Parasitic infections in an organic grazing cattle herd in Tuscany using geographic information systems to determine risk factors

Stefania Perrucci(1), Eleonora Pinello(1), Gianluca Fichi(1), Edoardo Ciardi(1), Paolo Bàrberi(2), Camilla Moonen(2), Giorgio Ragaglini(2) & Carlo Bibbiani(3)

Summary
An organic grazing cattle herd in Tuscany (Italy) was monitored for parasites between 2002 and 2006. Every two to three months, faecal samples from cattle of different breeds and age were collected and examined for endoparasites, using both qualitative and quantitative parasitological techniques. Several environmental parameters were monitored and data on biodiversity and field margin biodiversity of grazing areas were also collected. All data were geo-referenced and plotted on a vectorial map using geographic information systems (GIS) software. Soil was classified as silt and clay/sand. The hydraulic drainage was poor and water pooling was observed frequently. The biodiversity of field margins was relatively high. Cattle were infected by coccidia, gastrointestinal nematodes, cestodes and trematodes. Prevalence and intensity of infestations were highly variable. In most cases, this variability was related to cattle breed, age, season and meteorological data. The Pisana breed was most commonly infected by Fasciola hepatica and Paramphistomidae. These infestations were associated with more frequent flooding and water pooling in the areas grazed by this breed.

Keywords
Cattle, Climate, Endoparasites, Environment, Geographic information system, Grazing, Italy, Risk, Tuscany.

Endoparassitosi bovine in una azienda biologica toscana: utilizzo del sistema informativo geografico per la valutazione dei fattori di rischio

Riassunto
Nel periodo di tempo compreso tra il 2002 ed il 2006, una azienda biologica di bovini da carne della Toscana è stata monitorata per le endoparassitosi connesse con il pascolo. A tal fine, campioni fegali di bovini di razza ed età diversa sono stati raccolti ogni due-tre mesi ed esaminati per la ricerca di endoparasiti utilizzando tecniche parasitologiche sia quali che quantitative. Sono stati anche monitorati numerosi parametri ambientali e raccolti dati riguardanti la biodiversità vegetale e la biodiversità dei margini dei pascoli. Tutti i dati sono stati georeferenziati e disegnati su una mappa vettoriale utilizzando il software GIS. Il terreno è stato classificato come fangoso e argilloso/sabbioso. Il drenaggio idraulico è risultato insufficiente e la formazione di ristagni idrici è stata osservata frequentemente. La biodiversità dei margini dei

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Introduction

The development of methods for mapping diseases has progressed considerably in recent years. Geographic information systems (GIS) offer a new tool for epidemiological studies. GIS has been applied to study the spatial and temporal patterns of parasitic infestations (6, 9, 16).

Organic animal production relies heavily on grazing in pastures where soil-transmitted parasitic infections can be prevalent. These economically important diseases have negative effects on animal health, welfare and production (5, 7, 21). In organic cattle herds, the control of parasitic diseases is difficult because of grazing management and limitations on the amount and frequency of drug use (11, 19). For these reasons, continuous parasitological monitoring is essential for the efficacious control of parasitic diseases (17, 17). This data is most useful when linked with environmental parameters, including climatic data and soil and land characteristics. To obtain useful data for a large-scale parasite control programme of ruminants organically reared in Tuscany, an organic cattle herd was selected for a pilot study to monitor endoparasitic diseases and their spatial and temporal distribution.

Materials and methods

In this study, the La Sterpaia organic cattle herd in the Natural Park of Migliarino-San Rossore-Massaciuccoli (Pisa) was monitored from May 2002 to March 2006 to:

- evaluate the presence, prevalence and intensity of endoparasitic diseases and their association with age and breed of cattle, farm management, environmental parameters and season
- identify and evaluate the risks associated with endoparasitic diseases.

La Sterpaia organic farm

The farm and its grazing areas are part of San Rossore Park (Pisa), located around La Sterpaia buildings near the Serchio River in the northern part of San Rossore Park (1.607.400°E, 4.843.780 N, Gauss-Boaga projection).

The farm is composed of nine pasture units, each of 8-9 ha. The different cattle breeds present on the farm, namely: Limousine, Pezzata Rossa and Pisana in 2002-2003 and Chianina, Pisana and Limousine in 2004-2006 are allowed to graze in different areas (Figs 1 and 2). Plant species in these areas are both grazed species and a large number of ungrazed species. Grazing is integrated with supplemental feed during certain periods of the year.

Parasitological techniques

Between 2002 and 2006, about 300 cattle were examined every two to three months. Individual and pooled faecal samples were collected from naturally infected cattle. A description of the examination of samples is given below.

Qualitative methods

The following methods were used:

- flotation test, using a low specific gravity solution (sg: 1.200) for *Coccidia*, gastrointestinal nematodes and cestodes (2, 8, 12, 14)
- flotation test using a high specific gravity solution (sg: 1.450) for *Dicrocelium dendriticum* (2, 8)
- Baermann method for lung strongyles (2, 8)
- sedimentation for *Fasciola hepatica* and Paramphistomidae (2, 8, 12).
- faecal cultures for infective stage (L3) gastrointestinal strongyle larvae (8, 10) to identify the percentage of infestation of genera (8, 12, 22)
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- faecal cultures for Eimeria oocysts to identify species (8, 14).

The percentage of positive samples indicated the prevalence of each parasitic disease.

**Quantitative methods**

A modified McMaster method (2, 15) was used to estimate the faecal egg count (FEC) for gastrointestinal nematodes (eggs per gram of faeces [EPG]) and Eimeria spp. (oocysts per gram of faeces [OPG]). The FEC indicated the intensity of coccidial and gastrointestinal nematode infections.

**Statistical analysis**

EPG and OPG data were examined using analysis of variance (ANOVA). Associations with date of sampling, age and breed were tested. Data on strongyles and coccidia were transformed logarithmically \[y = \log(X + 25)\] to normalise variance (8).

**Environmental analysis**

Several environmental parameters, such as meteorological data (1) (Tables I and II), soil physical and chemical characteristics (3, 4), hydraulic and drainage parameters (4, 18) and land cover and land use were monitored. In addition, data on biodiversity and field margins (4, 13) of grazing areas were collected and classified using principal components analysis (PCA) using Canoco 4.5 (20), based on the boundary’s structural attributes recorded in the field (shrub and tree cover, maximum and average height of vegetation, width and depth of ditches and the presence of a fence or herbaceous field margin).

Land-use heterogeneity (H) was determined using the following formula: \[ H = -\sum p(i,j) \times \log p(i,j) \] where \( p(i,j) \) is the proportion of the total number of pixels and land use types \( i \) and \( j \) are adjacent. Seven different land-use types were defined, namely:

- sown annual crops
- alfalfa
- pasture
- natural woodland
- poplar plantation
- rivers
- constructions.
Six transects of 2.25 km were divided into 10 pixels each and H was calculated based on land-use sequences between the pixels in these transects. Maximum land-use heterogeneity in the six transects was 1.45.

Table I
Monthly mean temperature, rainfall and relative dampness in San Rossore Park, March 2002-May 2003

<table>
<thead>
<tr>
<th>Date</th>
<th>Mean temperature (°C)</th>
<th>Rainfall (mm)</th>
<th>Relative dampness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mar 2002</td>
<td>11.2</td>
<td>2.2</td>
<td>71%</td>
</tr>
<tr>
<td>Apr 2002</td>
<td>13.3</td>
<td>49</td>
<td>75%</td>
</tr>
<tr>
<td>May 2002</td>
<td>17.3</td>
<td>8.4</td>
<td>79%</td>
</tr>
<tr>
<td>Jun 2002</td>
<td>21.7</td>
<td>56.8</td>
<td>78%</td>
</tr>
<tr>
<td>July 2002</td>
<td>22.2</td>
<td>32</td>
<td>79%</td>
</tr>
<tr>
<td>Aug 2002</td>
<td>21.6</td>
<td>149.8</td>
<td>84%</td>
</tr>
<tr>
<td>Sept 2002</td>
<td>18.2</td>
<td>219.4</td>
<td>81%</td>
</tr>
<tr>
<td>Oct 2002</td>
<td>15.4</td>
<td>87.4</td>
<td>87%</td>
</tr>
<tr>
<td>Nov 2002</td>
<td>13.4</td>
<td>148</td>
<td>86%</td>
</tr>
<tr>
<td>Dec 2002</td>
<td>8.8</td>
<td>110.8</td>
<td>86%</td>
</tr>
<tr>
<td>Jan 2003</td>
<td>6.1</td>
<td>10.8</td>
<td>81%</td>
</tr>
<tr>
<td>Feb 2003</td>
<td>5.2</td>
<td>12.8</td>
<td>65%</td>
</tr>
<tr>
<td>Mar 2003</td>
<td>10.2</td>
<td>14.8</td>
<td>76%</td>
</tr>
<tr>
<td>Apr 2003</td>
<td>14</td>
<td>70.8</td>
<td>72%</td>
</tr>
<tr>
<td>May 2003</td>
<td>22</td>
<td>1.4</td>
<td>77%</td>
</tr>
</tbody>
</table>

Source: Agenzia Regionale per lo Sviluppo e l’Innovazione nel Settore Agricolo-forestale-Regione Toscana, Servizio Agrometeorologico Regionale

Table II
Monthly mean temperature, rainfall and relative dampness in San Rossore Park, October 2004-March 2006

<table>
<thead>
<tr>
<th>Date</th>
<th>Mean temperature (°C)</th>
<th>Rainfall (mm)</th>
<th>Relative dampness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct 2004</td>
<td>17.1</td>
<td>141.4</td>
<td>89%</td>
</tr>
<tr>
<td>Nov 2004</td>
<td>10.4</td>
<td>108.3</td>
<td>86%</td>
</tr>
<tr>
<td>Dec 2004</td>
<td>7.9</td>
<td>102.0</td>
<td>85%</td>
</tr>
<tr>
<td>Jan 2005</td>
<td>5.3</td>
<td>40.9</td>
<td>88%</td>
</tr>
<tr>
<td>Feb 2005</td>
<td>4.3</td>
<td>49.1</td>
<td>76%</td>
</tr>
<tr>
<td>Mar 2005</td>
<td>10.2</td>
<td>43.2</td>
<td>80%</td>
</tr>
<tr>
<td>Apr 2005</td>
<td>12.8</td>
<td>51.9</td>
<td>74%</td>
</tr>
<tr>
<td>May 2005</td>
<td>18.1</td>
<td>43.5</td>
<td>78%</td>
</tr>
<tr>
<td>June 2005</td>
<td>21.9</td>
<td>29.6</td>
<td>78%</td>
</tr>
<tr>
<td>July 2005</td>
<td>23.3</td>
<td>10.0</td>
<td>70%</td>
</tr>
<tr>
<td>Aug 2005</td>
<td>22.4</td>
<td>33.8</td>
<td>79%</td>
</tr>
<tr>
<td>Sept 2005</td>
<td>19.1</td>
<td>200.2</td>
<td>81%</td>
</tr>
<tr>
<td>Oct 2005</td>
<td>17.3</td>
<td>95.3</td>
<td>87%</td>
</tr>
<tr>
<td>Nov 2005</td>
<td>13.4</td>
<td>150</td>
<td>86%</td>
</tr>
<tr>
<td>Dec 2005</td>
<td>8.6</td>
<td>110</td>
<td>84%</td>
</tr>
<tr>
<td>Jan 2006</td>
<td>6.2</td>
<td>35.1</td>
<td>81%</td>
</tr>
<tr>
<td>Feb 2006</td>
<td>7.3</td>
<td>42.7</td>
<td>70%</td>
</tr>
<tr>
<td>Mar 2006</td>
<td>11.4</td>
<td>50.6</td>
<td>80%</td>
</tr>
</tbody>
</table>

Source: Agenzia Regionale per lo Sviluppo e l’Innovazione nel Settore Agricolo-forestale-Regione Toscana, Servizio Agrometeorologico Regionale
Results

Grazing areas were classified as silt and clay/sand soil, with a mean percentage of organic material around 2%. The hydraulic drainage was poor; thus many ditches in the drainage system were occluded by dead vegetation. For these reasons, water pooling was frequently observed in some areas, especially during rainy periods. The biodiversity of field margins was relatively high (Figs 1 and 2), but land use for natural and cultivated pastures for animal nutrition reduced the landscape heterogeneity. Land-use heterogeneity was 1.12, 77% of $H_{max}$; the matrix surrounding cultivated areas – which consists of planted poplar wood, pine wood, semi-natural woodland and a river – contributed most to land-use heterogeneity in the study area. Within the cultivated area, little natural vegetation was observed. The main part of the Serchio area is used as meadows or pasture and consequently the soil is covered all year, with low land-use diversity related to the cultivated area only.

Eight boundary structure classes were identified by ordination analysis, based on the structural characteristics of the boundaries recorded in the field (Table III). The boundary structure was not correlated with land use of the adjacent fields because of the wide variety of boundary structure types and the relatively homogeneous land-use types (mainly meadows and pastures in the Serchio area). Most boundaries were characterised by the presence of a fence and this was possibly due to the presence of animals in all of the fields at some point in the crop rotation.

Regarding floral diversity, 127 plant taxa (between genus and species) have been recorded in the Serchio area. Species richness varied from 11 and 48 species per boundary. The average species richness per boundary structure class was variable but the structurally simpler boundaries belonging mostly to classes 4 and 8 had the lowest plant species richness (Table III). Distribution of the boundary classes was evenly spread over the entire area, indicating good spatial heterogeneity of the boundaries (Fig. 1). No correlation between plant species richness and type of disturbance (grazing, cutting) or land use was found (Fig. 2).

During all study periods, several species of parasites were identified. However, prevalence and, in the case of coccidia and gastrointestinal strongyles, intensity were highly variable. In most cases, this variability was associated with breed (Figs 3, 4, 5, 6, 7 and 8), age, season and meteorological data. In the first period examined (May 2002-May 2003), coccidiosis (mean prevalence 74%) was highly prevalent, with decreasing intensity during the year. Gastrointestinal strongyles (mean prevalence 96.7%) showed a higher prevalence and intensity in autumn and spring, while *Strongyloides papillosus* (mean prevalence 1.6%) and *Neoscaris vitulorum* (mean prevalence 3.2%) were found only in May 2002 and 2003. The highest prevalence of *Moniezia* spp. (mean prevalence 11.4%) was found in October 2002 and *F. hepatica* (mean prevalence 28.6%) between January and May 2003. *D. dendriticum* prevalence (mean prevalence 31.3%) increased between July 2002 and March 2003. During the first two years of monitoring, the Pisana breed was the only breed infected by *F. hepatica* (Fig. 7) and Paramphistomidae (mean prevalence 5.8%), with higher prevalence in bulls; infestation was associated with more frequent flooding and water pooling observed in the area used for the grazing of this cattle breed (Figs 1 and 2). The frequently observed water pooling is most probably due to the absence of ditches in the surrounding field boundary elements (Table III; Figs 1, 7 and 8). Decreased prevalence of coccidia (over than 90% in unweaned calves) and gastrointestinal helminths (gastrointestinal strongyles, *S. papillosus, N. vitulorum* and *Moniezia* spp.) were associated with increased age and the increase of liver fluke infestation was also observed in older animals. The former (coccidia and gastrointestinal helminths) were considered an important cause of production...
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Table III
Partitioning of the eight boundary structure classes identified in the Serchio area and their average plant species richness

<table>
<thead>
<tr>
<th>Class</th>
<th>Boundary structure description</th>
<th>Boundaries (%)</th>
<th>Average plant species richness (n°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Boundary with shrubs and a fence</td>
<td>23.5</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>Large herbaceous margin next to a fence</td>
<td>14.7</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>Boundary with shrubs and at least one ditch</td>
<td>2.9</td>
<td>29</td>
</tr>
<tr>
<td>4</td>
<td>Herbaceous field margin</td>
<td>2.9</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>Ditch with or without fence</td>
<td>2.9</td>
<td>48</td>
</tr>
<tr>
<td>6</td>
<td>Hedge with at least one ditch</td>
<td>8.8</td>
<td>29</td>
</tr>
<tr>
<td>7</td>
<td>Hedge with a fence</td>
<td>35.3</td>
<td>35</td>
</tr>
<tr>
<td>8</td>
<td>Small herbaceous margin under a fence</td>
<td>8.8</td>
<td>26</td>
</tr>
</tbody>
</table>

Figure 3
Mean prevalence of Coccidia (Eimeria spp.) in the different cattle breeds in an organic beef herd, 2002-2003
Grazing areas are identified by the capital letter of each breed
Other areas (land use units) represent areas to use, in turn, as pastures in subsequent years or as supplemental feed

Figure 4
Mean prevalence of coccidia (Eimeria spp.) in the different cattle breeds in an organic beef herd, 2004-2006
Grazing areas are identified by the capital letter of each breed
Other areas (land use units) represent areas to use, in turn, as pastures in subsequent years or as supplemental feed

Losses in young animals and the latter (flukes) in adult animals. Between 2004 and 2006, breed (Chianina, Limousine and Pisana) was associated with gastrointestinal strongyles EPG and coccidian OPG. Growth phase (cow, unweaned calf, weaned calf and bull) was associated with OPG (p=0.006), while EPG was associated with season (p=0.046). The prevalence of flukes (mean prevalence 28.6%, 14% and 9.5% respectively for F. hepatica, D. dendriticum and Paramphistomidae) increased between the autumn of 2005 and spring of 2006 and was higher in Pisana and in cows of all breeds. The prevalence of coccidian (mean prevalence 67.4%) was higher in Limousine, especially in unweaned calves (90%). The prevalence of gastrointestinal strongyles (mean prevalence 77.5%) was
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Figure 5
Mean prevalence of gastrointestinal strongyles for the different cattle breeds in an organic beef herd, 2002-2003
Grazing areas are identified by the capital letter of each breed
Other areas (land use units) represent areas to use, in turn, as pastures in subsequent years or as supplemental feed

Figure 6
Mean prevalence of gastrointestinal strongyles for the different cattle breeds in an organic beef herd, 2004-2006
Grazing areas are identified by the capital letter of each breed
Other areas (land use units) represent areas to use, in turn, as pastures in subsequent years or as supplemental feed

Figure 7
Mean prevalence of Fasciola hepatica for the different cattle breeds in an organic beef herd, 2002-2003
Grazing areas are identified by the capital letter of each breed
Other areas (land use units) represent areas to use, in turn, as pastures in subsequent years or as supplemental feed

Figure 8
Mean prevalence of Fasciola hepatica for the different cattle breeds in an organic beef herd, 2004-2006
Grazing areas are identified by the capital letter of each breed
Other areas (land use units) represent areas to use, in turn, as pastures in subsequent years or as supplemental feed
higher in unweaned calves (>80%) of all breeds. The prevalence of *S. papillosus* (mean prevalence 11.2%) was highest in Limousine cows. *N. vitulorum* (mean prevalence 5.6%), gastrointestinal strongyles and *S. papillosus* showed higher prevalence and intensity of infestations in autumn. The prevalence and intensity of coccidiosis increased during the period under study. *Eimeria bovis* and *E. zuernii* were predominant in unweaned calves of all breeds, while *E. alabamensis* and *E. ellipsoidalis* occurred only in Limousine cattle. The gastrointestinal strongyles present in the herd were as follows: *Ostertagia* spp., *Haemonchus* spp., *Cooperia* spp., *Trichostrongylus* spp., *Nematodirus* spp., *Bunostomum* spp., *Oesophagostomum* spp. The greatest variability was recorded in the Chianina breed. The highest prevalence of *Moniezia* spp. (mean prevalence 4.5%) was found in March 2006, particularly in Chianina.

**Discussion**

Times and methods of sampling and the number of samples collected were determined by several factors related to the management system used on the study farm. Sampling was only possible when livestock, normally grazing in different pastures and dispersed from one another, were moved and contained for other management reasons (for example, vaccination and treatment). Thus, some sampling bias was present. In addition, there were changes in some farm management practices during the last period of the study, possibly resulting in higher rates of contact among the different breeds. Despite these deficiencies, the data obtained showed a clear picture of the parasitological problems present in the farm.

As reported in previous studies (21), prevalence, intensity and number of endoparasites isolated in the *La Sterpaia* farm demonstrate the importance of endoparasitic diseases in organic farms. Coccidia (*Eimeria* spp.), nematodes (gastrointestinal strongyles, *S. papillosus* and *N. vitulorum*), flukes (*F. hepatica, D. dendriticum*, Paramphistomidae) and cestodes (*Moniezia expansa* and *M. benedeni*) were identified. The absence of the lung-worm (*Dictyocaulus viviparous*) during all study periods (2002-2006) confirms previous observations of its absence from this area (2).

Among the endoparasitic diseases detected, coccidiosis and gastrointestinal strongylosis were the most prevalent. Highly pathogenic coccidian species, such as *E. bovis, E. zuernii* and *E. alabamensis*, and the most pathogenic genera of gastrointestinal strongyles (2, 19) were isolated. Higher prevalence and infection loads for these two parasitic diseases were observed in younger animals and in warmer months (spring and autumn). For example, acute clinical coccidiosis was observed in May 2006 in Chianina and Limousine calves, at the same time as high OPG counts. In the case of gastrointestinal strongyles, qualitative and quantitative data obtained in this study showed high breeding risks (2) in all breeds, mainly in the final period of the study. Although the prevalence and intensity of *S. papillosus, N. vitulorum* and *Moniezia* spp. infestations were generally low, a high risk (especially for *N. vitulorum*) was observed: the prevalence in bulls was similar to that found in calves and young stock (2).

The prevalence of flukes was higher in winter and in older cattle, representing an important health problem and a cause of reduced production. During the first two years of monitoring, *F. hepatica* (Figs 7 and 8) and Paramphistomidae infestation was only detected in the Pisana breed; infestation was associated with frequent flooding and water pooling, mostly due to the absence of ditches in the surrounding field boundary elements observed in the area used for the grazing of this cattle breed. However, in the final part of the study, infestation in the two other breeds present on the farm (Chianina and Limousine breeds) was also observed (although at a lower prevalence). This infestation might have been linked to the higher contact rates between cattle of the different breeds during this period, probably enabling the spread of these two parasites through all breeds on the farm. During the first few months of 2006, several cases of mortality in Chianina cows and young
stock due to heavy *F. hepatica* infestations were observed on the farm. The absence of clinical infestations in the Pisana cattle, despite higher prevalence of *F. hepatica* infestation, could indicate a higher resistance of this breed to the pathogenic effects of *F. hepatica*.

**Conclusion**

Results obtained in this study show that in the organic *La Sterpaia* cattle herd, prevalence and intensity of endoparasitic diseases, and the health and breeding risks linked thereto, were associated with factors such as age and breed and with several environmental and management factors (grazing area, season and meteorological data, field boundary structures and hydraulic and soil drainage variables).

**References**