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First isolation of *Salmonella enterica* serovar Napoli from wild birds in Italy

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Abstract

Salmonella enterica serovar Napoli (*S.* Napoli) is an emerging serovar in Italy. It accounts for 2-4% of all serovars isolated from human infections. The zoonotic origin of this serovar is still unknown and this makes difficult to apply any control intervention. We report here the isolation of *S.* Napoli from a river nightingale (*Cettia cetti*, Temminck 1820) which represents the first description of this serovar from wild birds. This finding adds knowledge to the ecology of *S.* Napoli and addresses further studies aimed to assess the epidemiologic link between *S.* Napoli isolated from wild birds, food, environmental sources and human infections.

Key words
- *Salmonella* Napoli
- zoonoses

INTRODUCTION

Salmonellosis was the second most commonly reported cause of foodborne diseases in the European Union (EU) in 2011 [1]. More than 2500 serovars of *Salmonella enterica* have been described but only a limited number of serovars are associated to most human infections. The most commonly reported serovars are *Salmonella* Enteritidis and *Salmonella* Typhimurium, which cumulatively account for up to 70% of all serovars isolated from human infections.

In Italy, basic epidemiologic and microbiologic information on *Salmonella* isolates from human infections and from samples of veterinary origin (animals and foodstuffs) are collected through the laboratory based surveillance systems Enteric Pathogen Network – EnterNet (www.iss.it/ente) and EnterVet (www.izsvenezie.it) networks, respectively. In addition, EnterNet collects information on *Salmonella* strains isolated from environmental sources, mainly surface water.

EnterNet and EnterVet networks share diagnostic procedures and databases in order to monitor the trends and the emergence of *Salmonella* serovars over the time.

In the framework of the EnterNet surveillance, an increase of human infections due to *Salmonella enterica* serovar Napoli (*S.* Napoli) was observed at the beginning of this century and, since then, this serovar is reported with a frequency of 2-4% each year (*Figure 1*) [2, 3]. Although a seasonal pattern is consistent for most serovars, *S.* Napoli shows a marked seasonality with human cases occurring primarily in the period June-October. Among environmental samples, *S.* Napoli represents 2% of all serovars, and as for human isolates 70% of the annual total for this serovar is identified in the period June-October. Finally, data from EnterVet network show that *S.* Napoli is rarely found in farm animals and foodstuffs of animal origin (www.izsvenezie.it/images/stories/Pdf/salmonellosi/report_Enter_Vet_2010.pdf).

On the basis of these findings, it can be hypothesized that the environment and in particular surface water can be a key factor for the direct or indirect transmission of this serovar to humans. However, the source of environmental contamination by *S.* Napoli and the zoonotic origin of this serovar remain unknown.

With the aim to evaluate a possible role of wild birds as a reservoir of *S.* Napoli, a "pilot study" was performed in Italy in 2012.

MATERIAL AND METHODS

The study was conducted in 2012, in a protected natural area in Central Italy, during the period May-October which coincides with the highest prevalence of *S.* Napoli infections in humans and with the bird migration in Central Italy.

Fifty-six wild birds including migratory and resident species were captured, by a transparent nylon mesh net, and identified by the staff of the authorized Ornithological Stations. Depending on the bird size, rectal swabs or stools, by dropping method, were collected. All birds

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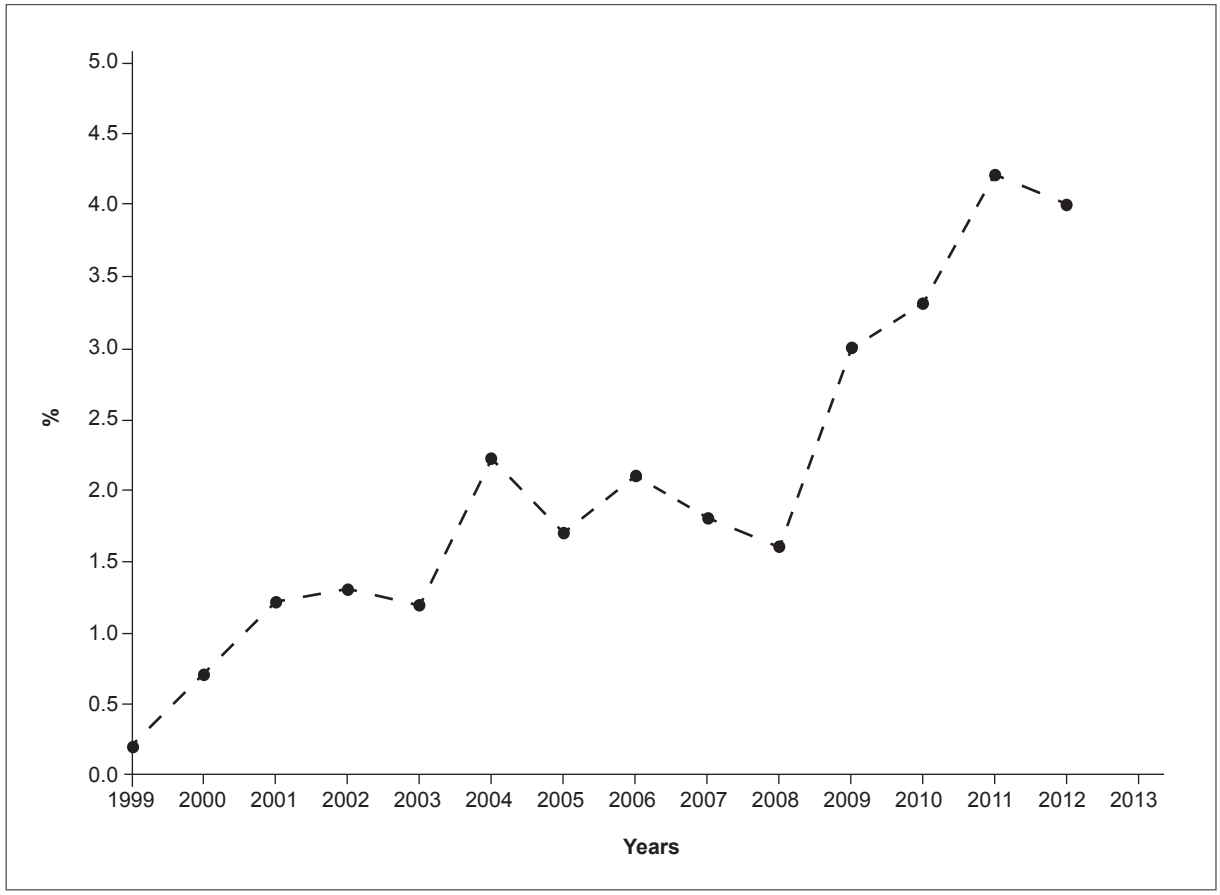

Figure 1
Proportion of *S.* Napoli strains isolated from human infections. Italy 1989-2012.

were released immediately after the sampling.

Each fecal sample was processed within 24 hours from collection. Bacteriological analyses for *Salmonella* detection were performed according to standard protocols [4, 5]. Typical *Salmonella* colonies were selected, confirmed with the API 20E system (bioMerieux, Marcy l'Etoile, France), and then were serotyped by using Statens Serum Institut diagnostic antisera (Copenhagen, Denmark) following Kauffman-White serotyping scheme [6].

RESULTS AND DISCUSSION

Salmonella spp. was isolated from 3 of the 56 captured birds (5.3%). *S.* Typhimurium was isolated from one out of 13 night herons (*Nycticorax nycticorax*, Linnaeus 1578), *S.* Livingstone from one out of 8 blackcaps (*Sylvia atricapilla*, Linnaeus 1578), and *S.* Napoli from 1 out of 6 river nightingales (*Cettia cetti*, Temminck 1820).

Several studies in different countries have been undertaken to assess the prevalence of *Salmonella* in wild birds and a wide variety of serovars has been reported [7] underlying how the contact with wild or captive birds pose a possible threat to human health even though many epidemiologic details remain to be defined. To the best of our knowledge this is the first report of isolation of *S.* Napoli from wild birds and this result underpins the importance of wild birds as potential as spreaders of this serovar in the water and in the environment in general.

In Italy, the isolation of *S.* Napoli from wild animals have been reported from intestinal content of lizard [8] and, more recently, from intestinal content of boars hunted in Northern and Central Italy [9, 10]. On the whole, these findings suggest an important role of wild in the diffusion of *S.* Napoli in environment and in the possible transmission to humans trough contaminated water and food.

Foodborne outbreaks due to *S.* Napoli and associated with food of Italian origin have been reported in Europe. In 1982, a large outbreak occurred in England and Wales was associated with the consumption of chocolate bars manufactured in Northern Italy [11] and more recently, in 2008 and 2009, strong evidence foodborne outbreaks due to *S.* Napoli have been reported in Sweden and, in both outbreaks, fresh vegetables produced in Italy were implicated, [12, 13]. In addition, frequent international alerts concerning the presence of *S.* Napoli in fresh vegetables produced in Italy have been notified by the EU, Rapid Alert System for Food and Feed (RASFF) (http://ec.europa.eu/food/food/rapidalert, 2004.644; 2006.0331; 2008.1399; 2009.0673; 2013.0939; 2013.0644).

Furthermore, a case control study carried out in Northern Italy showed that occurrence of infections

due to *S.* Napoli was significantly more common among individuals with exposure to surface water, as swimming and recreational activities [14].

In this context, the results of this "pilot study" add further knowledge to the eco-epidemiology of *S.* Napoli that constitutes an emerging relevant public health concern in our country. This deserves to be dealt via a multidisciplinary, cross-sectoral approach and intervention.

Future longitudinal studies conducted on a larger number of wild birds, in different geographical areas and in different periods of the year will assist in acquiring a better understanding of the relationships between birds and emerging zoonotic diseases for planning sound interventions in respect of the environment while improving public health.

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Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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