

Influence of season and host age on wild boar parasites in Corsica using indicator species analysis

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Abstract

The indicator value (*Ind Val*) method which combines measures of fidelity and specificity has been used in a study on wild boar parasites in Corsica during 2001–2003. Because of its resilience to changes in abundance, *IndVal* is a particularly effective tool for ecological bioindication. The *Ind Val* method showed how season can influence the occurrence of parasite species in the wild boar, and also identified parasites as bioindicators relative to host age. The randomization test identified five parasite species having a significant indicator value for the season (the ticks, *Hyalomma aegyptium* and *Rhipicephalus sanguineus*, the louse, *Haematopinus suis* and the nematodes *Globocephalus urosubulatus* and *Ascaris suum* and two indicator species of an age class (the nematodes *G. urosubulatus* and *Metastrongylus* sp.). Data on species composition and infection levels would help improve the monitoring and management of parasitism in Suidae populations.

Introduction

The maintenance of a good health is a major problem in animal populations. The monitoring of helminth parasites is considered an essential element of the management of the health of animals (Cabaret, 2004). The present survey aims to establish the seasonal evolution of parasites and their importance for animal health for an improvement in the monitoring and management of populations of Suidae. All animals are not equally infested, according to their age; the management of parasitism will allow us to identify the most sensitive animals (Cabaret, 2004).

There are many publications on the parasitofauna of the wild boar (*Sus scrofa*) in various European areas, mainly in Central Europe where hunting represents a significant economic activity (Gerbaldi, 1975; Humbert & Ferté, 1986). Despite the historical and commercial activities related to wild boar in Corsica, very few

studies have been proposed on their parasites. Most investigations draw up an inventory of intestinal helminths of wild boar from a certain locality, evaluate parasite distribution (Forrester *et al.*, 1982; Elsami & Farsad-Hamdi, 1992; Fernandez-de-Mera *et al.*, 2003) or epidemiology (Magi *et al.*, 2002), or discuss public health significance (Solaymani-Mohammadi *et al.*, 2003).

However, the present study aims to use parasites as indicators of season, and of the age of their host by means of a method described by Drufrène & Legendre (1997), an indicator value index (*IndVal*). Despite the obvious value of this approach, the *IndVal* is not widely used in ecology (Hufnagel *et al.*, 1999; Detsis *et al.*, 2000; Zimmer *et al.*, 2000; Mikusinko *et al.*, 2001; Renjifo, 2001). The methods used are generally multivariate analyses or more specific methods for indicator species (Mouillot *et al.*, 2002).

The objective of the present study is twofold: (i) to relate the occurrence of parasites to climatic conditions (or seasonal variation) and (ii) to evaluate the ability of parasite species as bioindicators of a change of an age group of their host, in this case the wild boar (*Sus scrofa meridionalis*).

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Materials and methods

Collection of samples

The study was carried out on 160 wild boars, collected during the hunting period in eight different Corsican areas (fig. 1). These areas were selected according to their altitude, in order to have areas more diversified and a more complete global study. Samples were collected in each area during two successive hunting periods (between 15 August 2001 and 15 January 2003). From each area, every year, ten wild boars have been caught and the alimentary tract, lungs, heart and liver have been examined. Most of the worms were preserved in ethanol 95%, others were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer for a future electron microscopic study.

Indicator species

Species are identified as bioindicators when their occurrence (instead of presence) and/or abundance within sites of a particular habitat or a conservation area are high (Mouillot et al., 2002). In this work, the *IndVal* combined the species' relative abundance (specificity) with its relative frequency of occurrence (fidelity) for a given variable (season or host age in the present study). Specificity is defined as the mean abundance of a given parasite in a given group of wild boar (this group being an area or an age class depending on the classification criterion) divided by the abundance of the same parasite species infesting all wild boars. Fidelity is the percentage of wild boars of a given group (this group being an area or an age class depending on the classification criterion) where the parasite is present. These two components were included because a species can be defined as an indicator of a group when this species is abundant in this group (relative to other groups) and when this species is present in the majority of constituents (often considered as replicates) of this group (Drufrene & Legendre, 1997).

The *IndVal* values were calculated for each species j and for each group k according to the formula (Drufrene & Legendre, 1997):

$$IndVal_{kj} = 100 \times A_{kj} \times B_{kj}$$

where A_{kj} is a measure of specificity and B_{kj} is a measure of fidelity according to the formulae:

$$A_{kj} = N \text{ individuals}_{kj} / N \text{ individuals}_{+j}$$

$$B_{kj} = N \text{ wild boar}_{kj} / N \text{ wild boar}_{k+}$$

In the present study, $N \text{ individuals}_{kj}$ represents the mean abundance of the parasite species j for the wild boars examined in the group k . $N \text{ individuals}_{+j}$ is the sum of the mean abundances of the species j within the total of wild boars. $N \text{ wild boar}_{kj}$ is the number of wild boars belonging to the group k where the parasite species j is present. $N \text{ wild boar}_{k+}$ is the total number of wild boars within the group k .

The indicator value is maximum (100) for one group when all individuals of a parasite species j are observed in all wild boars within this group while this parasite species is absent from the boars belonging to other groups. Conversely, when a parasite species j is absent from all wild boars within this group, its indicator value is 0. Many intermediate cases are possible. If we consider two groups of wild boar with 100 individuals each. In the first group G1, the parasite species j is only in one host but with an abundance of 10000 individuals. In the second group G2, the same parasite species j is present in every host with an abundance of 1. The fidelity of this parasite to the group G1 is very low ($B_{G1j} = 1/100 = 0.01$) while the fidelity of parasite j to the group is maximum ($B_{G2j} = 100/100 = 1$). The specificity of parasite j to group G1 is $A_{G1j} = 1000/1100 = 0.91$ and to group G2 is $A_{G2j} = 100/1100 = 0.091$. Finally, the indicator value of this parasite is ten times higher for the group G2 than for the group G1: $IndVal_{G1j} = 100 \times 0.91 \times 0.01 = 0.91$ and $IndVal_{G2j} = 100 \times 0.091 \times 1 = 9.1$. This simple example illustrates the capacity of the indicator value to include both specificity and fidelity in the same index. Even though parasite j was found 1000 times in one host of

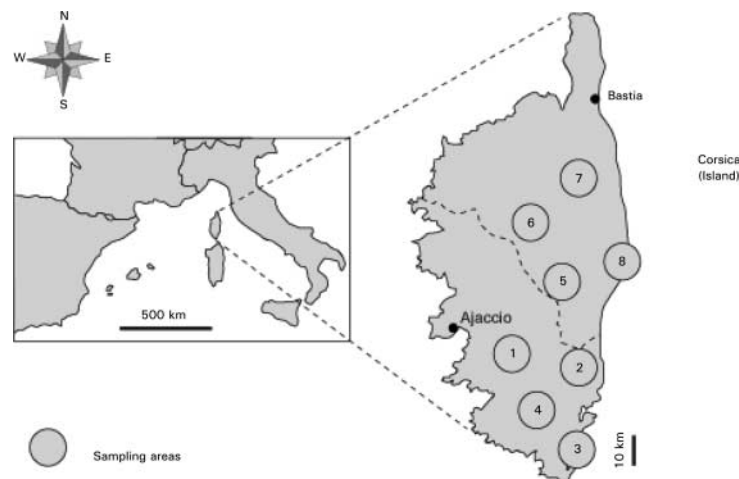


Fig. 1. Location of the sampling areas for wild boars in Corsica. 1, Taravo; 2, Ospedale; 3, Sainte-Lucie-de-Porto-Vecchio; 4, Alta-Rocca; 5, Venacais; 6, Bozio; 7, Ponte-Leccia; 8, Plaine Orientale.

group 1, the indicator value for this group was very low because the 99 remaining hosts were not infested. This last point constitutes an advantage over classical statistical tests such as ANOVA when we seek indicator species in highly variable communities such as fish or parasites and in both cases 1000 or 0 individuals can be sampled in two successive replicates (Mouillot *et al.*, 2002).

An attempt was made to identify indicator species for two variables. The variable k used to calculate the $IndVal$ was thus represented successively by season and host age.

The indicator value of a parasite species j ($IndVal_j$) for a k variable corresponds to the higher value of $IndVal_{kj}$ observed through the criteria which compose the variable. These different criteria are randomly permuted within the variable in order to obtain a distribution for $IndVal_j$. The statistical signification of $IndVal_j$ is evaluated by differences between observed and mean values obtained by aleatory random permutations. Ten thousand permutations have been carried out and differing degrees of significance have been obtained: 5%; 1%; 1%. Calculations of $IndVal$ values and associated tests were performed using the PC-ORD 4.0 for Windows software (McCune & Mefford, 1999).

Results

To determine which parasite species can be considered as an indicator species of season or age, an $IndVal$ has been calculated for each of these criteria.

Indicator species test for season

Of ten parasite species studied, the randomization test identified five species having a significant indicator value (<5% level of significance) for season (table 1). Three arthropod species (the ticks *Hyalomma aegyptium* and *Rhipicephalus sanguineus* and the louse *Haematopinus suis*) and one nematode (*Globocephalus urosubulatus*) have a significant $IndVal$ for spring, whereas the $IndVal$ value of another nematode (*Ascaris suum*) is significant for winter.

Globocephalus urosubulatus, an intestinal parasite, had a fidelity more pronounced in spring (70%), compared with

the three other seasons where its fidelity was 30–41%. In return, similar specificities were recorded between summer, autumn and winter periods.

Regarding the second intestinal parasite, *A. suum*, winter appeared as the season in which this species was an indicator due to its high specificity. Weak specificity and fidelity were observed in autumn.

The fidelity of *H. aegyptium*, an external parasite, was slightly higher in summer than in spring, whereas its specificity was higher in spring, thus making it an indicator for this season ($P = 0.0045$).

The fidelity and more especially the specificity of *R. sanguineus* species, an external parasite, were very high in spring.

Specificity was higher for the other species of external parasite, mainly for the louse, *H. suis*.

Indicator species test for age

Only two species were retained among the ten studied regarding the significance of their indicator values. *Globocephalus urosubulatus* was more an indicator of wild boars whose age was more than one year ($P = 0.0375$), whereas *Metastrongylus* sp., a pulmonary parasite, was an indicator species of hosts less than one year old ($P = 0.0002$) (table 2).

Discussion

In the present study, various patterns of parasite indicator species of one season have been observed. The combination of specificity and fidelity allowed us to identify some parasite species as indicators of season. The higher the specificity and fidelity are, the more likely the parasite species is to be a significant indicator. The study has shown that five parasite indicator species can occur in a given season and two parasite indicator species relative to host age class can be identified in wild boar. Indeed, this statistical test identified some relationships between parasite species and seasonal variation, and between parasites and host age.

Indicator species test for season

If the hookworm *G. urosubulatus* and the roundworm *A. suum* are compared, these two nematodes can be seen to be indicator species for two different seasons. Furthermore, *G. urosubulatus* is characterized by a high fidelity value for the spring season while *A. suum* exhibits an elevated specificity

Table 1. Specificity (%) and fidelity (%) (pooled data from 2001–2003) of five parasite species in the wild boar over four seasons during 2001–2003.

Parasite species	Spring	Summer	Autumn	Winter
<i>Globocephalus urosubulatus</i>				
Specificity	53	17	14	17
Fidelity	70	30	41	32
<i>Ascaris suum</i>				
Specificity	0	0	12	88
Fidelity	0	0	16	34
<i>Hyalomma aegyptium</i>				
Specificity	49	40	11	0
Fidelity	70	73	25	0
<i>Rhipicephalus sanguineus</i>				
Specificity	83	17	0	0
Fidelity	70	6	4	0
<i>Haematopinus suis</i>				
Specificity	73	17	10	0
Fidelity	50	18	14	0

Table 2. Specificity (%) and fidelity (%) of two parasite species in the wild boar, relative to host age.

Parasite species	Host age	
	> 1 year	≤ year
<i>Globocephalus urosubulatus</i>		
Specificity (%)	83	17
Fidelity (%)	43	15
<i>Metastrongylus</i> sp.		
Specificity (%)	6	94
Fidelity (%)	6	31

for winter but not a high fidelity. According to Roepstorff & Jorsal (1989) during winter, low outdoor temperatures were not the limiting factor for the embryonation of *Ascaris* eggs. Even after reaching the embryo stage, eggs of *Ascaris* survive for several months under favourable conditions of humidity (Ineson, 1954) and this is likely to be the reason why adult stages are found in winter.

Globocephalus urosubulatus occurs more frequently in wild boar than in pigs (Gadomska, 1981). The infective larvae of *G. urosubulatus* resist cold and freezing conditions (Meirhaeghen, 1998). The parasitic cycle of this nematode is monoxenous (Anderson, 2000) and once introduced into the host, larvae reach the adult stage in the intestinal mucosa. In the present study, adult *G. urosubulatus* were mainly found in the spring, unlike *A. suum* which was more prevalent in winter. Thus competitive exclusion was to be expected between these two species (Combes, 2001).

The three external parasites appear to be characteristic of the spring period. The presence of ticks is the result of the movement of wild boar into the forest habitat (Labruna *et al.*, 2002). *Hyalomma aegyptium* is more indicative of spring, although a high specificity and fidelity also characterize it in summer (table 1). The hard tick *R. sanguineus* is a cosmopolitan parasite and probably the most prevalent ixodid species (Pegram *et al.*, 1987). In tropical areas, *R. sanguineus* is present all year-long without a marked seasonal prevalence, whereas in temperate areas, it appears in April, disappears in September and hibernates as engorged nymphs or as adults without being engorged. (Brumpt, 1978). The high *IndVals* of *H. aegyptium* and *R. sanguineus* are based on specificity and fidelity values obtained for the spectrum of seasons and, despite the inevitable abundance changes between seasons, these two species remain specific and with a high fidelity to the spring season. Thus they can be identified as indicative.

Indicator species test for host age

Magi *et al.* (2002) found no host age-related difference in the infection of the wild boar with *G. urosubulatus*. However, in the present case, using a novel statistical approach, this gastrointestinal parasite was identified as an indicator species of wild boar of more than one year of age. These results are in agreement with other studies in which the infection with *G. urosubulatus* increased with host age (Rajkovic-Janje *et al.*, 2002) (30.9% in piglets, 100% in animals older than two years (Takacs, 1997)). The possible death of these animals would imply the death of many parasites, although the parasite species survives in most of these hosts (Magi *et al.*, 2002). *Globocephalus* helminthoses could also cause clinical symptoms and death (Takacs, 1996). Thus an ecological equilibrium is likely to exist between the parasites and their hosts with no risk of extinction of both species.

Metastrongylus sp., a nematode parasite of the respiratory tracts of pigs and wild boars, has been the subject of many investigations in the United States, and also in Europe due to its effect on the mortality of Suidae populations (Sale, 1971; Fraczak, 1974; Smith *et al.*, 1982). Humbert & Ferté (1986) discovered the importance of this nematode in various areas of France whilst studying the helminths of the wild boar. For *Metastrongylus*, high

intensities of lungworms have been found in young compared with adult wild boars (Humbert & Henry, 1989) and the present results are in agreement with these observations. Indeed, a high specificity (94%) has been recorded in young wild boar. Humbert & Drouet (1990) suggested that this difference in intensity of infection between the two age classes is related to changes in the diet of wild boars, with the quantity of food items, including earthworms (intermediate hosts), taken in by young boars being greater than that in adult boars (Dardaillon, 1984; De-la-Muela *et al.*, 2001). Furthermore, the availability of food items such as earthworms will depend upon climatic factors as low temperatures and humidities will reduce the number of oligochaetes available for predation (Humbert, 1992). The development of immune responses by wild boars to infection with helminths will also be a factor in regulating parasite populations in hosts, relative to age (Anderson, 1987). Wild boars which survived the initial infection would develop an acquired natural immunity protecting them against any potential second infection by parasites (Humbert, 1992).

Data obtained from the present study will thus serve as baseline information on parasite infestation in wild boars in Corsica, which would be essential for veterinary authorities to develop cost-effective treatment schemes.

The *IndVal* method has numerous advantages over other measures used for ecological bioindication (McGeoch & Chown, 1998). This statistical approach allows the identification of an indicator species among parasite species having similar specificity and fidelity but can also indicate a scarce species as an indicator species (Mouillot *et al.*, 2002). The usefulness of this method is ultimately dependent on the degree to which species maintain high and significant indicator values when tested in different locations and times of year (McGeoch & Chown, 1998), as the abundance of all species in an assemblage may change with weather and seasonal conditions (Byong-Seol *et al.*, 1979; Uriarte *et al.*, 2003; Latha *et al.*, 2004) However, the *IndVal* method may accommodate such abundance changes because *IndVal* compares the frequency of occurrence of parasite species between habitat types.

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