



Letter to the Editor

Occurrence of the tetracycline resistance gene *tetA(P)* in Apennine wolves (*Canis lupus italicus*) from different human–wildlife interfaces



Sir,

The proliferation of antimicrobial resistance genes (ARGs) in the environment is considered an important threat to human and animal health because it may contribute to the selection of multidrug-resistant bacteria, included clinically relevant pathogens [1]. Indeed, the One Health approach to this issue should consider the role of various environmental sources of ARGs such as soil, water and wild animals. Free-ranging wildlife often occupies habitats lacking human activities, with minor exposure to the contamination of resistance determinants. Despite that, many wild species are dependent on anthropogenic food sources or are less able to avoid anthropogenic inputs because of the expansion of urban and agricultural areas into fragmented wildlife habitats [1]. In this regard, an apex predator such as the Apennine wolf (*Canis lupus italicus*), which may live in habitats with different ecological features and food availability, may be considered a sensitive indicator of the environmental resistome.

In order to identify the genetic determinants of antimicrobial resistance, the application of culture-independent approaches (PCR or metagenomics assay) are powerful and quick tools to investigate the ARGs occurring in wildlife.

During the year 2017, a sanitary survey of two wolf packs of Majella National Park (Abruzzi, Italy) was carried out collecting stool specimens by means of a innovative non-invasive GPS-based telemetry protocol [2]. Briefly, two female adults were captured in different sites with evidence of resident and breeding wolf packs and were fitted with a GPS-GSM collar (Followit, Sweden) programmed to take 48 localisations/24 h for 10 days/month. Based on the GPS data, along with video data obtained from camera traps, the monitored wolves belonged to two different packs, named Majella Centrale (MC) and Bassa Valle dell'Orta (BVO). The MC pack was a recent formation unit, organised with a few animals (down to four wolves), whose home range (28 km²) is entirely included in the total reserve area of the park, located between 800–2200 m above sea level, where only a few activities (i.e. research investigations and grazing) are allowed. In contrast, the BVO pack appeared to be a stable and reproductive nuclear family (at least seven individuals) with a larger home range (40 km²), partially outside the park boundaries, comprising villages and other human infrastructures, up to 800 m (Fig. 1). For each pack, 10 fresh faecal samples were collected by visiting suitable kill or rest sites. Total DNA was extracted using a Brief Protocol Exgene™ Stool DNA Mini Kit (GeneAII, South Korea). A set of single and multiplex PCRs for tetracycline, lincomycin, chloramphenicol, aminoglycoside and

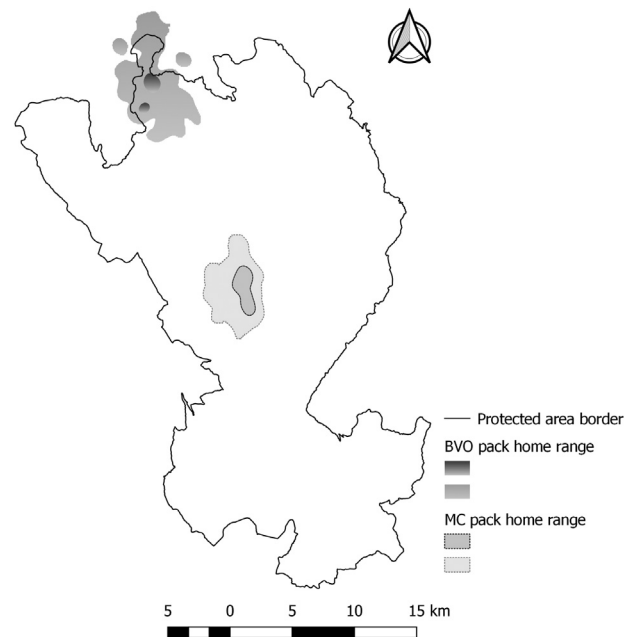


Fig. 1. Geographic distribution of Majella Centrale (MC) and Bassa Valle dell'Orta (BVO) packs home range in Majella National Park (Abruzzi, Italy).

colistin resistance genes was applied (Supplementary Table S1) and the relative products were analysed on 1.5% (w/v) agarose gels. No amplification was obtained for the investigated ARGs except for the *tetA(P)* gene, detected in seven samples belonging to the BVO pack. In order to verify the specificity of the reactions, sequencing analysis of one fragment was performed (GenBank accession no. **MT077858**), highlighting an identity up to 99.85% with the analogous tetracycline resistance gene identified in *Clostridium* spp. (GenBank accession no. LR607381).

A previous metagenomics study has underlined the dominance of tetracycline resistance genes in animal microbiomes, probably explained by the historical and current exposure to tetracycline in animal husbandry [3]. Indeed, environmental contamination may explain the results obtained in our study, considering that in animals up to 75–80% of tetracycline doses are excreted in the urine and faeces and the tetracycline molecules are characterised by long-term persistence in soils, with a half-life of 578 days [4]. In addition, detection of the *tetA(P)* gene could be related to the predation activity of the wolves on domestic or wild ungulates, as previously suggested for the Iberian wolf population [5]. However, during our study a total of nine kill sites (four for the BVO pack and five for the MC pack) with evidence of wolf predation on wild ungulates (six carcasses) and domestic small ruminants (three

carcasses) was identified for both packs, whilst the tetracycline resistance gene was detected only from BVO samples. The density of animals and the characteristics of their home range, rather than the predation activity, could have affected the distribution of genes in the investigated packs. Indeed, the BVO home range included villages, pig farms, a slaughterhouse and a meat processing plant, suggesting a relationship between spatial distance to human infrastructures and detection of the *tetA(P)* resistance gene, due to a major anthropogenic pressure for the BVO pack compared with the MC pack. The results revealed herein confirm that the ecological context in which wildlife lives can provide potential information about the environmental pathways by which antimicrobial resistance may be acquired and dispersed [1]. The molecular investigations, based on culture-independent PCR, appear to be an alternative approach useful to study ARGs and their potential role as environmental contaminants.

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Ethical approval

Not required.

Conflict of interests

None declared.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jgar.2020.09.011>.

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