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# Low Serologic Prevalences Suggest Sporadic Infections of Hepatitis E Virus in Chamois (*Rupicapra rupicapra*) and Red Deer (*Cervus elaphus*) in the Italian Alps

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ABSTRACT: Hepatitis E virus (HEV) is a worldwide public health concern, with an increase in human autochthonous cases in Europe. Although domestic pigs and wild boar (*Sus scrofa*) are the main reservoirs of HEV, the constant expansion of wild ruminants increases the potential for HEV transmission. We investigated HEV infection in chamois (*Rupicapra rupicapra*) and red deer (*Cervus elaphus*) in the Italian Alps using an enzyme-linked immunosorbent assay (ELISA). We detected HEV antibodies from 2013 to 2015 in both host species, with seroprevalences of 1.2% and 0.8% in chamois and red deer, respectively. All serum samples that were positive to HEV antibodies by ELISA were negative when tested by real-time reverse-transcriptase PCR to detect HEV RNA. The observed low seroprevalence of HEV suggested a sporadic circulation of HEV in the alpine environment, and it was consistent with the low seroprevalence observed in wild boar in the Alps. Our observations supported the role of chamois and red deer as spillover hosts of HEV infections in the Italian Alps.

*Key words:* Chamois, hepatitis E virus, Italy, red deer, seroprevalence.

Hepatitis E virus (HEV) of the *Hepeviridae* family represents a worldwide public health issue, with an increase of human cases acquired in Europe (Aspinall et al. 2017). Fecal-oral transmission is the most common route of HEV transmission and infections in humans may occur by means of virus-contaminated water, consumption of raw or undercooked meat, and by direct contact with infected animals (Rutjes et al. 2010; Van der Poel 2014). Moreover, HEV is very resistant under environmental conditions, providing high persistence (Parashar et al. 2011).

Currently, eight genotypes (1–8) of HEV are recognized (Al-Sadeq et al. 2018) and characterized by different geographic distribution (Zehender et al. 2014; Hakim et al. 2017). The spectrum of receptive hosts is unknown and the ever-expanding host range poses a significant concern for understanding the source of zoonotic HEV infections (Meng 2016). The different ecologic interactions among its numerous host species make HEV epidemiology difficult to understand, and the reservoirs and spillover hosts are yet to be defined (Pavio et al. 2017; King et al. 2018). Domestic pig and wild boar (*Sus scrofa*) are considered to be the main reservoirs of HEV while deer have been reported as spillover hosts in European countries (Boadella 2015; Anheyer-Behmenburg et al. 2017; Spancerniene et al. 2018).

The seroprevalence of HEV in wild boar in the Alps and in prealpine areas ranges from 4.9% to 10.2% (Caruso et al. 2015; Martinelli et al. 2015), likely related to scattered and irregular presence of this host. In the central Italian Apennine mountain range, where wild boars are abundant, high HEV seroprevalences were detected reaching 48.7% (Martinelli et al. 2015). No information is available for HEV infection in alpine wild ruminants, and the relationship between HEV infection in wild ruminants and wild boars needs investigation. In particular, red deer (*Cervus elaphus*) are expanding their distribution and have a spatial overlap with wild boar. Alpine chamois (*Rupicapra rupicapra*) are the most abundant wild ungulates in the Alps (Carnevali et al. 2009), but their high mountain habitat limits interactions with wild boar and a lower exposure to HEV is expected. We aimed to serologically investigate HEV in chamois

and red deer in order to provide basic epidemiologic patterns of this infection in alpine wild ruminants.

Sampling was carried out from 2013 to 2015 during autumnal hunting seasons and depopulation plans in three study areas. Area 1 was in the Lepontine Alps (northwestern Italian Alps), where populations of investigated species have an estimated density of 6.7 chamois/km$^2$ and 2 red deer/km$^2$ (VCO2 2014). Area 2 was the Orobic Alps (southeastern Italian Alps), with a density of 8.2 chamois/km$^2$ (De Liberato et al. 2015). Area 3 was in the Stelvio National Park (central eastern Italian Alps) with a mean density of 9.5 red deer/km$^2$ (Raganella Pelliccioni et al. 2013). In Areas 1 and 2, there was a low, but strongly increasing, density of wild boar (0.1–0.5 animals/km$^2$; Carnevali et al. 2009). No wild boars were present in Area 3 (Stelvio National Park 2019). Chamois were sampled in Areas 1 and 2 while red deer were sampled in Areas 1 and 3. The age for each animal was estimated by tooth eruption patterns, identifying three age classes: kid (<1 yr), yearling (1 yr), and adult (>2 yr).

Blood samples were collected in the field from major blood vessels during animal bleeding or from the cardiac clot at the hunting control centers. After centrifugation at 242 × G for 15 min, highly hemolyzed sera were excluded and the remaining samples were stored at –20 C.

A total of 172 chamois and 254 red deer sera were collected. Samples were collected from 128 chamois from Area 1, 44 chamois from Area 2, and 81 and 173 red deer from Areas 1 and 3, respectively. Sera were tested by a species-independent enzyme-linked immunosorbent assay (HEV ELISA 4.0v, MP Diagnostics-Biomedicals, Singapore), previously used in wild boar and cervids (Rutjes et al. 2010; Thiry et al. 2017), which simultaneously detects immunoglobulins M, G, and A formed against HEV recombinant protein ET2.1 (Hu et al. 2008). The ELISA was performed according to manufacturer recommendations using the cut-off values they recommended. We calculated seroprevalence and confidence limits using the Wilson method (Brown et al. 2001). Positive sera for HEV antibodies were individually tested for the presence of HEV RNA using a one-step real-time reverse-transcription PCR (Jothikumar et al. 2006).

Chamois and red deer showed a HEV seroprevalence of 1.2% (2/172; 95% confidence interval: 0.3–4.1 exact Wilson) and 0.8% (2/254; 95% confidence interval: 0.2–2.8), respectively. Seropositive chamois were two females, 2 and 7 yr old from area 1, while seropositive red deer were from area 1 (one female 7–9 yr old) and area 3 (one female over 15 yr old). The mean optical density and SD values for negative and positive samples were 0.02 (±0.03) and 0.52 (±0.17), respectively, for chamois; 0.01 (±0.03) and 0.25 (±0.02) for red deer. All samples tested negatively for HEV RNA.

We report HEV circulation in chamois and red deer in alpine environments, showing a low prevalence of HEV antibody. The low prevalences were observed during a 3-yr monitoring plan, suggesting that HEV has a sporadic presence in both hosts in the Alps. Although wild boar are known to be a reservoir of HEV, this species shows a wide range of seroprevalences in Italy, ranging from 4.9% to 48.7%, with the highest values in central Apennines (Martinelli et al. 2015) where this host is abundant. Red deer share habitat in this area with an increasing number of wild boar and had the highest HEV seroprevalence (13.9%; Di Bartolo et al. 2015). On the other hand, our finding of a sporadic presence of HEV in red deer is consistent with the low prevalence observed in wild boar in alpine and prealpine environments (Caruso et al. 2015; Martinelli et al. 2015). These observations support the role of red deer as a spillover host from wild boar population in the Alps.

However, we detected HEV-positive sera in a red deer in Stelvio National Park, where wild boar are not present, and in a chamois, a host species that usually lives in remote, high-altitude habitats where interactions with wild boars and humans are limited. Therefore, we cannot rule out other, unknown sources of infection for alpine wild ungulates besides

wild boar. Currently, data about seroprevalence in livestock within the study area are not available.

The ongoing expansion of wild boar populations throughout a wide spectrum of habitat types, including alpine grasslands over 2,000 m in elevation (Massei et al. 2015), could lead to more interspecific interactions and thus to the increase of HEV spread. The HEV detection in alpine ruminants should be further considered in relation to the public health implications linked to the hunting activity and game meat consumption, considering the high number of chamois and red deer (about 13,000 and 10,000, respectively) culled in Italy every year (Raganella Pelliccioni et al. 2013).

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