Spatial Distribution and Epidemiology of Echinococcus Granulosus Infection in Sheep and Goats Slaughtered in a Hyperendemic European Mediterranean Area

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Research

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Spatial distribution and epidemiology of *Echinococcus granulosus* infection in sheep and goats slaughtered in a hyperendemic European Mediterranean area

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Abstract

Background: Cystic echinococcosis (CE) is a parasitic zoonosis caused by the larval stage of *Echinococcus granulosus*, highly widespread in livestock, particularly sheep and goats. This study aimed to evaluate the spatial distribution of CE in sheep and goats slaughtered in a hyperendemic Mediterranean area.

Methods: A survey was conducted in Basilicata region (southern Italy) from 2014 to 2019. A total of 1454 animals (1265 sheep and 189 goats) from 824 farms were examined for hydatid cysts detection by visual inspection, palpation and incision of target organs. All the CE cysts were counted and classified into five morphostructural types (unilocular, multisepted, calcified, caseous and hyperlaminated). The molecular analysis was performed on 50 cysts. For spatial analysis, kriging interpolation method was used to create risk maps, while the clustering was assessed by Moran's I test.

Results: CE prevalence of 72.2% (595/824) and 58.4% (849/1454) were observed at the farm and animal level, respectively, with higher values in sheep (62.9%) than goats (28.0%). The liver and lungs were the most frequently infected organs both in sheep and goats. Most of recovered cysts belonged to the calcified and multisepted morphotypes. All the isolates were identified as *E. granulosus sensu stricto* (genotypes G1-G3). Spatial distribution showed a moderate clustering of positive animals.

Conclusions: The findings of this study can be used to better understand the eco-epidemiology of echinococcosis and to improve the CE surveillance and prevention programs in regions highly endemic for CE.

Keywords

Echinococcus granulosus, Cystic echinococcosis, Sheep, Goats, Spatial distribution, Cysts

HIGHLIGHTS

- An overall CE prevalence of 72.2% and 58.4% were observed at farm and animal level
- Spatial distribution showed a moderate clustering of positive animals
- A higher value of CE was found in sheep (62.9%) than in goats (28.0%)

Background

Cystic echinococcosis (CE) is a parasitic zoonosis caused by taeniid tapeworms, belonging to the *Echinococcus granulosus sensu lato* complex [1]. The domestic life cycle of this infection involves dogs as definitive hosts and a

broad spectrum of mammals (e.g. sheep, goat, water buffaloes, cattle) as intermediate hosts. Briefly, intermediate hosts become infected through ingestion of pasture grass contaminated with *E. granulosus* eggs released by infected dogs. The cycle is completed when definitive hosts ingest cysts (metacestodes) present in different organs (e.g. liver, lungs, spleen, heart) of intermediate hosts, particularly sheep and goats. Although frequent, human infection is considered an accidental event [2].

Currently, *E. granulosus sensu lato* complex is composed by *E. granulosus sensu stricto* (genotypes G1-G3), *E. equinus* (G4), *E. ortleppi* (G5), *E. canadensis* (G6/G7, G8 and G10) and *E. felidis* [1, 3]. Undoubtedly, the G1 is the most widespread genotype and it associated to sheep has been detected in the majority of human CE cases (88.4%) [4]. CE constitutes a significant financial constraint in the public health field and the livestock industry. The global burden of CE has been estimated at approximately 1 million Disability Adjusted Life Years (DALYs) and the world's livestock industry loss has been estimated around $3 billion a year [5, 6].

Echinococcus granulosus is a cosmopolitan species, but it is mainly widespread in rural areas of central Asia, South America, and southern and eastern Europe [5, 7, 8]. The distribution of CE in different parts of the world is related to environmental and anthropogenic factors. Deplazes et al. [5] showed a heterogeneous geographic distribution in the European Mediterranean area with prevalence values < 0.1% in the coastal regions of France and Spain, reaching values > 50.0% in Italy, with a higher prevalence in the southern (Basilicata and Campania regions) and insular (Sardinia and Sicily) part of the country [8, 9, 10]. However, the reported prevalence of CE in livestock is widely underestimated, because the surveillance system based on reports recorded at slaughterhouses is still inefficient [9, 11]. In addition, the data of surveillance systems are usually obtained for wide geographic areas that assume a homogeneous prevalence [12]. Therefore, this study aimed to evaluate the spatial distribution of CE in sheep and goat farms uniformly distributed in a hyperendemic region of the European Mediterranean.

Methods

Study area and sampling

This study was carried out from 2014 to 2019 in Basilicata region, southern Italy. This region comprises an area of about 10,000 km^2 where the provinces of Potenza (40° 38' N; 15° 48' E) and Matera (40° 39′ N; 16° 36′ E) are located. The area presents a climate Mediterranean with dry summers and rainfall concentrated between October and March. Precipitation is abundant, about 1200 mm per year [13]. The average temperature in the coldest month (January) is about +8 °C and the warmest month (August) about +28 °C, with an annual average of +14 °C.

A Geographic Information System (GIS) of the Basilicata region was constructed using as data layers the administrative boundaries at the provincial and municipal levels. In order to uniformly sample the farms throughout the study area, the region was divided into 100 quadrants, by overlaying a grid of 10 x 10 km. In each quadrant about 15 small ruminants aged 3-7 years from 7-8 farms were involved. A total of 1454 animals (1265 sheep and 189 goats) from 824 farms were examined. The geographical coordinates of each sheep and goat farms were obtained referring to the farm code of each farm.

Postmortem examination

The animals were transported to an abattoir for slaughter and postmortem inspection. For each animal slaughtered, CE detection was performed by visual inspection, palpation and incision of heart, kidneys, liver, lungs and spleen. For each positive sheep the CE cysts were counted and classified into five morphostructural types (unilocular, multisepted, calcified, caseous and hyperlaminated) in accordance with Conchedda et al. [8].

When cystic lesions were attributable to CE, the animal and consequently the farm of belonging were classified as positive.

Molecular analysis

The molecular study was carried out on 50 cysts. The germinal membrane and the cystic liquid of the cysts were collected and stored at −20 °C until DNA extraction. Genomic DNA was extracted from the germinal layers of cysts using the Qiamp DNA mini kit (Qiagen, Hilden, Germany) [14]. The PCR for the CO1 gene was performed as reported in Capuano et al. [14], while the PCR for the 12S rDNA gene as described in Rinaldi et al. [15]. PCR products were detected on a 2% ethidium bromide-stained low melting agarose gel (BIO-RAD, Spain) for both PCR reactions. Bands were cut from the gel under UV exposure and the amplified DNAs were purified by QIAquick Gel Extraction KIT (Qiagen, Germany). The PCR products were sequenced and analyzed using the Chromas version 2.6.6 software. DNA sequences comparison was achieved using GenBank with the BLAST system and ClustalW.

Geostatistical analysis

All georeferencing and data were expressed in geographical ETRS89 format and were projected to UTM zone 33N at reference datum WGS84, as specified by RSDI Basilicata Geoportale [16].

Indicator kriging to access continuous area probability

Disease incidence detection and probability mapping were performed in three steps.

The first step produced empirical semi-variograms, which represented half of the mean square difference between pairs of sampling locations (Equation 1).

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [z(x_i + h) - z(x_i)]^2 \qquad (1)$$

where $N(h)$ is the number of data pairs for the lag h, while h is the distance between animal sampling sites and $z(xi)$ is the location of the animal sample.

The stable semi-variogram function [17] (Equation 2) was used to fit the semi-variogram model to the empirical data. It has a nugget effect, which consists of variance of lag distances, in which sample points are smaller than the typical sample spacing plus measurement error. The upper limit of the semi-variogram model is called the sill, which represents the variance of the variable. The distance to the sill or correlation between lag distances is called the range.

$$\gamma(h;\theta) = \theta_s \left[1 - \exp\left(-3\left(\frac{\|h\|}{\theta_s}\right)^{\theta_e}\right)\right] \quad \text{for all } h \qquad (2)$$

Where partial sill ($\theta_s \geq 0$), range ($\theta_r \geq 0$) and power ($0 \leq \theta_e \leq 2$) parameters are to be estimated. If $\theta_e = 2$, the semi-variogram model is Gaussian. This model is more flexible and retains desirable properties.

The second step involved estimation mapping to predict the presence or absence of disease in an unknown location. Indicator kriging was used to estimate mapping distributions under a given threshold (z_k) [18]. The resulting data were interpreted as values between zero and one. If the value is nearly one, it is considered to be positive and, conversely, if the value is nearly zero, it is considered to be negative. The indicator kriging function used is given in Equation 3.

$$I(x_i : z_k) = \begin{cases} 1, & \text{if } z(x) = z_k \\ 0, & \text{if } z(x) \neq z_k \end{cases} \qquad (3)$$

The last step consisted of estimation mapping for the probability of presence or absence in the range [0; 1], as described in Adhikary et al. [19].

Local Moran's I statistics for spatial autocorrelations and clustering

Local spatial autocorrelations were used to calculate the significance levels of local indicators of spatial association (LISAs). Additionally, local Moran's I statistics were used to analyze the degree of spatial difference between each area and the surrounding region. The analysis steps were as follows. The spatial weight matrix was established by:

$$W_j = \frac{1}{d_{ij}^m}$$

Where m is the power and d_{ij} represents the distance between region i and region j.

The global spatial autocorrelation index, Moran's I, was then calculated. There were n area units in the study area, and the observed values on the I unit were X_i. The mean value of the observation variable in the N unit was X. W_{ij} was a spatial weight matrix. Thus, Moran's I was defined as:

$$I = \frac{n\sum_{i=1}^{n}\sum_{j=i}^{n} W_{ij}\,(X_i - \bar{X})(X_j - \bar{X})}{\left(\sum_{i=1}^{n}\sum_{j=i}^{n} W_{ij}\right)\sum_{i=1}^{n}(X_1 - \bar{X})^2}$$

The value of Moran's I statistics are in range [− 1, 1]. $I > 0$ shows that there is a positive spatial correlation between research objects (the incidence of streets), which means that 0 is irrelevant, while $I < 0$ shows negative spatial correlation.

In this study, LISA was used to reflect the degree of correlation between the incidence of disease among animals on a given farm and the incidence among animals on nearby farms. The local Moran's I index was defined as:

$$I_j = \frac{n(X_i - \bar{X})\sum_{i=1}^{n} W_{ij}\,(X_j - \bar{X})}{\sum_{i=1}^{n}(X_i - \bar{X})^2}$$

Where n is the number of space units involved in the analysis; X_i and X_j represent the observational values of a phenomenon (or an attribute characteristic) x on the I and j of the space unit; and W_{ij} is the spatial weight.

If $I_i = 0$, there is no spatial autocorrelation. This shows that there is no aggregation around the area, thus implying random distribution; if $I_i < 0$, there is a spatial negative correlation; if $I_i > 0$, there is a positive spatial correlation. The greater the absolute value of I_i is, the higher the degree of aggregation around the area is. When the I_i value is positive, this area presents high incidence. When I_i is negative, this area has low incidence.

All analyses were performed using the ESRI ArcGIS ArcMap 10.6 software.

Results

Overall, a CE prevalence of 72.2% (595/824) and 58.4% (849/1454) was found at the farm and animal level, respectively. CE was higher in sheep (796/1265, 62.9%) than goats (53/189, 28.0%) ($p < 0.01$).

There were animals with one (39.7%), two (59.4%) or three (0.9%) infected organs. Regarding the organ distribution of CE, the liver and lungs were the most frequently infected visceral organs in sheep (53.0% and 49.6%, respectively) and goats (18.5% and 13.2%, respectively). Very few sheep and goats (< 1%) had cysts in other organs

(heart, spleen and kidneys) (Table 1). A total of 4579 cysts recovered from infected sheep and 229 cysts from infected goats were examined (Fig. 1). In the liver and lungs, the majority of the cysts belonged to the calcified and multisepted morphotypes (Table 2). The molecular study allowed to identify the presence of G1 (GenBank Accession number: U50464 for CO1 and GenBank Accession number: AY462129 for 12S), G2 (GenBank Accession number: M84662 for CO1 and GenBank Accession number: DQ822451 for 12S) and G3 (GenBank Accession number: M84663 for CO1 and GenBank Accession number: DQ822451 for 12S) strains from ovine and caprine isolates.

Table 1 Anatomical localization of cystic echinococcosis (CE) cysts in sheep and goats slaughtered

Organ	No. positive animals; prevalence (%) (95% CI)	
	Sheep (No.=1265)	Goats (No.=189)
Liver	671; 53.0 (50.3-55.8)	35; 18.5 (13.6-24.7)
Lungs	627; 49.6 (46.8-52.3)	25; 13.2 (9.2-18.8)
Spleen	11; 0.9 (0.5-1.6)	0; 0
Kidneys	8; 0.6 (0.3-1.2)	0; 0
Heart	4; 0.3 (0.1-0.8)	1; 0.53 (0.1-2.9)
Total	796; 62.9 (60.3-65.5)	53; 28.0 (22.1-34.8)

Table 2 Frequency of cystic echinococcosis (CE) cysts morphotypes recovered from each organ of sheep and goats slaughtered

Animal species	Organ	No. cysts (%)					
		Unilocular	Multisepted	Calcified	Caseous	Hyperlaminated	Total
Sheep	Liver	214 (7.9%)	592 (22.1%)	1099 (40.9%)	241 (8.9%)	536 (19.9%)	2682
	Lungs	168 (8.9%)	449 (23.9%)	729 (38.9%)	205 (10.9%)	321 (17.1%)	1872

	Spleen	0 (0%)	0 (0%)	0 (0%)	0 (0%)	11 (100%)	11
	Kidneys	1 (10.0%)	8 (80.0%)	0 (0%)	0 (0%)	1 (1.0%)	10
	Heart	0 (0%)	0 (0%)	3 (75.0%)	0 (0%)	1 (25.0%)	4
	Total CE cysts						4579
Goats	Liver	12 (9.3%)	29 (22.5%)	48 (37.2%)	18 (13.9%)	22 (17.1%)	129
	Lungs	5 (5.1%)	19 (19.2%)	40 (40.4%)	14 (14.1%)	21 (21.2%)	99
	Spleen	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0
	Kidneys	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0
	Heart	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1
	Total CE cysts						229

The higher prevalence of positive animals was found in Potenza province. The spatial distribution of positive animals is shown in the Fig. 2. Warmers colors indicated higher prevalence. Spatial distribution showed a moderate clustering of positive animals.

Discussion

The data on the prevalence of CE in some Italian regions are scarce. In Basilicata prevalence ranging between 5-28% were reported in sheep from 1996 to 2002 [20] and of 12% from 2010 to 2015 [9]. No previous data were available for goats. The prevalence of CE found in this study in Basilicata region was of 62.9% in sheep and 28.0% in goats. These values are higher than those reported in sheep and goats have been in other countries of Mediterranean area, respectively: 30.2% and 7.6% in Greece [21]; 16.4% and 2.9% in Tunisia [22]; 6.9% and 1.6% in Algeria [23]; < 0.1% for both in Spain [24]; and < 0.002% and absence of infected goats in the last national census conducted in France [25]. The variation in the prevalence of CE in different parts of the world may be associated not only with environmental factors such as cool temperatures, high rainfall and shade that increase the probability of egg survival in the environment and favor the transmission of CE in livestock, but also with control measures and breeding systems, numbers of dogs in each location, education level and economic status of the population[26]. In the Mediterranean area, echinococcosis is predominant particularly in countries with large number of grazing sheep. Moreover, the transmission is favored by farmers which feed shepherd dogs with infected viscera as well as the lack of knowledge of the population about good prevention practices for this parasitosis [5, 27].

196 The results from the present study showed that the prevalence of CE was higher in Potenza than in Matera
197 province. However, all the Basilicata region has a high sheep and goat farming tradition, usually based on extensive
198 management using broad pastures. Moreover, there is a shepherd dog population of 92208 animals. Therefore, the
199 potentially infected dogs with *E. granulosus* can contaminate the grazing pastures with faeces containing eggs,
200 contributing to the high prevalence of CE in livestock. For these reasons, the infection of small ruminants in this
201 area is probably associated with different optimal conditions for the transmission of this parasite (e.g. high density
202 of canine population, lack of the dog deworming program, inappropriate animal management practices by farmers).
203 Lastly, the higher prevalence of CE in sheep than in goats can be attributed to where these animals graze,
204 such that sheep eat more grass from contaminated pastures [28]. Regarding the distribution of CE according to organ,
205 the liver and lungs were the visceral organs most frequently infected among both sheep and goats, following by the
206 heart, spleen and kidneys. These findings are in agreement with other authors, who found that the liver and lungs of
207 sheep were commonly infected with CE [29, 30, 31]. However, some authors indicated that the lung parenchyma
208 has a spongy consistency and a greater capillary bed, which supports a higher presence of cysts in this organ, whereas
209 the compact tissues of the liver resist the development of larger cysts [32, 33]. Molecular results showed the presence
210 of G1, G2 and G3 genotypes. According to other studies [15, 34, 35, 36, 37], *E. granulosus s.s.* is widespread in
211 ruminants worldwide and it must be rigorously controlled due to its recognized infectivity in humans.
212 Therefore, the areas with low and high clusters of cases identified in the present study (Fig. 2) can serve to
213 identify not only which areas are hotspots for transmission of *E. granulosus* among sheep and goats but also for
214 human infection. In this way, the results from this spatiotemporal analysis on echinococcosis in sheep and goats
215 revealed moderate clustered patterns for the period 2014-2019.
216 This study is part of a research project concerning the disease mapping caused by viral, bacterial and other
217 parasitic infections found in ruminants in the Basilicata region using GIS. These maps are intended to be used in
218 control programs to prevent and control CE in ruminants. In this context, a multidisciplinary program using a One
219 Health perspective is required in order to control the transmission of *E. granulosus*. Over eight years, the
220 EchinoCamp project demonstrated that the reduction of *E. granulosus* infection rates of dogs, humans and livestock
221 (e.g. a decrease of up to 30% was observed in sheep) is feasible in Campania region, an endemic area of the
222 Mediterranean [10].
223

Conclusions

The present study provides evidence of the persistence of CE in a hyperendemic European Mediterranean area. Moreover, the identification of these disease hotspot areas is important in relation to understand the eco-epidemiology of echinococcosis and the persistence of infection, and thus, to improve the echinococcosis prevention programs and surveillance that will be important to reduce CE not only in animals, but also in humans.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article. The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LCA, MPM, LR and GC conceived, designed and coordinated the study. AB, PC, AA, PP and MEM performed inspection of organ, cyst classification and molecular analysis. EFM, KRS and RANR performed geostatistical analysis. All authors contributed to data analysis and preparation of the manuscript. All authors read and approved the final manuscript.

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341

FIGURE CAPTIONS

Fig. 1 Cystic echinococcosis (CE) cysts morphotypes recovered of sheep and goats slaughtered. Unilocular cysts in liver (**A**) and lung (**B**) of sheep; multisepted cysts with cavity divided by septa into spheroidal chambers of widely variable number in liver (**C**) and lung (**D**) of goat; calcified cyst showing almost virtual internal chambers in liver (**E**) and lung (**F**) of sheep; caseous cyst with cavity filled with a thick matrix of cheesy consistency in liver (**G**) of sheep; hyperlaminated cyst with the virtual cavity filled with sheets of laminated tissue in lung (**H**) of goat.

Fig. 2 Local Moran's I statistics for spatial autocorrelations and clustering in sheep (**A**) and goat farms (**B**)

Figures

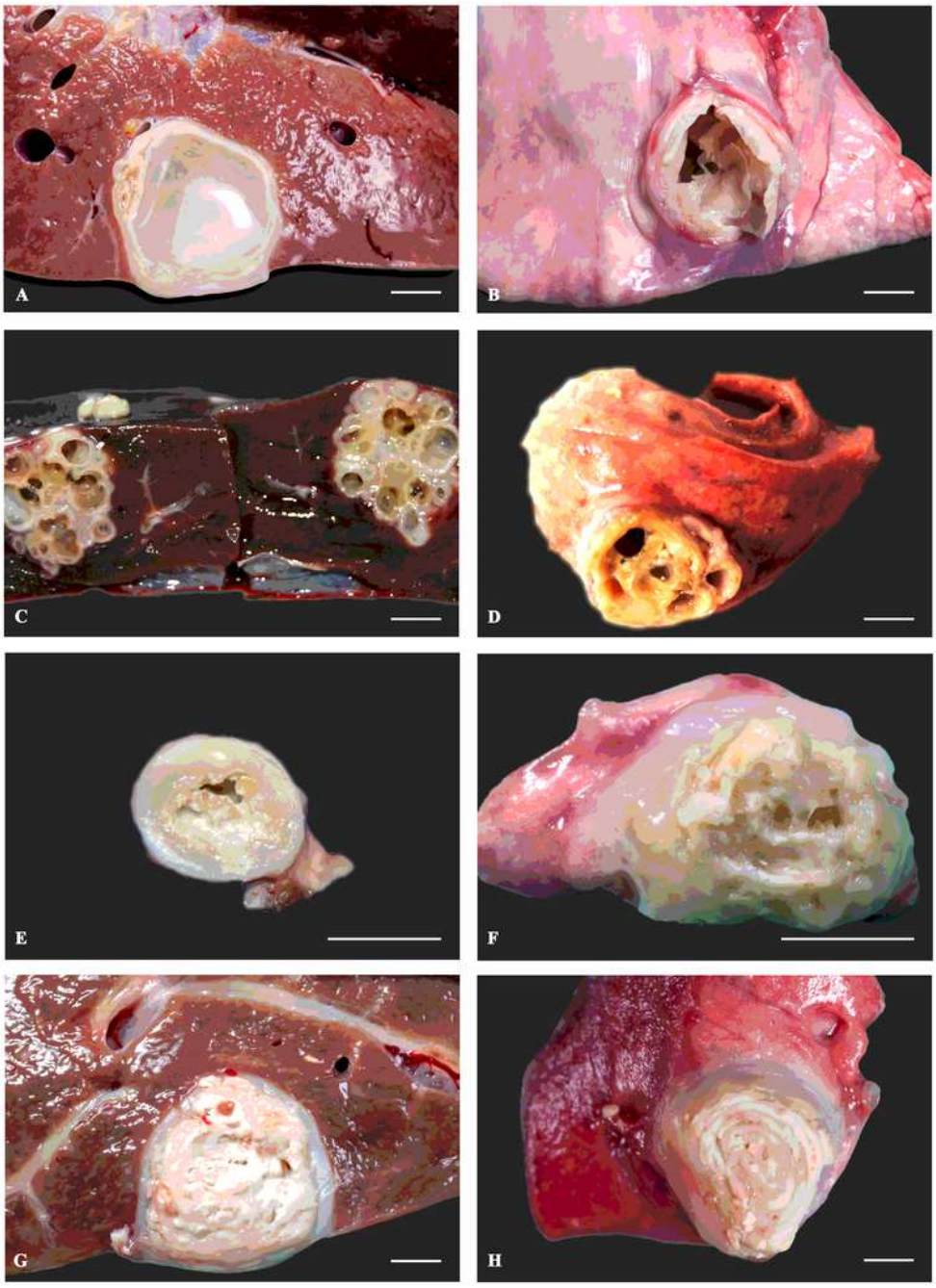

Figure 1

Cystic echinococcosis (CE) cysts morphotypes recovered of sheep and goats slaughtered. Unilocular cysts in liver (A) and lung (B) of sheep; multisepted cysts with cavity divided by septa into spheroidal chambers of widely variable number in liver (C) and lung (D) of goat; calcified cyst showing almost virtual

internal chambers in liver (E) and lung (F) of sheep; caseous cyst with cavity filled with a thick matrix of cheesy consistency in liver (G) of sheep; hyperlaminated cyst with the virtual cavity filled with sheets of laminated tissue in lung (H) of goat.

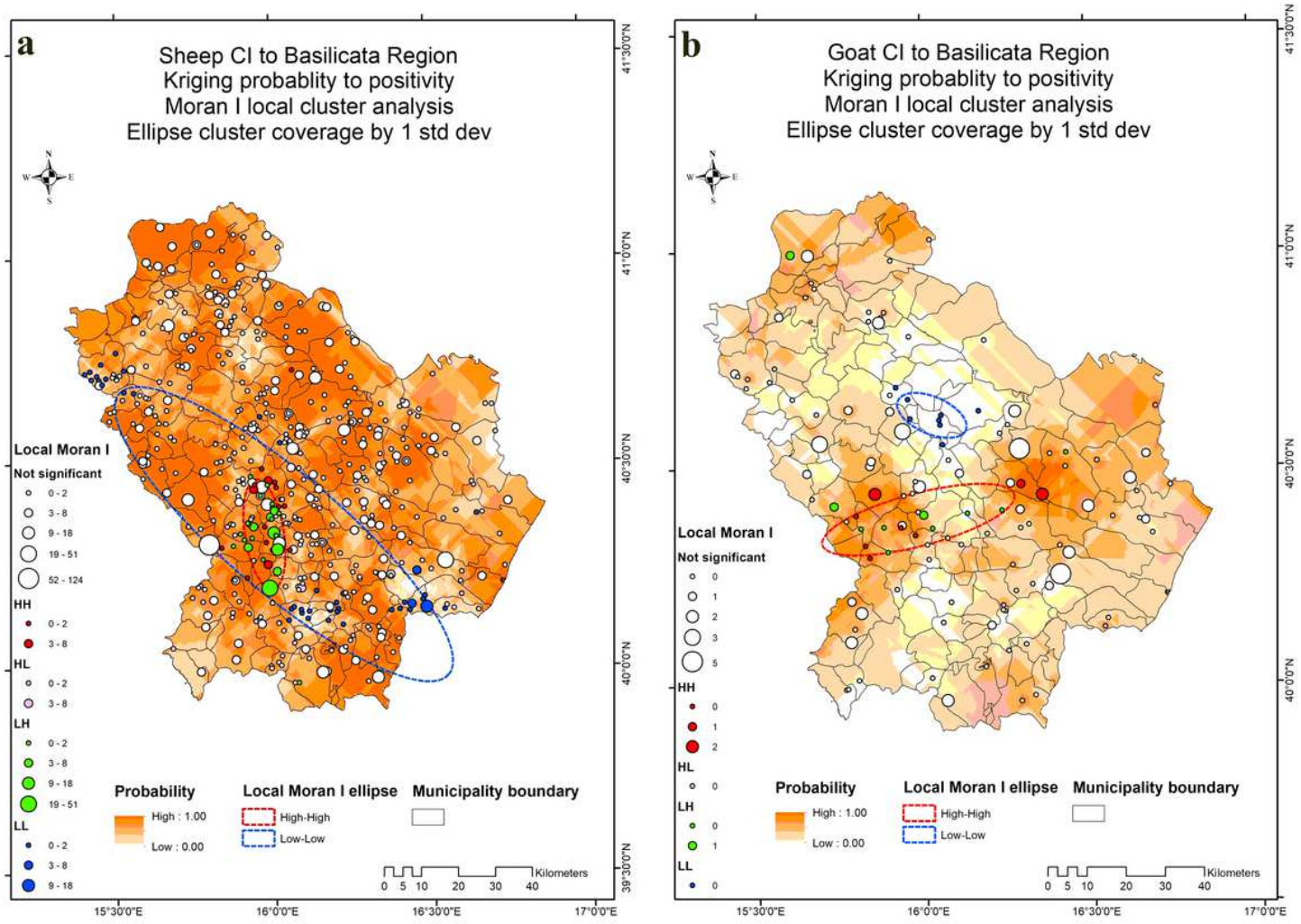

Figure 2

Local Moran's I statistics for spatial autocorrelations and clustering in sheep (A) and goat farms (B) Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

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