Emergence of leishmaniasis in northern Italy

Marina Gramiccia, Luigi Gradoni, Trentina Di Muccio, Gioia Bongiorno and Michele Maroli

Unit of Vector-borne Diseases and International Health, MIPI Department
Istituto Superiore di Sanità, Rome, Italy

Background – Canine leishmaniasis (CanL) is traditionally endemic in central-south Italy, whereas northern regions (Padana plane and Alpine territories) have been considered Leishmania-free (Pozio et al., 1985) until early 1990’s, when CanL foci were firstly identified in Veneto and Piedmont. Because of the appearance of autochthonous human cases (VL), a national project was launched in 2003 to systematically collect CanL sandfly data in 5 northern regions of Italy: Valle d’Aosta, Piedmont, Lombardy, Veneto, Trentino-Alto Adige and Emilia Romagna. A 2.7% seroprevalence was revealed among some 6,500 dogs from 18 scattered foci, with 118 proven autochthonous infections. *Phlebotomus perniciosus* was recorded in almost all new foci, characterized by hilly sub-Apennine and sub-Alpine environments. *P. neglectus*, *P. ariasi* and *P. perfiliewi* showed more limited diffusion in the territories investigated (Capelli et al., 2004; Rossi et al., 2005; Ferroglio et al., 2005). In the frame of the subproject EDEN-LEI we aimed to study and monitor leishmaniasis spreading in two selected areas of the northern Italy, trying to answer the following questions: (i) Is *Leishmania* transmission spreading northward into Alpine valleys? and (ii) Which environmental conditions limit the diffusion of *P. ariasi* and *P. perfiliewi* towards northern latitudes and, in general, the spreading of all *Leishmania* vectors in the large Padana plane?

Materials and methods

Study locations – Of the 2 selected study places (Fig 1A), one is a sub-Alpine area (1800 Km²) of Lombardy region. Here, 28 sandfly collecting sites were established in a lacustral area at south (Iseo Lake) and in three narrow valleys of Brescia and Bergamo provinces (Camonica, Seriana and Brembana valleys) at north, with a linear grid of about 10 km per valley (Fig. 1B). The second study place is a sub-Apennine area (1500 Km²) including parts of Piedmont, Lombardy and Emilia Romagna regions. Sandfly collections were carried out during in 2006 in 20 sites distributed in flat and hilly areas (with a grid of about 10 km) including provinces of Alessandria, Lodi, Milano, Pavia and Piacenza (Fig. 1B).

Sandfly collection - Sandflies specimens were collected by sticky traps (20x20 cm, castor-oiled paper) settled in a variety of sandfly diurnal resting sites (scarp wall cracks, animal shelters and houses). All phlebotomine specimens were identified to the species level (Maroli et al., 1994). Site information was recorded in the Arcview GIS database adopted by the EDEN-LEI UK-French-Spanish teams in summer 2005, modified according to the relevant Italian habitats.

Human leishmaniasis surveillance – Both active and passive recording of human leishmaniasis cases from northern Italy were performed and analysed as regards the putative origin of infection.

Canine leishmaniasis investigations - CanL screening activities were performed by serology through IFAT according to procedures following the protocol of the Office International des Epizooties (Gradoni and Gramiccia, 2004). The cut-off dilution was set in the range of 1:80 (suggestive) - 1:160 (confirmatory). From IFAT-positive dogs a lymph node aspirate material was cultured in Evans modified Tobie’s medium and *Leishmania* strains were identified by starch gel isoenzymes electrophoresis and/or PCR-RFLP according to Gramiccia (2002) and Minodier et al (1997) procedures, respectively.

Leishmania natural infection - Natural infections by *Leishmania* were investigated in sandflies by nested PCR (Velo et al., 2005).

Results and Discussion

Presence and distribution of phlebotomine vectors - In the sub-Alpine area, 13/28 sites monitored were positive for sandflies, for a total of 169 specimens collected. Four species were identified of which two were *Leishmania* vectors, *P. perniciosus* and *P. neglectus*. No previous sandfly records are available for this area before our survey (Maroli et al., 2006). Sandflies were found at altitudes from 206 to 570 m a.s.l. The records of *P. neglectus* in Camonica and Brembana valleys add further data to the recent findings of this sandfly vector in Italy (Maroli et al. 2002). In the sub-Apennine area only 3/20 collecting sites were found negative for sandflies, of which 2 in the Padana plane. Four species were identified at altitudes ranging from 52 to 440 m a.s.l., including 2 proven *L. infantum* vectors: *P. perniciosus* (12/17 positive sites) and *P. perfiliewi* (2/17, both in Piacenza province). By comparing the entomological data from both areas, it can be
concluded that the most competent vector of *L. infantum, P. perniciosus*, is widespread in all the environments investigated. On the contrary, the secondary vectors *P. neglectus* and *P. perfiliewi* show limited distribution, being the first present only in the sub-Alpine area and the second in the sub-Apennine one. Apparently, the large Padana plane (see Fig. 1) seems to be free from phlebotomine sandfly colonization, and its peculiar environment could also represent a limit for the southward/northward diffusion of the above species. Finally, the other *Larroussius* vector recorded in Italy, *P. ariasi*, was apparently absent in both areas, confirming previous recording of this species in a limited territory bordering France, namely some sites of Liguria and Piedmont regions (Biocca et al., 1977; Maroli et al., 1997).

**Human leishmaniasis.** The general trend of VL in Italy was confirmed to settle on the average of 200 cases/year. As for the northern regions, cumulative incidence records from 1998 to present indicate that VL cases are diagnosed in all of them, and some are undoubtedly autochthonous. However, most of the 73 cases recorded in high-incidence regions (Piedmont, Lombardy and Trentino-Alto Adige) should be classified as imported ones.

**Canine leishmaniasis** – Serological investigations for CanL were carried in 2004-2005 in 9 sites of northern Italy. Autochthonous cases were detected for the first time in Alessandria, Pavia, Piacenza, Brescia and Lecco provinces. A *Leishmania* strain was obtained and identified as belonging to *Leishmania infantum* zymodeme MON-1. In 2006, serosurveys were performed in localities at the entrance of Brembana and Seriana valleys, which revealed 4/69 positive dogs (5.80%), and in different places of Camonica valley (0/52). Therefore, in the whole selected sub-Alpine area a total of 4 autochthonous dogs resulted positive, out of 121 examined (3.31%).

**Data management** - A retrospective collection and analysis of published papers and unpublished records from 1965 concerning CanL and its vectors in all Italian regions, was performed to complete 2 databases, respectively for CanL and sandflies.

**Activities planned for the next years**
1. Study of the seasonal dynamics and distribution of sandfly populations, with particular reference to the altimetry of each species.
2. Study on the natural *Leishmania* infections of sandflies.
3. Molecular tools to investigate on the association of *L. infantum* and *P. perniciosus/P. neglectus* populations in the area surveyed and comparisons with neighbouring areas.
4. Updating of the information on notified cases of human leishmaniasis from northern Italy.
5. CanL serological survey will start in the sub-Apennine area in order to assess the presence of autochthonous CanL cases. A similar survey will continue in the sub-Alpine area.
6. Depending on results obtained in 5), parasitological and molecular surveys will be planned in order to identify *Leishmania* from dogs by biochemical and molecular techniques.
7. In collaboration with the EDEN-LEI consortium, GIS framework and horizontal statistical plan will be planned to evaluate the risk for the leishmaniasis spread.

REFERENCES