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# Being prepared for an avian influenza epidemic with a One Health approach: a cartographic study to identify animal carcasses burial sites in central Italy

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#### Abstract

During epidemics, pandemics, or animal disease outbreaks, the large-scale disposal of carcasses presents greater environmental and biosecurity challenges. In Europe, disposal through a rendering plant is the preferred option, but the on-site carcasses burial may be authorised due to logistical and economic advantages. This study utilised a comprehensive GIS-based approach and focuses on the challenges and strategies for large-scale carcass disposal, particularly in the context of avian influenza outbreaks in the Lazio Region of Italy. Integrating data from official geospatial sources regarding presence of environmental restrictions and regulations, factors affecting susceptibility to groundwater contamination, factors affecting soil stability over time, potential burial sites were identified. The resulting map provides decision-makers with valuable information for prompt and efficient response during disease outbreaks. The study underscores the importance of a multidisciplinary approach involving veterinarians, epidemiologists, GIS experts, and geologists. Further research and international consensus are essential to standardize the selection of geographic variables/layers to use in similar projects. This study significantly contributes to the preparedness of environment, health and animal/human interface events.

#### **Keywords**

Avian influenza, burial sites, cartography, epidemic, GIS, outbreaks

## Introduction

During epidemics, pandemics and animal disease outbreaks, competent authorities implement measures to control and prevent the disease spread, in accordance with international and national regulations or disease-specific control plans. The availability of specific procedures or guidelines is crucial to ensure a prompt response and the proper disposal of dead animals. Mortality is an inevitable consequence in livestock, and in some cases may be due to high mortality diseases or massive culling as part of a disease control strategy. The large-scale disposal of carcasses presents greater environmental and biosecurity challenges compared to routine animal mortality (Baba et al. 2017; Koh et al. 2019) and requires thorough preparedness, including vigilant planning. Effective disposal minimizes costs and issues when conducted promptly, safely, following strict biosecurity measures, and with consideration for public perception (Miller et al. 2019; Chowdhury et al. 2019). European and Italian legislation requires that carcasses of dead and culled animals are sent to rendering plants (Italian Veterinary Contingency Plan, 2014). Over time, strict regulations had limited disposal options primarily to rendering and incineration (Regulation EC 1069/2009, Commission Regulation EU 142/2011). This led farmers in different countries to abandon traditional methods of onfarm burial and burning (Gwyther et al. 2011). Large-scale burial worldwide (disposal of carcasses in graves, ditches or open pits) is typically prohibited due to concerns about potential environmental impacts (e.g., on surface and groundwater). Although some studies suggest that leachate infiltration may pose a threat to groundwater (Hseu et al., 2016; Chowdhury et al., 2019), no studies have reported adverse effects in humans or other animals linked to animal burial, and no significant environmental issues have been reported, thus the actual threat to human health remains

unclear (NABC, 2004; Gwyther et al. 2011; Yuan et al. 2013; Baba et al. 2017; Vithanage et al. 2021). Despite bans, burial may be authorised in some cases due to logistical and economic advantages, particularly on farms lacking access to engineered landfills, rendering plants, or controlled incinerators (Vithanage et al. 2021). Each disposal method has advantages and disadvantages that should be carefully considered when the mass to be disposed of can range from hundreds to tens of thousands of tons (NABC, 2004; Gwyther et al., 2011). The drawbacks of mass burial include the need for proper site planning, long-term site monitoring, and the potential for groundwater contamination (Chowdhury et al., 2019). Disadvantages of rendering include transport costs, the risk of residue contamination during transport, high energy consumption and cost, and the need for skilled personnel (Chowdhury et al., 2019). The International Organization for Standardization (ISO) has provided guidance for the burial of animal carcasses (ISO 28901:2011) to prevent soil contamination. The Food and Agriculture Organization of the United Nations (FAO) has recently issued guidelines for proper disposal (including burial) on small to medium-sized farms (Costa et al., 2019; Miller et al., 2020). The decomposition time of buried carcasses can depend on a number of factors, including temperature, burial depth, soil type (salinity, pH), drainage, animal species, humidity/aridity (Gwyther et al., 2011; Dalziel et al., 2016; Schmitz et al., 2020; USDA-APHIS, 2023). Identifying suitable burial sites is one of the main objectives of epidemic preparedness. Delays in selecting such sites have been shown to prolong disease eradication efforts and increase economic burdens (Carpenter et al., 2011). To minimize environmental impact and improve logistical feasibility and community acceptance, a burial site must meet legal and environmental requirements. Buried animals can take over a decade to decompose; some authors found incompletely decomposed carcasses in excavated burials after six years, while others suggest that complete decomposition can occur within two years (Chowdhury et al., 2019; Hseu et al., 2016). Over time, the environment must be protected from a variety of biological and chemical agents, including bacteria, viruses, spores, decomposition by-products (e.g., nitrates, chlorides, gases), pharmacological residues, and disinfectants (special attention must be paid to chemicals used for pit disinfection and their environmental toxicity). To prevent the release of leachate to the environment, pits are typically lined with impermeable or semi-permeable, wear-resistant materials. This study focuses on avian influenza, a disease affecting both wild and domestic birds with increasing potential to infect mammals, thus posing pandemic risks. Avian influenza viruses (AIV) belong to the Orthomyxoviridae family and are relatively unstable under natural conditions, remaining harmful in the environment for only a few days. These viruses are inactivated at pH levels below 6.5 and can persist for four days at 22°C in water and up to 30 days at 0°C (Farina et al., 1998; Castrucci, 2005; Dalziel et al., 2016). A classic 20th-century study on AIV in duck feces suggests that the viruses retain infectivity in fecal material for at least 30 days at 4 °C and for 7 days at 20 °C (Webster et al., 1978). In a study to assess the persistence of AIV in slurry from different duck production sites, the results indicated viral infectivity for 4 weeks in Muscovy or Pekin duck breeders and for 2 weeks in ducks for foie gras production. Persistence of infectious after lime treatment at pH 10 or pH 12 was less than 1 week (Schmitz et al., 2020). The Lazio Region in central Italy has a significant poultry industry. with over 650 commercial farms housing about 3,800,000 birds, and 2,403 backyard poultry farms (National Animal Registry, Italian Ministry of Health), primarily located in the Viterbo and Rome provinces. Additionally, several wild bird species susceptible to avian influenza are present in the region as residents or during migration (mainly Anseriformes and Caradriiformes). During an epidemic, it is invaluable to have a large-scale map that summarizes information relevant to the response, including environmental constraints or regulations (e.g., landscape restrictions, protected areas), natural soil characteristics that affect susceptibility to contamination (e.g., hydrogeology), soil stability over time (e.g., landslide risk, flooding), and social acceptability (e.g., proximity to urban areas). A mapping project that summarizes all the above information was the primary objective of this study, with the aim to support decision-makers in identifying suitable locations for potential mass carcass burial during an epidemic. The secondary objective was to create a map that could be updated, shared, and navigated (zoom, address search, information display) by different stakeholders.

## Material and methods

#### Study area and parameters identification

The entire territory of the Lazio Region underwent a comprehensive examination to identify features that could aid in selecting a suitable burial site. All identified features were overlaid onto a cartographic project created using ArcGIS Pro© software (ESRI Release 3.1).

The study area was classified based on the following criteria:

• Presence of environmental constraints and regulations governing land use (e.g., protected areas, parks, archaeological sites, coastal zones);

- Factors influencing groundwater susceptibility to pollutants (e.g., water table depth, hydrogeological vulnerability);
- Considerations related to logistical issues (e.g., proximity to urban areas, road network);
- Factors affecting long-term stability (e.g., risk of landslides, floods);
- Parameters collection and data source.

All features collected in this project were extracted from official or public available geodata web services, including:

- geoportal of the Institute of Environment Research and Protection (Istituto Superiore per la Ricerca e la Protezione Ambientale - ISPRA);
- geoportal of the Ministry of the Environment (Ministero dell'Ambiente e della Sicurezza energetica -MASE);
- geoportal of Lazio Region (Open Data Lazio);
- Lazio Region Environment Information System (Sistema Informativo Regionale Ambientale Sira);
- free online geodata.

In assessing the eligibility of sites, this project applied the principle of extreme caution: sites were considered ineligible if there was at least one legal constraint, limitation or environmental risk (exclusion criteria in Table 1). Any areas not affected by these constraints or risks were considered potentially suitable. Characteristics indicating different levels of groundwater vulnerability to contamination (e.g. depth to water table, hydrogeological vulnerability) and logistical feasibility (e.g. road network, proximity to urban areas) were also included in the design to better evaluate the areas preliminarily identified as suitable (evaluation criteria in Table 1).

Informative laver	Criteria	Reference
Main hydrographic network (main rivers)	constraint or legal restriction/susceptibility to pollution (Exclusion criterion)	Public waters respect. Regional Territorial Landscape Plan (PTPR), approved by Regional Council Resolution no. 5 of 21 April 2021, Supplement 2. Corrected with DGR 228 of 21/04/2022; Corrected with DGR 670 of 02/08/2022. (Table B). Map release: 2022
Detailed hydrographic network (ditches and minor watercourses)	constraint or legal restriction/susceptibility to pollution (Exclusion criterion)	European catchments and Rivers network system (Ecrins), rivers - version 1, Jun. 2012
Drainage channel	constraint or legal restriction (Exclusion criterion)	Drainage channels. Regional Territorial Landscape Plan (PTPR), approved by Regional Council Resolution No. 5 of 21 April 2021, , Supplement 2. (Table B). Map release: 2021
Hydrogeological Vulnerability Map	susceptibility to pollution (Evaluation criterion)	Vulnerability extrapolated be Hydrogeological Complexes of Hydrogeological map of the territory of the Lazio region. La Sapienza' and Roma Tre' Universities. Release date: 2023
Water sources	constraint or legal restriction/susceptibility to pollution (exclusion criterion)	Map of springs, resurgences and natural fountains from the "Inland and transitional waters", of the "Hydrography" layer of the Regional Geotopographic Database (code 040104 - AF_ACQ), scale 1:5000, v. 2017. <u>Map release: 2021</u> Water springs from the Hydrogeological map of the territory of the Lazio region. La Sapienza' and 'Roma Tre' Universities. Release date: 2023 "Water abstraction for public water supply, from springs or wells". Vectorial map of Sistema Informativo Regionale Ambientale del Lazio (SIRA). ARPA Lazio. Cartography. Accessed on: January 2023
Natura 2000 (SICs and ZPSs)	constraint or legal restriction (Exclusion criterion)	Natura 2000 network sites (SIC/ZPS) under the Habitats Directive 92/43/EEC and the Birds Directive 2009/147/EC
Parks	constraint or legal restriction (Exclusion criterion)	Official list of protected areas: national parks, regional nature parks, state nature reserves (EUAP) extracted from World Database on Protected Areas (WDPA). Update: 2023.
Woodlands	constraint or legal restriction/ logistic feasibility (Exclusion criterion)	Woodlands from the Regional Territorial Landscape Plan (PTPR), approved by Regional Council Resolution No. 5 of 21 April 2021, , Supplement 2. (Table B). Map release: 2021
Archaeological areas	constraint or legal restriction (Exclusion criterion)	Typified archaeological points from the Regional Territorial Landscape Plan (PTPR), approved by Regional Council Resolution No. 5 of 21 April 2021, , Supplement 2. (Table B). Map release: 2021
Areas at risk of landslides	environmental risk/stability over time (Exclusior criterion)	Inventory of Franous Phenomena in Italy (IFFI) by Istituto Superiore per la Protezione e la Ricerca ambientale (ISPRA) with the collaboration of IdroGeo platform.
Flood hazard	environmental risk/stability over time (Exclusior criterion)	Extent of Floodable Area. Flood risk management plan (PGRA, 2021). Directive 2007/60/EC. Geoportale Nazionale.
Cavities map	environmental risk/ susceptibility to pollution (Exclusion criterion)	Archive of natural cavities. Speleological Federation of Lazio (FSL). Protection of the Karst Landscape and Enhancement of Speleology Regional Law 22/99. Map release: 2021
Lake basins	constraint or legal restriction (Exclusion criterion)	Coastal lakes from the Regional Territorial Landscape Plan (PTPR), Approved by Regional Council Resolution no. 5 of 21 April 2021, Supplement 2. (Table B). Map release: 2021
Coastal areas	constraint or legal restriction (Exclusion criterion)	Sea Coast from the Regional Territorial Landscape Plan (PTPR), approved by Regional Council Resolution No. 5 of 21 April 2021, Supplement 2. (Table B). Map release: 2021
Mountain areas	constraint or legal restriction (Exclusion criterion)	Altimetry 1200 m (above sea level) from the Regional Territorial Landscape Plan (PTPR), approved by Regional Council Resolution No. 5 of 21 April 2021, Supplement 2. (Table B). Map release: 2021
Ramsar Wetlands	constraint or legal restriction (Exclusion criterion)	Ramsar Wetlands. Ramsar Convention, signed in Ramsar, Iran, on 2 February 1971. Map release: 2021
Identitary Agricultural Areas	constraint or legal restriction (Exclusion criterion)	Identitary agriculture from the Regional Territorial Landscape Plan (PTPR), approved by Regional Council Resolution No. 5 of 21 April 2021, Supplement 2. (Table B). Map release: 2021
Nitrate Vulnerable Zones	constraint or legal restriction (Exclusion criterion)	Nitrate Vulnerable Zones. Sistema Informativo Regionale Ambientale del Lazio (SIRA). ARPA Lazio. Cartography. Accessed on: January 2023
Water table depths	susceptibility to pollution (Evaluation criterion)	Hydrogeological Map - Isopieze. Hydrogeological map Hydrogeological map of the territory of the Lazio region. La Sapienza' and 'Roma Tre' Universities. Release date: 2023
Urbanised Areas	logistic feasibility (Evaluation criterion)	Urbanised areas from the Regional Territorial Landscape Plan (PTPR), approved by Regional Council Resolution No. 5 of 21 April 2021, Supplement 2. (Table B). Map update: 2021
Historic Centres	constraint or legal restriction (Exclusion criterion)	Historic Centres (respect buffer 150 m) from the Regional Territorial Landscape Plan (PTPR), approved by Regional Council Resolution No. 5 of 21 April 2021, Supplement 2. (Table B). Map update: 2021
Road Network	logistic feasibility (Evaluation criterion)	Italy roads from the free OpenStreetMap.org & MapCruzin.com.

Table I. List of the informative layers for the selection of suitable sites for mass burial of animal carcasses.

#### Identification of information layers - reasons and criteria

The reasons and criteria used to identify informative layers for the assessment of burial suitability are outlined as follows:

 Main Hydrographic Network (Main Rivers): Major watercourses serve as primary receptors and pathways for environmental pollutants. In hydrogeological terms, subsurface flow lines near riverbanks converge toward the riverbed, facilitating the transport of leachate during rainfall events. A buffer zone of 150 meters around watercourses was considered, aligning with the Regional Territorial Landscape Plan (PTPR, 2021).

- Detailed Hydrographic Network (Ditches and Minor Watercourses): This map encompasses small watercourses such as streams, canals, and ditches, which may be perennial, seasonal, or active only during rainfall. A buffer zone of 50 meters was established for the same reasons mentioned above (PTPR, 2021).
- Drainage Canals: Areas within a 150-meter buffer of agricultural drainage canals, particularly in the Pontina area of South Lazio Region, were excluded due to environmental and landscape preservation concerns. These areas, once marshy floodplains, were not considered suitable for the purposes of this study, as their shallow water table poses problems for both groundwater contamination and soil excavation (the bottom of the excavation may be exposed to water).
- Water sources: In order to protect the quality of water intended for human consumption, the law (Decreto Legislativo 3 aprile 2006, n.152) establishes protection zones around springs, including absolute protection zones within 10 meters, respect zones that must be at least 200 meters and sometimes larger protection zones around these buffers if necessary (respect and protection zones decided by local authorities). A conservative buffer of 500 meters around each water source was considered ineligible in the absence of alternative information.
- Natura 2000 areas: These areas are Europe-designated key sites that require habitat and species conservation plans (Council Directive 92/43/EEC and Directive 2009/147/EEC, respectively). An Environmental Impact Assessment (EIA) is required by law (D.P.R. 8 settembre 1997, n. 357) for any project (such as a landfill site) that could alter these restricted areas. In the absence of an Environmental Impact Assessment, we considered these area ineligibles.
- Parks: Nature reserves and parks under national or regional legislation, listed in the Italian Official List of Protected Areas, were considered ineligible for this project (PTPR).
- Wooded areas: Forested areas, crucial for biodiversity and ecology (PTPR; Decreto Legislativo 22 gennaio 2004, n. 42), were considered ineligible also due to their lack of accessibility and road infrastructure.
- Archaeological Areas: Buffer zones of 50 and 100 meters around archaeological sites, as required by the PTPR, were considered ineligible for adequate protection.
- Areas at Risk of Sliding: fragile slopes prone to landslides were deemed ineligible for safety reasons.
- Flood hazard: in accordance with Directive 2007/60/EC, Italy produced flood hazard and risk maps (Decreto Legislativo 23 febbraio 2010, n. 49). The hazard maps indicate three levels of hazard: low, medium and high probability. All areas at risk of flooding (low to high risk) were considered ineligible.
- Underground cavities: the Lazio Region identified the natural cavities, as defined in a Regional Law (Legge Regionale 1 settembre 1999, n. 20). Caves, tunnels and fractured rock that receive leachate from the surrounding area can rapidly transport contaminants for several kilometres without the filtration or degradation that normally occurs in porous soils. The layer shows areas interested by natural caves in square cells with a side of 250 m. The areas within a 500 meter buffer around these cells were considered ineligible.
- Lake basins: buffer zones of 300 meters around lake basins were considered ineligible, as required by the PTPR.
- Hydrogeological Vulnerability: The intrinsic or natural vulnerability of aquifers is defined as the specific susceptibility of an aquifer to the absorption and diffusion of a contaminant, in such a way that the quality of the underlying groundwater may be adversely affected (Civita, 1987). It depends on hydro-lithological characteristics (e.g. relative permeability, percolation rate of the aquifer), physical and chemical characteristics of the soils (e.g. porosity, fracture index), connection to surface water bodies, as indicated by specific Italian guidelines (ANPA, 2001). For the purposes of this project the hydrogeological vulnerability was classified as high, medium and low.

- Coastal areas: Areas within 300 meters from the coast were considered ineligible due to landscape significance and habitat preservation (PTPR Table b, "Coastal Sea") or other existing regulations (Decreto Legislativo 22 gennaio 2004, n. 42).
- Mountain areas: Mountain areas above 1200 meters were considered ineligible to protect landscape interests (PTPR; Decreto Legislativo 22 gennaio 2004, n. 42).
- Ramsar wetlands: according to the Ramsar Convention on Wetlands of International Importance, wetlands are marshes, bogs, fens with stagnant or flowing water, natural or saline, including areas of sea water where the depth at low tide does not exceed six meters. To date, six sites have been identified in Lazio Region. These areas were considered ineligible.
- Areas of agricultural identity: These areas (14 in total) of historical significance for literary, pictorial and landscape evidence (PTPR) were deemed ineligible.
- Nitrate vulnerable zones: Areas identified as vulnerable to nitrate pollution were considered ineligible due to their potential impact on water quality (Council Directive 91/676/EEC, Decreto Legislativo 3 aprile 2006, n. 152).
- Water table depth: It is the depth of the water table from the surface of the ground. Dissolution and mobilization of contaminants is facilitated by the water in the pores of saturated soils. Shallow groundwater levels increase the risk of groundwater contamination. Because of the low accuracy of this layer (due to the scale), we recommend this layer only for a better understanding, but not as an exclusive criterion.
- Urbanised areas: the presence of densely urbanised areas is a limitation for the practice of massive burial sites, both in terms of population exposure, social acceptance and logistics (transport, loading and unloading). The proximity to urbanised areas was used to better assess the suitability of eligible areas, but not as an exclusive criterion.
- Historic centres: these areas are identified in the PTPR. They are ancient or recent (20th century) urban areas that have preserved their traditions. The perimeter of these places was coming by the historic plans (Catasto Gregoriano 1820-40, IGM 1:25,000 cartography of 1873/83). Buffer zones of 150 meters around historic centers (PTPR) were considered ineligible.
- Road network: The road network was considered for logistical feasibility. In some countries, a distance of 60 to 800 meters from roads has been indicated as adequate for burial sites (Freedman & Fleming, 2003). Roads should be suitable for lorry passage, possibly paved and not heavily trafficked. This informative layer was suggested not for inclusive or exclusive purposes but to better evaluate potential suitable areas.

## Results

A mapping project has been drawn to identify potential mass burial sites across the Lazio Region. All areas deemed ineligible have been consolidated into a single recognizable layer highlighted in a distinct color (Figure 1). Based on the criteria used in our study, approximately 77% of the total regional area was found to be ineligible. An interactive map interface has been created to enable users to navigate and visualize all relevant information used for classification. This interface allows users to analyze any potential issues affecting specific areas (Figure 2). Users can access information by selecting topics from a dedicated menu, with data displayed in the legend using appropriate symbols and classification labels. The map is accessible through a dedicated website, either as a web application or through specific links. Alternatively, users can access the project's shapefiles/layers for further analysis.

The mapping project is designed to be utilized by the following stakeholders:

- Regional authorities
- Municipalities

- Veterinarians of local health units
- Poultry farmers and other relevant stakeholders



Figure 1. The map of the entire territory of the Lazio Region, showing all legal constraints and environmental risks on a single colored, ineligible layer (uncolored areas: potentially suitable).



Figure 2. A 1:200,000 scale map showing the different layers that can be viewed simultaneously on a specific area to analyze all potential ineligibility issues.

# Discussion

The disposal of a large volume of organic material of animal origin raises significant biosecurity concerns. We outlined a methodology that considers multiple factors, including environmental, legal, and urban aspects, while adopting a One Health approach to facilitate decision-making. The final product is a navigable map that allows users to zoom in, search by address, explore information, and share insights among stakeholders, particularly regional authorities. The GIS methodology enables the simultaneous visualization of various environmental aspects to assess their interactions and potential impacts on environmental risks. In scenarios that require the selection of a mass burial site, consulting on such a project can help prevent the further spread of pathogens and subsequent exposure of humans or animals. Using a conservative approach, only a small part of the Lazio Region has been identified as suitable (about 23 %) for the burial of large quantities of animal carcasses, supporting the current legislation that favors the transport of carcasses to authorized incinerators. However, further in-depth studies could better identify suitable areas beyond the preliminary boundaries drawn by this project (Figure 1, 2), and the project can be enriched with any additional information required by the authorities to improve decision making on specific sites (e.g. detailed water depth, urban plan, geological survey). Further investigations at local level are also needed to avoid other constraints (e.g. plan for new urban areas) not covered in this study.

The identification of suitable sites does not absolve operators from the need to take appropriate biosecurity measures to prevent leakage, including treatment of both sides and the bottom of the pit, as also explained by Chowdhury et al. 2019. Although avian influenza was the target for which the project was designed, the result is a useful tool in all epidemic cases with a large number of dead or culled animals. The project emphasized the need for a multi-sectoral approach involving many different professionals, including veterinarians, epidemiologists, GIS and environmental specialists in all similar cases where there is a need to consider infectious risk and environmental risk at the same time. Further research and international consensus may be helpful to standardize the selection of geographic variables/layers to be used in similar projects. This study significantly contributes to the preparedness and management of environment, health and animal/human interface events.

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