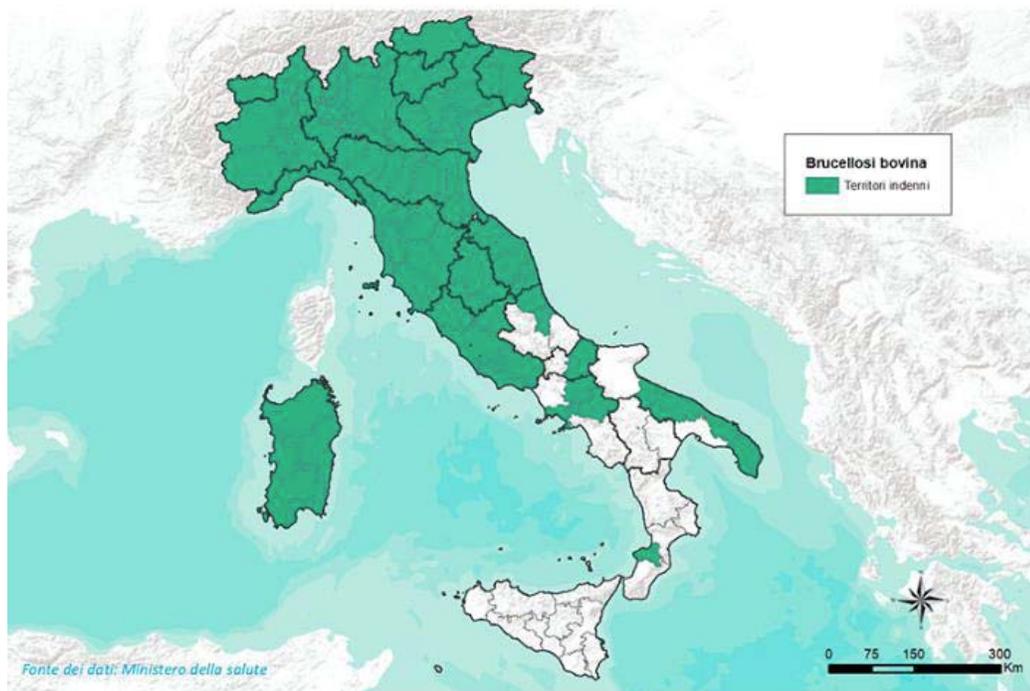
persists; two Italian Regions do not have Brucellosis-Free Provinces [2,3], (Table 1, Figure 1).

Currently, the European Commission (EC) assigns the status of "Member State or zone Free from infection with *Brucella abortus*, *B. melitensis*, and *B. suis*" for buffaloes/cattle when at least 99.8% of the establishments holding buffaloes/cattle, equivalent to at least 99.9% of the buffalo/cattle population, have maintained a status of free from infection with *Brucella abortus*, *B. melitensis*, and *B. suis* in the absence of outbreaks and without vaccination over the last three years (annual incidence rate lower than 0.1%). In Italy, the highest prevalence of brucellosis in buffaloes is

\*Address correspondence to this author at the Department of Agricultural, Environmental and Food Sciences, University of Molise, Via Francesco de Sanctis s.n.c - 86100 Campobasso (IT), Italy; Tel: +390874404874; E-mail: alessandramazzeo@unimol.it

**Table 1: Italian Regions and Provinces that Acquired or not the Disease-Free (DF) Status from Infection with *Brucella abortus*, *B. melitensis*, and *B. suis* in Bovine and Buffalo Populations [2]**

ITALIAN REGIONS	PROVINCES IN REGIONS
Non-DF ABRUZZO Region	DF Provinces: Pescara and Teramo
Non-DF BASILICATA Region	DF Provinces: none
Non-DF CALABRIA Region	DF Province: Vibo Valentia
Non-DF CAMPANIA Region	DF Provinces: Avellino, Benevento and Napoli
DF EMILIA ROMAGNA Region	(all the Provinces are DF)
DF FRIULI VENEZIA GIULIA Region	(all the Provinces are DF)
DF LAZIO Region	(all the Provinces are DF)
DF LIGURIA Region	(all the Provinces are DF)
DF LOMBARDIA Region	(all the Provinces are DF)
DF MARCHE Region	(all the Provinces are DF)
Non-DF MOLISE Region	DF Province: Campobasso
DF PIEMONTE Region	(all the Provinces are DF)
Non-DF PUGLIA Region	DF Provinces: Bari, Barletta-Andria-Trani, Brindisi and Lecce
DF SARDEGNA Region	(all the Provinces are DF)
Non-DF Sicilia Region	DF Provinces: none
DF TOSCANA Region	(all the Provinces are DF)
DF TRENTO ALTO ADIGE Region	(all the Provinces are DF)
DF UMBRIA Region	(all the Provinces are DF)
DF VALLE D'AOSTA Region	(all the Provinces are DF)
DF VENETO Region	(all the Provinces are DF)

**Figure 1: Disease-Free status (green areas) from infection with *Brucella abortus*, *B. melitensis*, and *B. suis* of the bovine population in Italian Regions and Provinces [3].**

reported in the Province of Caserta (where 43% of the national buffalo heads are concentrated).

Data concerning brucellosis in cattle and buffaloes in the Province of Caserta, in cattle establishments of the adjacent Molise Region, in Italy, and in the EU are reported and correlated with the rules in force at the various EU, national, and regional levels and with the environmental characteristics, in order to highlight the need of aligning the control of brucellosis with the One Health approach and the EU green policies.

### **THE NEW EU ANIMAL HEALTH LAW AND ITS RULE CONCERNING BRUCELLOSIS IN CATTLE AND BUFFALOES**

The EU's recent animal health legislation Regulation (EU) 2016/429 entered into force on April 21, 2021 [4]. This regulation establishes, among other things, rules for controlling and preventing transmissible animal diseases - including zoonoses - by outlining responsibilities for animal health, early detection and reporting of diseases, and implementing programs for surveillance and eradication. The regulation also covers the registration and approval of establishments and transporters, as well as the traceability of animals, germinal products, and products of animal origin within the EU. Additionally, it covers the import and export of animals, germinal products, and products of animal origin, as well as the non-commercial movement of pet animals. In the event of a disease emergency, the regulation also outlines emergency measures to be taken. The ultimate goal of this legislation is to improve animal health and support sustainable agriculture and aquaculture production in the EU while also promoting the effective functioning of the internal market and reducing the negative impacts of certain diseases on animal health, public health, and the environment.

Animals and related animal diseases of EU concern are listed in proper annexes.

The regulation takes into consideration the economic, social, cultural, and environmental effects of disease control and prevention measures, as well as international standards and the relationship between animal health and factors such as public health, the environment, food and feed safety, animal welfare, antimicrobial resistance, and food security.

Regulation (EU) 2016/429 refers, for many aspects, to the issuance of delegated acts and implementing acts. EC may also adopt immediately applicable

implementing acts if a listed disease spreads rapidly and poses a significant risk to animal or public health, the economy, or society. Biosafety and biosecurity measures are of paramount importance, with biosecurity involving management and physical measures to reduce the risk of disease introduction, spread, and development within animal populations, establishments, zones, or compartments. Biosecurity measures for terrestrial animals may include physical protection measures, such as enclosing, fencing, roofing, netting, cleaning, disinfection, and control of insects and rodents, as well as management measures, such as procedures for entering and exiting establishments, use of equipment and conditions for movement, the introduction of animals or products, quarantine, isolation, or separation of newly introduced or sick animals, and a system for safe disposal of dead animals and other animal by-products.

The Commission Implementing Regulation CIR (EU) 2020/2022 details compulsory notification and annual reporting requirements from Member States to the Animal Disease Information System [5].

In the Commission Implementing Regulation CIR (EU) 2018/1882 [6], the Commission carried out a systematic evaluation that took into account various factors such as the receptive species to certain listed diseases, the disease vectors, whether the listed disease is currently present or not in the EU and its modes of transmission between animals and from animals to humans, as well as its potential impact on human and animal health, including morbidity and mortality rates, the broader impact of such listed diseases, such as their effects on the economy, society, animal welfare, environment, and biodiversity. Based on this evaluation, the diseases have been "categorized" into:

- "Category A disease", which includes diseases listed in Regulation (EU) 2016/429 that do not normally occur in the EU and for which immediate eradication measures must be taken as soon as detected;
- "Category B disease", which includes listed diseases that must be controlled in all Member States with the goal of eradicating them throughout the EU;
- "Category C disease" includes listed diseases that are of relevance to some Member States and for which measures are needed to prevent it

from spreading to parts of the EU that are disease-free or that have eradication programmes for the listed disease concerned;

- “Category D disease” includes listed diseases for which measures are needed to prevent it from spreading on account of its entry into the EU or movements between Member States;
- “Category E disease” includes listed diseases for which there is a need for surveillance within the EU.

The disease "infection with *Brucella abortus*, *B. melitensis*, and *B. suis*" is classified as follows:

- in the listed species *Bison* spp., *Bos* spp., *Bubalus* spp., *Ovis* spp., *Capra* spp., as categories B+D+E;
- in the listed group *Artiodactyla*, in species other than *Bison* spp., *Bos* spp., *Bubalus* spp., *Ovis* spp., *Capra* spp., as categories D+E;
- in the listed groups *Perissodactyla*, *Carnivora*, and *Lagomorpha*, as category E.

In summary, in 2021, the EU's regulatory framework for brucellosis in animals underwent significant changes with the implementation of Regulation 2016/429. This regulation mandates eradication programs for infection with *B. abortus*, *B. melitensis*, and *B. suis* in bovines, sheep, and goats and control programs in *Artiodactyla*. CIR (EU) 2018/1882 designates *Bos* spp., *Bubalus* spp., *Bison* spp., *Ovis* spp., and *Capra* spp. as species subject to notification, surveillance, prevention, certification, and mandatory eradication of infection with the *Brucella* mentioned above species.

The delegated regulation 2020/689 of December 17, 2019 [7] - which supplements Regulation (EU) 2016/429 - contains rules on surveillance, eradication programmes, and DF status for certain listed and emerging diseases, including brucellosis.

### **THE ITALIAN RULES CONCERNING BRUCELLOSIS IN CATTLE AND BUFFALOES**

Tighten measures were put in place in 2012 to prevent the spread of brucellosis in southern Italy, and they were strengthened in 2015 [8] to cover the entire country, with a prohibition on possession and use - with certain exceptions - of reagents for the diagnosis of *Brucella abortus*, *B. melitensis*, and *B. suis* infection.

These measures were renewed annually up to 2023, with the most recent being the Italian Minister of Health's Ordinance issued on 14.06.2022 [9], which adopts the official analytical methods listed in CIR (EU) 2018/1882 for the detection of the disease in blood and milk samples. The Ordinance requires that the General Managers of local health authorities must work towards eradicating tuberculosis, brucellosis in cattle and buffaloes, ovine and caprine brucellosis, and enzootic bovine leukosis in order to obtain DF status. The previous health status OBF is replaced with DF. In non-DF zones for infection with *B. abortus*, *B. melitensis*, and *B. suis* - with or without vaccination - animals whose blood sample tests result positive in the Complement Fixation Test (CFT) and positive or negative in the buffered *Brucella* antigen test/Rose Bengal Test (RBT) are considered "suspected"; they are "confirmed" if the etiological agent is isolated, or its DNA or an antigen is detected in the same animal, or if serological tests on animals from the same establishment or epidemiologically connected with positive or suspected cases and presenting clinical signs give positive results. The result is considered doubtful in the case of positive RBT and negative CFT results, and serological tests on the animal must be repeated. In the presence of suspected or doubtful cases, the DF status of the establishment is suspended, transfer (entry or exit) of receptive animals is forbidden - unless for immediate culling in a designated slaughterhouse - and molecular and bacteriological tests are carried out in milk and vaginal swabs of animals in *post partum*. The Italian Minister's Ordinance-reassesses the serological tests as heads that test positive for RBT are to be considered infected in case of an outbreak. In Disease-Free establishments without vaccination, the indirect-ELISA test in milk samples can be used as a screening test if at least 30% of dairy cattle are in lactation [9].

Furthermore, on September 27, 2022, Legislative Decree No. 136/2022, issued on August 5, 2022 [10], came into effect in order to align and reconcile national regulations on the prevention and control of animal diseases that are transmissible to animals or humans with the provisions of Regulation (EU) 2016/429.

The simultaneously issued Legislative Decree No. 134/2022 [11] greatly emphasizes animal identification, registration, traceability, and animal movement. The National Data Bank was established, which is fully accessible by health authorities and public administrations. Operators, transporters, and all professionals involved in animal identification and

traceability are responsible for the data entered into the National Data Bank. Also, suppliers of animal identification systems must provide a conformity certificate issued by the International Committee for Animal Recording for any type of tools used.

Recently, Italy adopted the EU Parliament Resolution of October 19, 2021, which calls for a 50% reduction in the total sales of antibiotics for farm animals and the aquaculture sector by 2030, in line with the EU's Farm to Fork Strategy and the EC Green Deal. To achieve this goal, Italy has developed the ClassyFarm system [12], which is included in the veterinary portal VETINFO of the Italian Ministry of Health [13]. The ClassyFarm system was financed and implemented by the Italian Ministry of Health in collaboration with the Experimental Zooprophyllactic Institute of Lombardy and Emilia Romagna (IZS LER) and the University of Parma. The ClassyFarm system is integrated into the national veterinary portal, and it allows the detection, collection, and processing of data in the following areas: biosecurity, animal welfare, health and production parameters, animal feed, consumption of antimicrobial drugs and lesions found at the slaughterhouse.

Obliging the registration of veterinary drug treatments in electronic format [14], Italian law fights antimicrobial resistance (AMR), the second ongoing pandemic [15]. The integrated ClassyFarm system also allows the categorization of livestock on the basis of risk development and diffusion.

## EPIDEMIOLOGY OF BRUCELLOSIS IN HUMAN BEINGS, CATTLE, AND BUFFALOES

### European Union

As per Directive 2003/99/EC [16], Annex I list A, reporting of any *Brucella* species is mandatory. Therefore, the European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) zoonosis reports include epidemiological data on brucellosis in the EU. The *EU One Health 2021 Zoonoses Report*, published on 13.12.2022 [17], highlights the state of infections with *Brucella* in the European Union. The report states that, in 2021, 162 cases of confirmed human brucellosis occurred in the EU, with Bulgaria, Estonia, Finland, Hungary, Ireland, Latvia, Lithuania, Malta, Romania, and Slovenia reporting no cases; only in 2 cases (3.4%) *B. abortus* was identified as the etiological agent. Italy did not report the origin of infection in human beings for 2021. Brucellosis in humans has

seen a significant decline in the EU, with a decrease confirmed in Germany, Greece, and Italy, which have non-Disease-Free Zones (non-DFZs). Greece, Italy, and Spain reported the highest number of confirmed brucellosis cases in humans, while Greece, Italy, and Portugal had the highest prevalence of *Brucella*-positive ruminant herds. In 2021, the prevalence of brucellosis-infected cattle herds remained very low in the non-DF zones of the six non-Disease-Free Member States, with 546 positive herds (0.43%), compared with 603 (0.38%) positive herds in 2020. In the EU, only eight infected cattle herds were reported in the DF zones (six in 2020), with Italy reporting seven positive herds and France one. The number of positive herds out of all herds reported by Italy in its non-DFS zones was 453 (504 in 2020), while this number was 24 in Portugal (27 in 2020). Greece reported 69 positive herds (72 in 2020), but the overall number of infected herds should be interpreted differently between countries, as the proportion of tested herds was highly variable between Member States.

In the EU, comparing data from 2012 to 2021, the overall annual number of reported infected cattle herds in non-DF zones decreased by 53.7%, from 1,181 to 546 herds, while the prevalence of infected cattle herds increased from 0.10% to 0.43% due to the drop in the total number of cattle herds of interest: from 1,162,978 to 127,000. No speciation of *Brucella* isolates was reported for cattle.

In the EU *Brucella* species were reported from a wide range of animal species; *B. suis* biovar 2 or unspecified *Brucella* species were reported in pigs, wild boars, and wild hares in seven Member States (Finland, France, Germany, Italy, Romania, Slovakia, and Spain); *B. melitensis* was isolated from Alpine ibex in France; unspecified *Brucella* species were found in wild bears, wild deer, and wild boars.

Eradication of brucellosis in cattle has been achieved in Croatia and Spain [17].

In the EU, brucellosis cases in humans and ruminants are on the decline, yet the disease remains a significant threat to both animal and public health. The data shows a clear trend of increasing positivity from the northern EU Member States, which are disease-free, to the southern Member States, particularly in the Mediterranean region.

### Italy

In Italy, 13 of the northern and middle Italian Regions and some middle and southern Provinces

included in the Regions where the infection persists acquired the Brucellosis Free status; Basilicata and Sicily Regions do not have Brucellosis-Free Provinces (Table 1, Figure 1).

In 2019, 99.55% of herds under the bovine brucellosis eradication and surveillance programmes were controlled. The national prevalence was 0.42%, with the highest rates in non-OBF regions at 1.25%. Sicily, in particular, continues to be a public health

**Table 2: The Reemerging Infection with *Brucella abortus* Biovar 3 in Cattle in Molise (15.10.2019 - 10.05.2021)**

OUTBREAK NUMBER	PROVINCE OF INSURGENCE	No. of CULLED CATTLE/No. of FARMED CATTLE
REEMERGENCE		
1. 2019/400	IS (15/10/2019)	52/52 <sup>(*)</sup>
2. 2019/429	IS	116/129 <sup>(*)</sup>
3. 2019/433	IS	56/58 <sup>(*)</sup>
4. 2019/449	IS	91/96 <sup>(*)</sup>
5. 2019/450	IS	87/96 <sup>(*)</sup>
6. 2019/451	IS	3/31
7. 2019/455	IS	3/60
8. 2019/456	IS	3/24
9. 2019/457	IS	1/26
10. 2019/459	IS	18/18 <sup>(*)</sup>
11. 2019/469	IS	18/18 <sup>(*)</sup>
12. 2019/470	IS	65/65 <sup>(*)</sup>
13. 2019/471	IS	3/13
14. 2020/5	IS	1/12
15. 2020/10	IS	41/41 <sup>(*)</sup>
16. 2020/13	CB	2/46
17. 2020/17	IS	3/69
18. 2020/23	CB	2/2 <sup>(*)</sup>
19. 2020/24	IS	34/34 <sup>(*)</sup>
20. 2020/27	CB	2/2 <sup>(*)</sup>
21. 2020/29	IS	3/160
22. 2020/32	IS	8/8 <sup>(*)</sup>
23. 2020/83	IS	14/14 <sup>(*)</sup>
24. 2020/92	IS	1/69
25. 2020/93	IS	1/53
26. 2020/134	IS	1/27
27. 2020/186	IS	1/263
28. 2020/281	IS	40/40 <sup>(*)</sup>
29. 2020/354	IS	1/16
30. 2020/391	IS	1/20
31. 2020/445	IS	1/29
32. 2020/478	IS	1/34
33. 2021/135	IS (10.05.2021)	58/58 <sup>(*)</sup>

No. of CATTLE FARMED IN THE ESTABLISHMENTS WHERE OUTBREAKS OCCURRED: 1,683.

No. of CULLED CATTLE: 732 (43.49%).

(\*) stamping out operations.

threat due to the majority of Italian human cases (89%) being reported there and the highest prevalence (2.36%). All OBF regions and provinces maintained their status, with some provinces meeting the requirements for OBF status. In Campania, almost 100% of herds under the bovine brucellosis eradication and surveillance programs were controlled, with the Provinces of Napoli, Avellino e Benevento which acquired the OBF status [18-20].

In 2020, brucellosis in humans continued to decline; this is also due to the COVID-19 pandemic during which international travel was considerably reduced and, therefore, the risk for human beings of becoming infected in endemic areas traditionally suited to tourism is likely to have been much smaller.

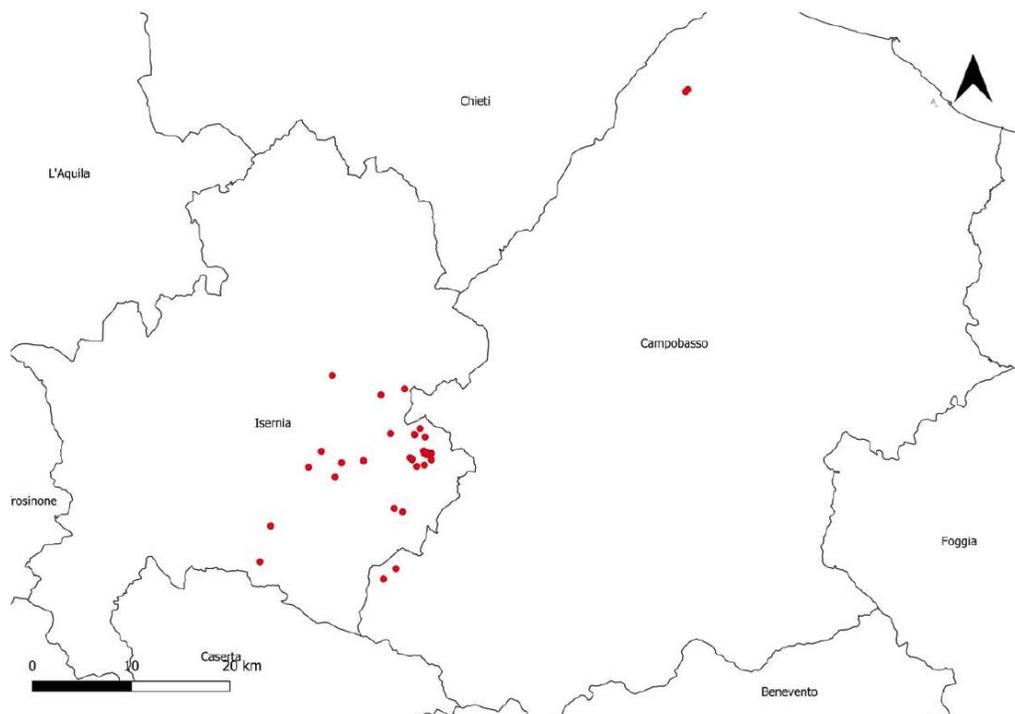
In 2021, the majority of problems were concentrated in the southern part of the Italian peninsula and in Sicily, with a focus on the Provinces of Salerno and Caserta in the Campania Region.

### Molise Region

In 2017 no outbreak of brucellosis in cattle occurred in the Molise Region, which includes the Province of Campobasso (CB) and the province of Isernia (IS), both bordering the Province of Caserta (CE) in the Campania Region. In 2018, CB had a reassuringly low number of cases of bovine brucellosis, with only 1

establishment having 4 positive heads of the 83 farmed cattle out of a total of 11,719 animals tested through the Rose Bengal Test (RBT) as part of the eradication programme. The data collected in IS were similarly low since only 1 outbreak occurred in cattle establishments in 2018 (in December), followed by another outbreak in January 2019. They induced the culling of 4 heads of a total of 128 housed in the 2 establishments. No outbreaks arose in the following nine months. This was in contrast to Sicily, where 1,972 cattle were culled, and to the neighboring regions such as Puglia, where 1,372 cattle were culled, and Campania, where 214 cattle were culled for brucellosis.

Additionally, there was a high incidence of the disease in buffaloes in Campania, resulting in the culling of roughly 5,000 heads [21-23]. Therefore, the data from Molise appeared to be a positive indication of the effectiveness of the brucellosis eradication efforts in the Region. In October 2019, a resurgence of cattle brucellosis started in IS, which also spread during 2020, reaching CB and inducing, in the following 19 months, a total of 33 outbreaks, of which 30 occurred in IS and 3 in CB (Table 2, Figure 2). It resulted in the culling of 732 cattle out of a total of 1,683 farmed animals in the affected establishments, equivalent to 43,49%. The stamping out operations concerned 16 cattle establishments; in the mixed establishments where infection of *B. abortus* was detected in sheep



**Figure 2:** Map of outbreaks that occurred in the Molise Region (01.01.2017-31.12.2021).

and goat heads, all the flocks were culled [24]. The causative agent was identified as *Brucella abortus* biovar 3 in all cases that occurred in Molise in 2019-2021 and in which molecular tests were performed. Compensation was provided to affected farmers. Notably, no human cases of brucellosis were reported. Outbreaks were quickly contained, but the economic impact was severe due to the concurrent COVID-19 pandemic [21]. In late 2021 no outbreak arose, nor outbreaks occurred in the only buffalo establishment located in Venafrò (IS), the nearest Molise municipality in respect of the Province of Caserta.

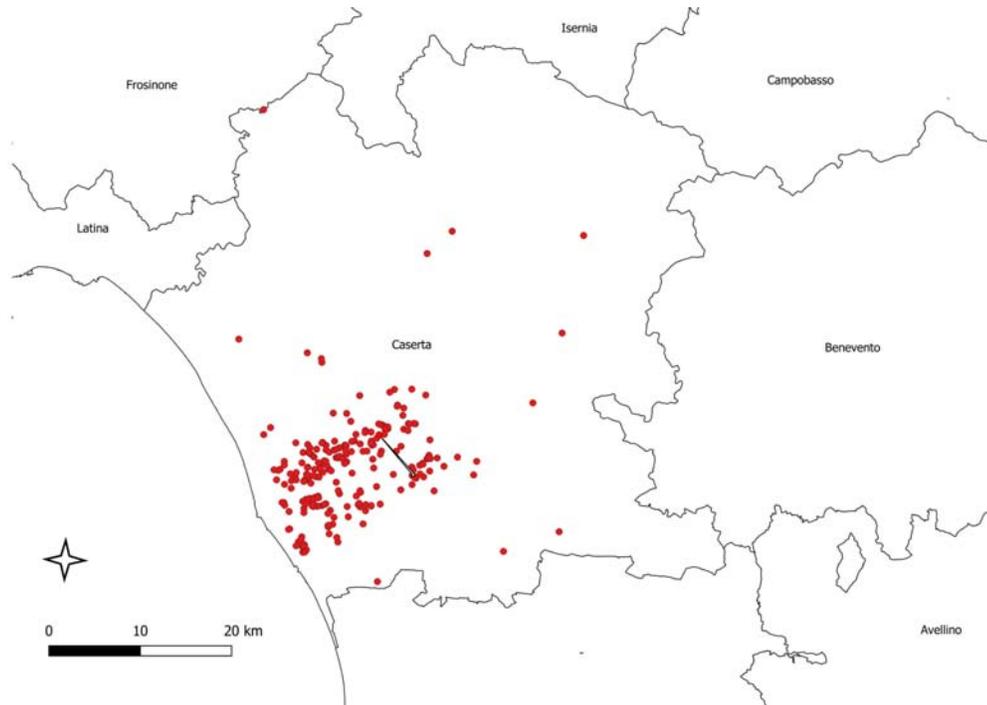
### Province of Caserta

The Province of Caserta shows significant prevalence and incidence of infection among buffaloes. In the period 01.01.2017 - 31.12.2021, in the Province of Caserta, 314 outbreaks of buffalo brucellosis

occurred (Figure 3), [24], in which 39,163 heads were positive to tests (Tables 3,4), and *Brucella abortus* biovar 1 was frequently isolated. This is of great concern as the majority of Campania's buffalo population, approximately 70%, is concentrated in Caserta (43,28%) and Salerno (25,58%) Provinces [25]. The buffalo livestock has undergone variations over time, as shown in Figures 4 and 5 [26].

### THE ONE HEALTH APPROACH TO TACKLE BRUCELLOSIS IN BUFFALOES

One Health recognizes the interdependence of human health, animal health, and the health of the environment. It is a holistic approach adopted worldwide to coordinate multi-sectoral efforts to prevent, respond and prepare for zoonoses and other diseases [27].



**Figure 3:** Map of outbreaks that occurred in the Province of Caserta (01.01.2017 - 31.12.2021).

**Table 3: Data on Brucellosis in the Buffalo Population in the Province of Caserta (01.01.2017 - 31.12.2021)**

YEAR	POSITIVE HEADS	PREVALENCE IN THE BUFFALO POPULATION (%)
2017	3,797	2.20
2018	5,727	3.80
2019	8,766	4.78
2020	11,930	6.63
2021	8,943	5.04

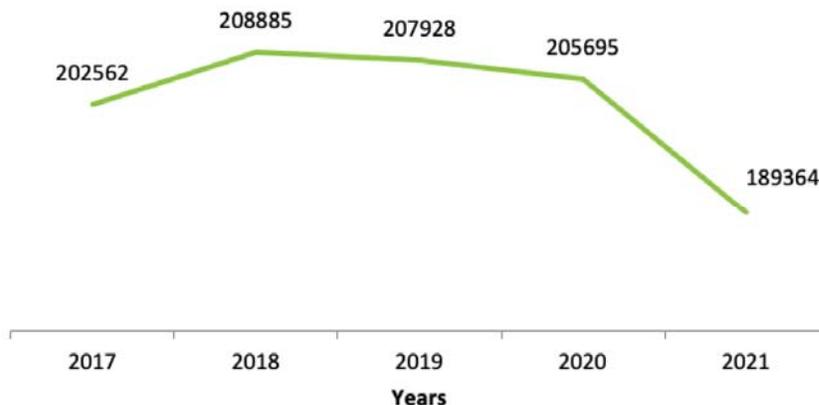
TOTAL NUMBER OF POSITIVE HEADS: 39,163.

**Table 4: Data on Brucellosis in Buffalo Establishments in the Province of Caserta (01.01.2017 -31.12.2021)**

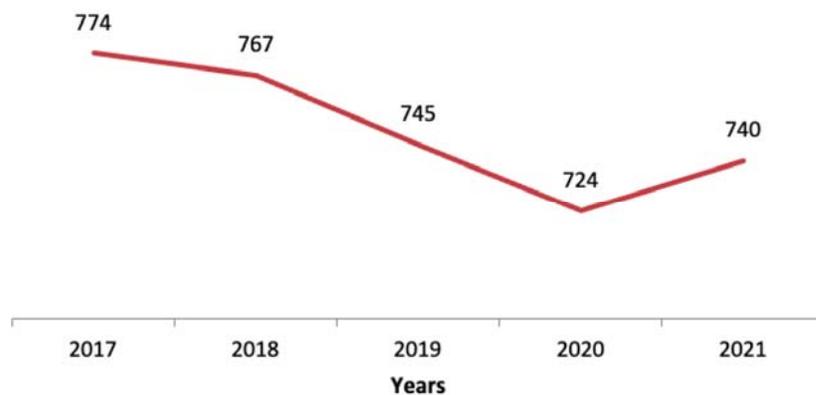
YEAR	PREVALENT OUTBREAKS <sup>1</sup>	INCIDENT OUTBREAKS <sup>2</sup>	PREVALENCE	INCIDENCE	STAMPING OUT
2017	40	32	5.19	4.15	10
2018	59	43	7.87	5.73	15
2019	85	63	11.47	8.50	17
2020	106	75	14.64	10.36	14
2021	131	87	17.70	11.76	30

<sup>1</sup>prevalent outbreaks: number of buffalo establishments where the brucellosis outbreak persists during the year under consideration.

<sup>2</sup>incident outbreaks: number of unaffected buffalo establishments where the first case of brucellosis is detected with the confirmed outbreak date in the year.



**Figure 4:** Number of farmed buffaloes in the Province of Caserta (01.01.2017 - 31.12.2021).



**Figure 5:** Number of buffalo establishments in the Province of Caserta (01.01.2017 - 31.12.2021).

The One Health approach to fight brucellosis in buffaloes in the Campania Region was imposed with "Decision of the Regional Government of Campania No. 104 of 08.03.2022 - mandatory program for the eradication of infectious diseases of bovine and buffalo species in the Campania Region" [28,29]. The Mandatory Programme for the eradication of infectious diseases in Cattle and Buffaloes in Campania aims to solve a long-standing problem affecting livestock - especially in the Province of Caserta - through a series of interdisciplinary interventions and activities aimed at achieving a very important objective: to have a stable model with very high biosafety standards.

The Mandatory Programme intervenes on:

- identification of Cluster Areas and Non-Cluster Areas, distinguished for the two diseases covered by the Programme: brucellosis and tuberculosis ("Cluster Areas" are defined as those falling within 2 km around each of the infected establishments, with at least 2 active outbreaks in the last 2 years, 1 of which repeated in the last 5 years, or 3 active outbreaks in the last 2 years, considering the positive establishment that generated the buffer; "Cluster infection municipality" is defined as

those including an area that covers around 50% of the municipal area; in these municipalities, all measures provided for infection cluster areas are applied);

- control modalities for tuberculosis and brucellosis, with related timing and prescriptions, differentiating interventions in Non-Cluster Areas from those in Cluster Areas;
- vaccination programme with RB51 vaccine for impuberal females (6 to 9 months of age);
- development of a self-checking system for operators/breeders, also through the valorization of the establishment's veterinary doctor;
- strengthening of biosecurity measures in the establishment;
- structural company parameters;
- management of effluents, wastewater, and digestate, for agricultural utilization;
- epidemiological surveillance on wildlife;
- support for investments in agricultural establishments dedicated to the buffalo sector;
- investments aimed at reducing nitrogen content and agronomic valorization of livestock effluents;
- mitigation of microbiological risk of water origin through intervention on the territory, at various levels, on canal management and territory maintenance.

Specific and extraordinary measures to fight tuberculosis and brucellosis are provided, especially for the Caserta Province and all areas of the Campania Region identified as infection Cluster Areas. In these cases, rules have been provided for:

- block or limitation of IN / OUT movements in Cluster Areas;
- positive/infected animals have to be culled within 4 working days;
- review of establishment codes present in infection Cluster Areas, with the attribution of a single code to the facilities, even if owned by different owners, attributable to a single epidemiological unit;

- in the intervals between the routine serological controls: milk-ELISA tests performed in bulk milk.

Regarding the personnel operating in livestock establishments, the Veterinary Service of CE adopts the following procedure: when an outbreak occurs, the Veterinary Service reports the company code to the Occupational Health Service and to doctors of the Collective Prevention; then doctors go to perform visits and exams on the personnel in the establishment site.

## DISCUSSION AND CONCLUSIONS

From the One Health perspective, special attention must be paid to the disproportionate impact of emerging zoonoses that may be linked to systemic inequities; in fact, the burden of zoonoses and other challenges – such as the climate crisis – are not evenly distributed [30]. The prevalence of human brucellosis differs between areas and has been reported to vary with standards of personal and environmental hygiene [31]. According to the World Health Organization (WHO), brucellosis is classified as one of the seven neglected zoonotic diseases involved in a high portion of poverty in developing countries, where control for brucellosis outbreaks could be valuable in the preservation of dairy herds [32]. The poverty level is highly associated with the occurrence of disease, which results in colossal economic losses worldwide in terms of animal health and production as well as from public health aspects in terms of the cost of human treatments and loss of animal productivity [33]. Although human brucellosis is the most widespread zoonosis worldwide, with more than 500,000 new cases annually, it remains severely neglected as a potential cause for chronic, debilitating maladies since the human case-fatality proportion is very low (< 1%) [34,35]. In a study of brucellosis incidence among pastoralist and agropastoralist communities in some areas of Kenya and Tanzania, a total of 1,140 cases were found in selected study areas with an overall incidence of 22.7% (n=1140). By country, the incidence rates were 28.2% (n=578) and 17.1% (n=562) in selected areas of Tanzania and Kenya, respectively. In a study assessing the status of brucellosis in and around Limpopo National Park, the brucellosis seroprevalence in buffaloes was found to be 17.72% and 27.42% using Rose Bengal Test (RBT) and ELISA, respectively. Serum samples from 13 of 133 (9.77%) tested positive for bovine brucellosis using Rose Bengal Test [36].

Since it would be counterproductive to jeopardize Disease-Free territories [34], causing the continuous

reemerging of outbreaks, the worldwide application of the One Health approach can improve the efficient use of resources (finances, infrastructures, and personnel) and the quality and timeliness of veterinary interventions; engagement, assessment, planning, implementation, monitoring, prioritization, evaluation and preparedness are then needed [37,38].

The Molise Region – one of the two Italian territories taken into consideration in the present study – is characterized by the low density of establishments subject to the brucellosis eradication programme, low number of animals per establishment (Table 2), a predominantly mountainous Apennine territory, absence of large-flow watercourses (Figure 6), [39] and almost exclusive family management of establishments, that allows to easily standardize the hygiene rules adopted both at the farm and personal level. For these reasons, the One Health approach to tackle the reemerged brucellosis in Molise could be limited to the observance of veterinary procedures in compliance with the legislative provisions in force on animal health, with particular attention to the timeliness of interventions, biosecurity, and the use of pastures. This allowed the rapid extinction of the outbreaks, despite the simultaneous imposition of sudden and

stringent restrictions caused by the onset of the COVID-19 pandemic [21].

In the Province of Caserta, instead, brucellosis cannot be considered solely as an animal health problem with zoonotic implications. It is also a human health problem - physical, mental, and economic - that is seriously endangered by the important epidemiological trend of brucellosis found in the area. In particular, the economic insecurity resulting from it could have a negative impact on the living conditions (such as the quality of housing, personal hygiene, attention to disease prevention, etc.) of establishment owners and workers, as highlighted in the EFSA-ECDC zoonosis report, which states that non-foodborne transmission of brucellosis to humans may still occur in non-Disease Free Zones through direct contact with infected animals, putting people who work with farm animals, such as farmers, livestock breeders, cheesemakers, butchers, abattoir workers, and veterinarians, at increased risk [17]. Probably, it is due to behaviors that are not attentive to prevention induced by mental and economic stress. All this leads to the need for financial aid that goes beyond compensation for slaughtered animals. The Campania Region is, in fact, evaluating the possibility of providing

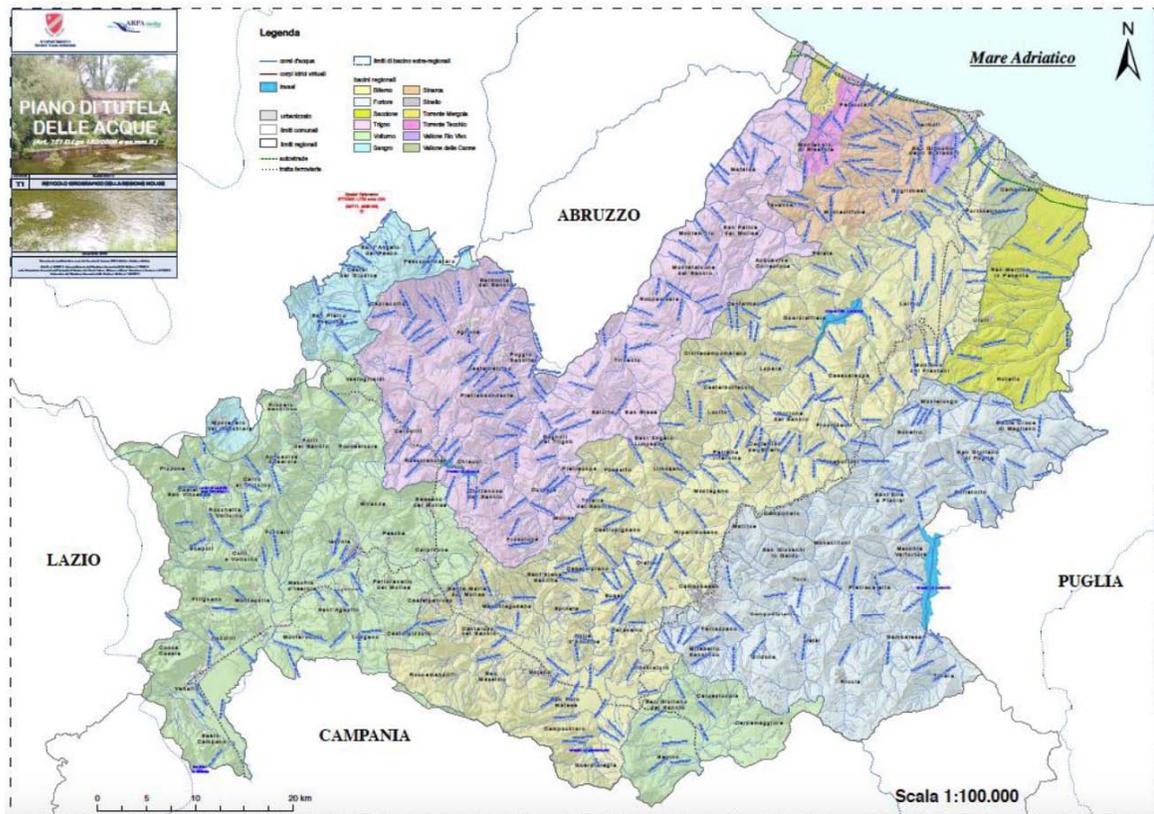


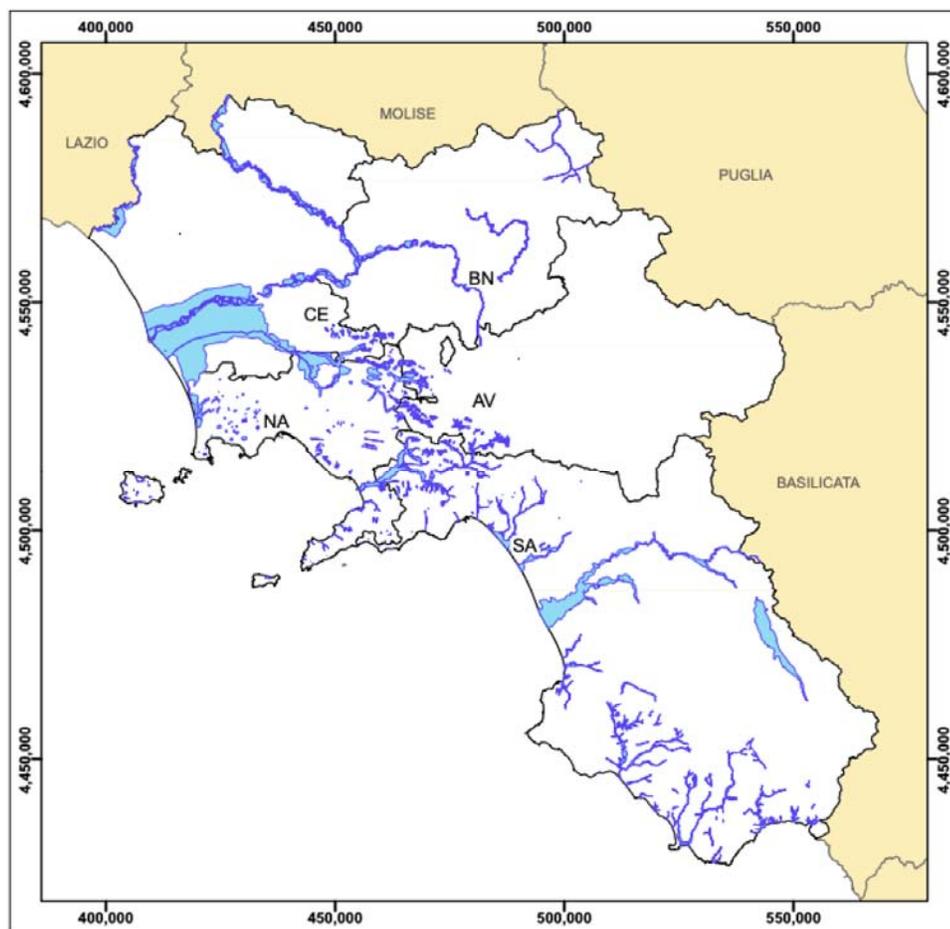
Figure 6: Hydrographic grid of the Molise Region [39].

contributions to revive businesses in crisis due to brucellosis economically, but this should be done with careful planning of: - animal load on the territory (where the highest concentration of buffalo population in Italy persists), [17]; - adequate spacing between companies; - accountability interventions for breeders, who must work in harmony with veterinary management, rather than in opposition; - public engagement interventions for citizens, who must be co-responsible for valuing the excellence product of their land, the Protected Designation of Origin (P.O.D.) cheese "Mozzarella di Bufala Campana" which is a significant source of income.

Since the success of the approach to tackle brucellosis depends on the willingness of the decision-makers to implement the necessary policies [37], in the Province of Caserta, significant efforts have been activated to eradicate brucellosis in buffaloes, with particular attention given to the One Health approach. By aligning the need to eradicate infectious diseases with the need to avoid contaminating the territory,

progress has been made in solving the long-standing brucellosis problem.

The Province of Caserta has hydrographic and orographic characteristics that induce critical issues: the presence of rivers, including the Volturno, which originates in Molise and crosses the Province of Caserta (receiving tributaries) up to its mouth and is the longest river and the main one by the flow of water from southern Italy; low altitude and slope, up to the presence of depressed areas near the mouth of the river (Lower Volturno). In the past, the Lower Volturno area was swampy and marshy, representing a favorable environment for buffaloes. Its reclamation was obtained with the construction of a capillary net of water outflow canals that flow into main collectors. The system is equipped with dewatering pumps, essential for the outflow of water from the depressed areas to the sea, but in periods of greater water flow, occasional flooding occurs in some areas adjacent to the waterways (Figure 7) [40]. In order to contain the environmental contamination by pathogens favored by



**Figure 7:** Floodable areas of the Province of Caserta (CE) and the Campania Region [40].

Floodable areas  .

the floods of the Lower Volturno territories, the Campania Region has financed exceptional interventions for cleaning the aforementioned canals and restoring the efficiency of the entire water outflow system, which have been entrusted to the General Consortium for the Reclamation of the Lower Volturno Basin [41] and are coordinated by the Extraordinary Commissioner for overcoming the emergency related to the eradication of infectious diseases of bovine and buffalo species in the Campania Region. The Consortium works in tandem with the local Veterinary Services to secure establishments in critical areas exposed to flooding. In compliance with the One Health approach, these interventions integrate the biosecurity measures of the establishments, the diagnosis on the farm and at the slaughterhouse (tests on animals), the indirect prophylaxis (vaccination), and the prompt screenings of human beings exposed to contact with infected animals.

Furthermore, the Extraordinary Commissioner has activated procedures approved by the Ministry of Health for the transport of manure also from infected establishments to biogas production plants, in line with EU green policies integrated into the One Health approach. For manure of infected establishments, operations will be conducted under sanitary restriction order, and the cost of transportation will be covered by the local health authority. All the measures described represent a significant model that can be adopted in similar territories.

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