

Self-Clearance of Pestivirus in a Pyrenean Chamois (*Rupicapra pyrenaica*) Population

Emmanuelle Gilot-Fromont,^{1,2,7} Mathieu Garel,³ Philippe Gibert,³ Sébastien Lambert,² Pierre Menaut,⁴ Brigitte Bonetti,⁵ Yvette Game,⁵ Gaël Reynaud,⁵ and Kévin Foulché⁶ ¹Université Lyon 1, Unité Mixte de Recherche du Centre National de la Recherche Scientifique 5558-Laboratoire de Biométrie et Biologie Evolutive, VetAgro Sup-Campus Vétérinaire de Lyon, 1 Avenue Bourgelat, 69280 Marcy L'Etoile, France; ²Université Lyon, Unité Mixte de Recherche du Centre National de la Recherche Scientifique 5558-Laboratoire de Biométrie et Biologie Evolutive, Bâtiment Mendel, Université Lyon 1, 43 Bd du 11 Novembre 1918, 69622 Villeurbanne Cedex, France; ³Office National de la Chasse et de la Faune Sauvage, Unité Faune de Montagne, Les Portes du Soleil, 147 Avenue de Lodève, 34990 Juvignac, France; ⁴Office National de la Chasse et de la Faune Sauvage, Délégation Régionale Occitanie, Cellule Technique, 1 Route du Puget, 09120 Crampagna, France; ⁵Laboratoire Départemental d'Analyses Vétérinaires de la Savoie, 321 Chemin des Moulins, 73000 Chambéry, France; ⁶Office National de la Chasse et de la Faune Sauvage, Délégation Régionale Occitanie, Cellule Technique, Impasse de la Chapelle, 31800 Villeneuve de Rivière, France; ⁷Corresponding author (e-mail: emmanuelle.gilotfromont@vetagro-sup.fr)

ABSTRACT: Understanding the dynamics of host-pathogen interaction is key to the management of epidemics. A pestivirus belonging to the border disease virus group 4 emerged around 2001 in Pyrenean chamois (*Rupicapra pyrenaica*) in Spain and France. The virus had significant demographic impact in some populations, but it was less harmful and more endemic in other places. The determinants of these local variations are still unclear. Here, we documented empirical evidence of self-clearance of the virus in a chamois population in France. This population has regularly been counted, and chamois were trapped and harvested each year, providing unique demographic and epidemiologic surveys of the population since 1984 and 1994, respectively. The virus was detected using direct (PCR) and indirect (antibody) testing. We showed that virus transmission declined in 2011–12 and likely ceased in 2013, leading to a decline in antibody prevalence since 2014. Self-clearance may be due to limited exchanges with other populations, decrease in population size after an epizootic, and herd immunity. The age structure of captured animals shifted to younger age classes after virus self-clearance, suggesting a return to a colonizing population structure. The possible consequences of virus re-entry are discussed. This observation suggests that pestivirus dynamics occurs at the scale of the metapopulation of Pyrenean chamois. Local self-clearance and re-emergence may help explain the variation of virus dynamics at the local scale.

Key words: Age structure, border disease virus, metapopulation, Orlu, *Rupicapra pyrenaica*

Pestivirus emerged as a health issue in Pyrenean chamois (*Rupicapra pyrenaica*) in 2001, when massive mortality was observed in Spain and France (Marco et al. 2009).

However, retrospective studies using serology (Marco et al. 2011), phylogenetic reconstruction (Luzzago et al. 2017), or mathematical modeling (Beaunée et al. 2015) suggested that the virus entered the chamois populations between 1989 and 1991. Since then, the pestivirus, a unique clade originating from the ovine border disease virus group 4 (Luzzago et al. 2017), has been among the most damaging infectious diseases in Pyrenean chamois populations (Serrano et al. 2015).

Chamois form metapopulations consisting of sets of local populations bounded by the strong landscape structure of mountain areas and connected by dispersal of individuals (Loison et al. 1999). The virus emerged in the east central part of Pyrenees and spread westward (Luzzago et al. 2017). However, its transmission and impact were not homogeneous: although major outbreaks associated with strong population declines were observed in some areas, other populations were chronically infected, with little or no clinical expression or impact on population dynamics (Fernández-Sirera et al. 2012). Several explanations have been proposed, including virus properties, host genetic or immune status, or environmental determinants (Marco et al. 2015); however, their respective importance and the mechanisms explaining virus persistence have not yet been identified. Here, we provide the first empirical evidence of self-clearance (i.e., elimination of a pathogen from

a chronically infected population without human intervention) and discuss possible mechanisms and consequences on the host population.

The Orlu National Game and Wildlife Reserve (Orlu) is located in a valley surrounded by high pastures in the eastern Pyrenees (42°39'29''N, 1°57'54''E) and covers 4,247 ha, with 55% laying above 2,000 m. Pyrenean chamois in Orlu have been continuously infected since the first serologic assays in 1994 (Pioz et al. 2007). Approximately 2,000 sheep, 100 cattle, and 70 horses graze there in summer.

The Pyrenean chamois population was monitored by annual single block count in late spring between 1984 and 2008 during which 16 teams of two observers each cover the complete study area in 1 d. Since 2005, these pedestrian counts were progressively replaced by transects performed by helicopter five to eight times each year to determine uncertainty around abundance estimates. Aerial counts were numerically scaled to block counts based on the two common years of sampling during which an average of 43% of animals seen during the ground survey were also seen during the aerial survey. Chamois were also captured annually, and autumn hunting yielded 14–27 samples per year. Most individuals were sampled once, 21 were sampled twice, and three were sampled three times in total. The ELISA BVD/Mucosal Disease p80 kit (Institut Pourquier, Montpellier, France) was used to detect antibodies—indicators of past infections—and estimate seroprevalence. According to the manufacturer, assay sensitivity and specificity were estimated at 100 and 99.2%, respectively. The presence of antigen or viral RNA was detected using a sandwich enzyme-linked immunosorbent assay antigen test (Synbiotics, Lyon, France) or reverse transcription PCR focused on the 5'-untranslated region (Pioz et al. 2007). We estimated virus prevalence as the proportion of chamois carrying antigen or viral DNA. In all, 718 serologic tests and 585 virologic assays were performed between 1995 and 2016, with a mean (SD) of 33.1 (9.46) individuals tested per year.

Seroprevalence and virus prevalence can vary markedly with age and season, respectively (Pioz et al. 2007). Because yearly samples differed in terms of season and age structure, we had to adjust these variables to obtain correct estimates of yearly variations. We built logistic models for the presence of antibodies (model age+year, with both effects being significant according to the likelihood ratio test [LRT], $P < 0.001$) and the virus (model season+year, LRT, $P < 0.001$ for both variables). We used the models to predict age-adjusted seroprevalence and season-adjusted virus prevalence (Fig. 1). Whereas seroprevalence fluctuated between 0.4 and 0.9 between 1995 and 2013, it sharply decreased to 0.21 in 2014–2016 (Fig. 1), leading us to suspect a change in epidemiologic dynamics. We hypothesized that virus transmission declined between November 2011 when the last direct detection of viral antigen occurred, and 2014 when seroprevalence declined. Because virus transmission has a seasonal pattern with levels higher in autumn and lower in spring (Pioz et al. 2007; Beaunée et al. 2015), the most probable season for clearance of the virus was winter or spring.

A decline in virus transmission is expected to modify the age-prevalence relationship, with cohorts alive during the period of high-level transmission having steeper slopes for the age-prevalence relationship than more recent cohorts. To identify which cohorts were involved in virus decline, we examined the presence or absence of antibodies in chamois according to year of birth, and we found that the proportion of seropositive results had declined since the 2009 cohort (Fig. 2A). We examined the age-prevalence relationship in all cohorts born since 2009 and compared it with the same relationship for individuals born beforehand that served as a reference for the period of endemic presence of the virus. This comparison was done with logistic models accounting for the effect of age and cohort interaction on antibody carriage, with various cohort groupings. Among models considering two or three groups of cohorts, the best model was obtained considering two groups of cohorts, 2000–10 and 2011–16 (Fig.

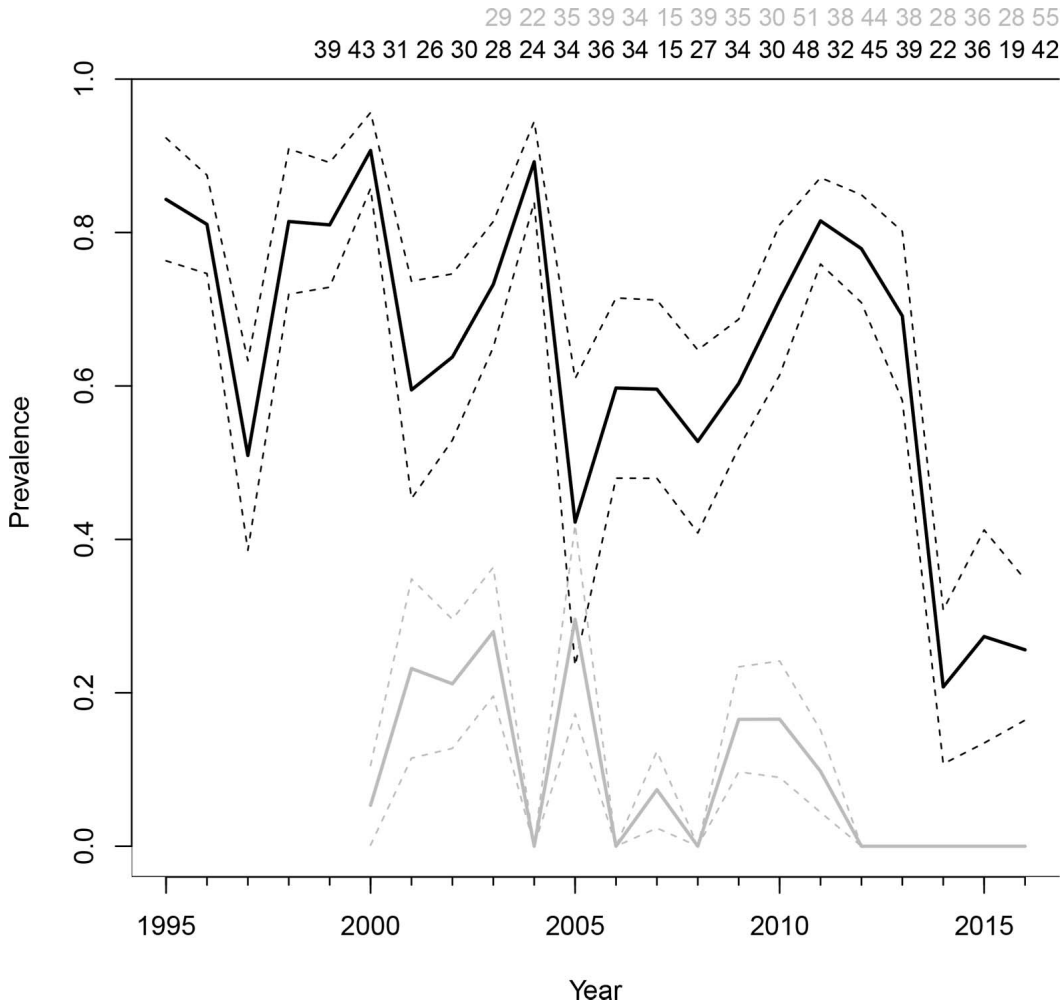


FIGURE 1. Age-adjusted antibody prevalence (seroprevalence, black) and season-adjusted antigen prevalence (virus prevalence, gray) for chamois pestivirus in the population of Pyrenean chamois (*Rupicapra pyrenaica*) of the Orlu National Game and Wildlife Reserve, France, between 1995 and 2016, with 95% confidence intervals (dashed lines). Seroprevalence was estimated for 5-yr-old individuals, and virus prevalence was estimated for the autumn season. Sample sizes are given above the graph.

2B). Specifically, only seven of the 115 chamois born since 2011 carried antibodies. Considering a given prevalence of 0.2, the probability that a positive result corresponded to a true positive case (predictive positive value) was 0.97; as such, these seven results were considered as true positives. Two were 1-mo-old kids captured in July 2012; thus, they probably carried maternally derived antibodies. Four were 1-yr-old individuals: two were born in 2011, one in 2012, and one in 2015. The first three cases suggested that

transmission occurred until autumn 2012. The case of the male hunted in 2016 was more intriguing, because it was born 2.5 yr after autumn 2012. This individual was hunter harvested at the extreme northern border of the reserve and may have been in contact with chamois from surrounding populations. Finally, a 5-yr-old female born in 2011 and hunted in 2016 carried antibodies, but this animal did not contradict the hypothesis of self-clearance in autumn 2012. All other individuals born in 2011 ($n=30$), 2012 ($n=26$), 2013 ($n=16$), 2014

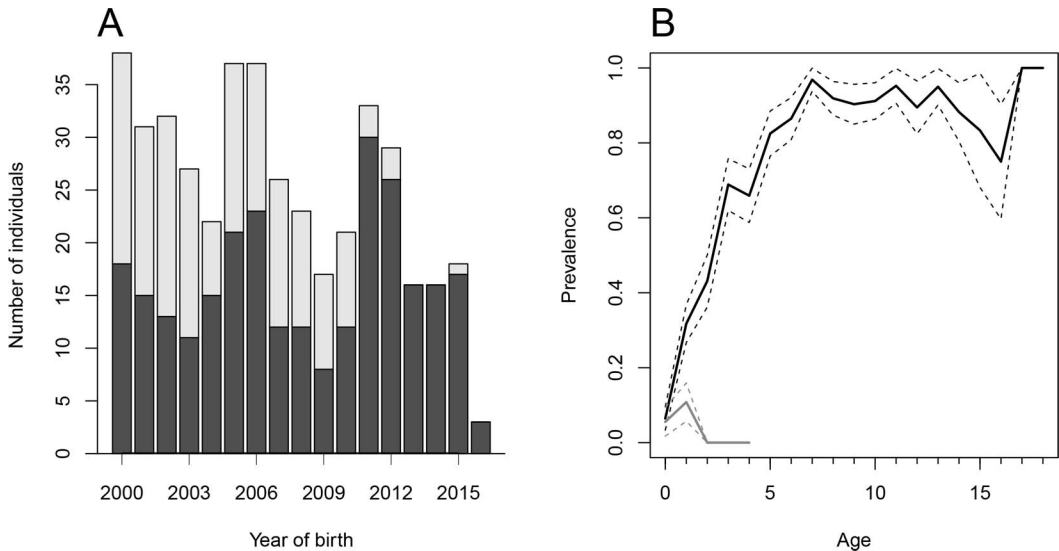


FIGURE 2. (A) Distribution of serologic results in Pyrenean chamois (*Rupicapra pyrenaica*) captured or hunted between 2000 and 2016 in the Orlu National Game and Wildlife Reserve, France, according to their year of birth, showing seronegative (dark gray) and seropositive (light gray) results for chamois pestivirus antibodies. (B) Age-prevalence relationship for chamois born up to 2010 (black) and born in 2011 and after (gray), with 95% confidence intervals (dashed lines).

($n=16$), 2015 ($n=17$), and 2016 ($n=3$) were antibody negative.

We propose the following mechanism. In 2011, high seroprevalence (Fig. 1) may have enabled sufficient herd immunity to stop virus transmission in some parts of the reserve. Virus transmission continued locally in 2012, as shown by 1-yr-old animals captured in 2012 and 2013, but stopped between autumn 2012 and spring 2013. Self-clearance first went unnoticed: seroprevalence did not decline until 2014 when the proportion of young chamois in the population increased (see below).

Due to the high lethality of pestivirus in naive hosts, as observed in Spanish populations (Marco et al. 2009) and estimated through modeling in Orlu (Beaunée et al. 2015), self-clearance should have strong demographic consequences. We therefore expected the survival of chamois to increase after virus disappearance, particularly in young classes (<2 yr old) that had previously experienced the highest incidence (Pioz et al. 2007). This increase should have, in turn, impacted population age distribution. Accord-

ingly, age structure of trapped animals was significantly biased toward young classes during 2013–16 compared to 1994–2012 (Fig. 3; $\chi^2=54.30$, 4 df, $P<0.001$). Proportions of kids, 1-yr-old, and 2-yr-old animals increased from 4% to 8%, 13% to 39%, and 11% to 16%, respectively. In contrast, population size estimates had not shown any obvious increase since 2013 (Fig. 4). This discrepancy may be explained by the delay between self-clearance and population increase. The Pyrenean chamois is a long-lived species for which demographic parameters are strongly age structured, with expected lagged effect. Because age at first reproduction is around 3 yr old in this population (Loison 1995), females born in 2013 were expected to reproduce in 2016 at the earliest.

Serologic and virologic monitoring suggested that partial, local extinctions may have occurred in the studied population earlier, but that was not followed by complete extinction (Figs. 1 and 3). Particularly in 2014, high seroprevalence combined with the absence of virus detection suggested that herd immunity may have driven partial self-clearance, but in

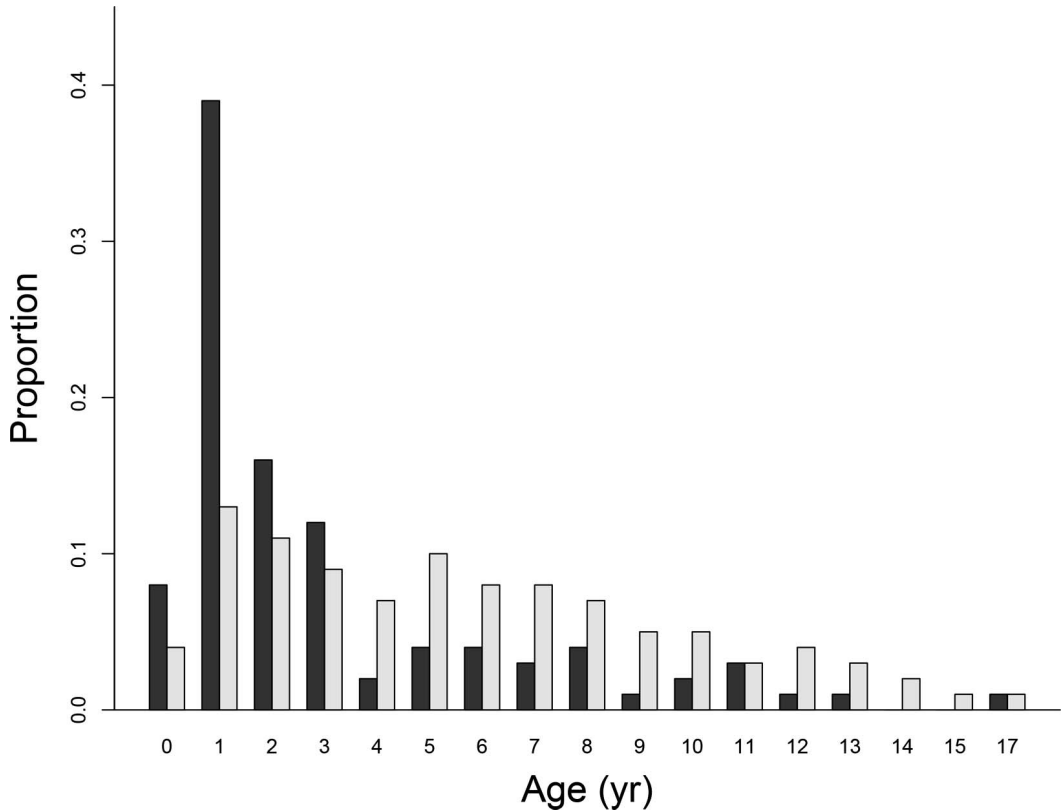


FIGURE 3. Age structure of Pyrenean chamois (*Rupicapra pyrenaica*) captured in the Orlu National Game and Wildlife Reserve, France, in 1994–2012 (presence of the virus, white bars, $n=393$) and in 2013–16 (after virus self-clearance, black bars, $n=101$).

2005, virus prevalence was high, suggesting that transmission had begun again.

The Orlu population is presently exposed to virus reintroduction from surrounding areas. With only 20% of the chamois carrying antibodies, reintroduction would likely cause a new epidemic, resulting in high mortality, especially in young chamois. However, the population size is much smaller than when the current epidemic first occurred (estimated as 1991; Beaunée et al. 2015), which should limit virus diffusion among social groups.

Self-clearance is rarely described in wildlife populations due to the lack of detailed empirical data. This case, along with observations of asynchronous and heterogeneous epidemiological patterns among populations (Marco et al. 2015), suggests that chamois pestivirus transmission is not locally stable but occurs on metapopulation level. Host

spatial distribution affects disease dynamics, with large connected populations serving as reservoirs, whereas small isolated populations have a high probability of disease self-clearance (Guivier et al. 2011). In the long term, metapopulation dynamics is expected to increase disease persistence, by connecting populations at different epidemic stages (Wood and Thomas 1996). Thus, in the Pyrenees, the connection between populations is expected to result in long-term viral persistence. Self-clearance of the bovine viral diarrhea virus is also commonly observed in cattle herds. Although self-clearance works in favor of virus control, reintroductions may make the virus endemic at the regional scale (Stahl et al. 2008). In Pyrenean chamois, a better understanding of metapopulation dynamics may help to identify the determinants of this large-scale

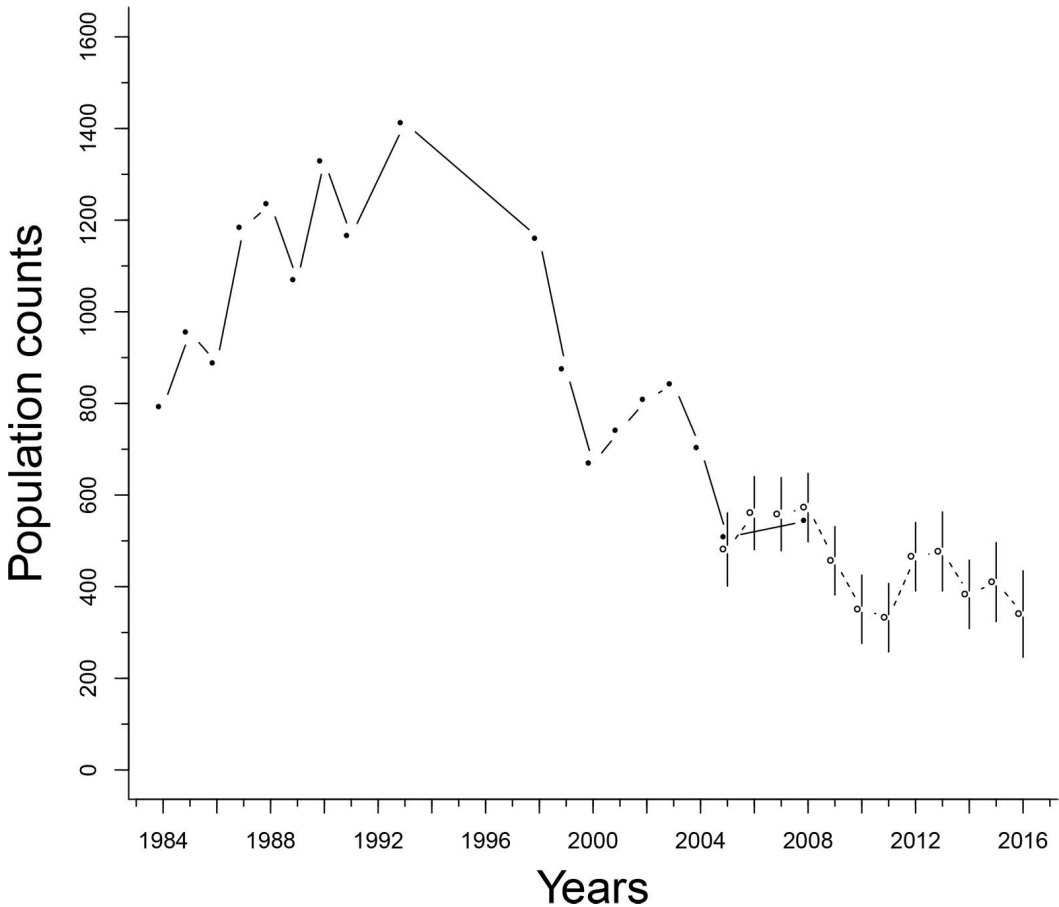


FIGURE 4. Estimated number of Pyrenean chamois (*Rupicapra pyrenaica*) in the Orly National Game and Wildlife Reserve, France, from 1984 to 2016: estimates issued from annual block counts (1984–2008, black circles) and from helicopter counts (2005–16, white circles).

pattern and to suggest possible management options.

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