

Gastrointestinal nematodes of goats: host–parasite relationship differences in breeds at summer mountain pasture in northern Italy

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Abstract

Introduction: The Orobic goat is a hardy breed native to the Orobic Alps (Lombardy, northern Italy). The aim of the study was the assessment of gastrointestinal nematode (GIN) egg excretion in Alpine and Saanen (cosmopolite breeds) and Orobic grazing goats, after a strategic treatment with eprinomectin in late June. **Material and Methods:** Individual faecal samples from a mixed flock of cosmopolite and Orobic goats were collected and analysed by the FLOTAC double technique every three weeks from June to September. **Results:** Strongylida was the primary GIN infection observed in goats that grazed on Alpine pastures; a strategic treatment with eprinomectin led to a prolonged reduction of egg excretion during the whole study period. Egg excretion was also influenced by breed. Pluriparous Orobic does were able to control reinfection better than the pluriparous cosmopolite does. Regarding *Nematodirus* sp. eggs per gram of faeces (EPG), the autochthonous Orobic breed presented higher values than the cosmopolite breeds. However, cosmopolite goats presented higher EPG values of *Strongyloides papillosus* than their Orobic counterparts in August. **Conclusions:** Further studies on genetic features of local autochthonous goats, such as the Orobic breed, are needed, since they could reveal peculiar characteristics of susceptibility, resistance or resilience to GIN infection, providing genetic resources for selection.

Keywords: goat, breed, gastrointestinal nematodes, resistance, Italy.

Introduction

Dairy goats are reared worldwide under a wide variety of management systems, ranging from intensive to extensive. In northern Italy and mainly in the pre-Alpine and Alpine environments, semi-extensive and extensive breeding systems are practiced; in this context, parasitic diseases represent an ineliminable sanitary risk, in some cases linked to sympatric wild animals (1, 28). Protozoa and helminths infecting goats can impair production, inflict economic losses and, in some cases, cause zoonotic diseases (14, 15, 27, 34). Gastrointestinal nematodes (GIN) are the most common parasites of goats in northern Italy (11), and an integrated approach should be adopted to their control. Regular deworming effectively controls internal parasites in grazing dairy goats, but it should be properly carried out to prevent or slow down the onset of anthelmintic resistance, which

has already been reported in northern Italy and neighbouring regions (30, 35, 36, 38). In this regard, in recent years strategic or targeted selective treatments have been suggested and other strategies have been developed to improve the management of gastrointestinal parasites in goats, such as nutritional supplementation with minerals, vitamins, condensed tannins, spores of *Duddingtonia flagrans*, and copper oxide wire particles (13, 20, 25, 29). Also, selection of animals with greater resistance or resilience to GIN enhances productivity under conditions of risk of infection by parasites and, as this leads to less frequent treatments, reduces selection pressure for anthelmintic resistance (26). Information on the potential for selecting resistant goats is very limited (21); however, genetic resources for selection of resistance or resilience against nematodes in goats could also be provided by autochthonous or local breeds (3, 6, 17).

Considering the lack of information on GIN dynamics in autochthonous goat breeds after treatment on Alpine ecosystem pastures, three aims seemed appropriate, and three were served by the present study. The first was to evaluate the quali-quantitative variations of GIN egg excretion due to reinfection in a naturally infected flock of dairy goats after an anthelmintic treatment, the research being intended to use the whole grazing season on a mountain pasture. The second was to investigate the influence of selected risk factors (sampling, breed, and number of births) on the faecal egg count, and the third was to find the differences in GIN reinfection between autochthonous and cosmopolite goats.

Material and Methods

The study was conducted in a flock composed of 55 lactating goats, of which 38 were pluriparous and the remaining 17 primiparous. The breed composition of the flock was 39 autochthonous (Orobic) and 16 cosmopolite (8 Alpine and 8 Saanen) animals. All were individually identified by ear tags, and the two groups of goats used the same pasture throughout the study.

The Orobic goat, a native breed of the Orobic Alps, can be found in the lower part of the Valtellina (Sondrio province), and in the provinces of Bergamo and Lecco (Lombardy, northern Italy). This goat has impressive horns and a long-haired coat in varying colours (grey, beige, black, brown, or dappled) (Fig. 1). The most common combination is black-grey in the hindquarters with a white-beige front. This hardy breed, suitable for mountain pastures, is a good source of meat and milk of high quality used for the production of traditional “violino” raw ham and raw-milk cheeses.



Fig. 1. Two Orobic goats in a pre-Alpine environment

The surveyed flock grazed freely from April to October on a mountain pasture (Alpe Giumello, Lombard Prealps, Lecco province, northern Italy) at an altitude of $\pm 1,600$ m above sea level. At the end of June 2014, the flock was treated with a pour-on formulation

of eprinomectin (EPRINEX Pour-on, Merial Italia S.p.A., Italy); extra-label dose of 1 mg/kg b.w.). The body weight of the visually heaviest goat of the flock was estimated to determine the anthelmintic dose. Individual faecal samples were collected from the rectum on the day of treatment but before it (June sampling) and subsequently four times every three weeks (July, Aug1, Aug2, and Sept samplings). In previous years to the study, treatments against GIN had been given once annually and were fenbendazole (2.5% PANACUR, MSD Animal Health S.r.l., Italy), in November or December depending on the dry status of does. All animal procedures used in this study were approved by the Milan University Institutional Animal Care and Use Committee.

Faecal samples were refrigerated and analysed within 48 h by the FLOTAC double technique, with an analytic sensitivity of two eggs per gram (EPG) of faeces (8). Analyses were performed using the FS2 flotation solution (NaCl, specific gravity 1,200). The eggs and oocysts per gram of faeces were not calculated for *Skrjabinema*, the cestode *Moniezia*, or *Eimeria* sp. At each sampling, eleven faecal pools consisting of five individual samples each were analysed by sedimentation and the Baermann technique to determine the presence of the first-stage larvae of lungworm and trematode eggs.

To analyse the selected risk factors, individual EPG values were introduced in generalised linear mixed models (GLMMs) as the dependent variables. The sampling (June, July, Aug1, Aug2 or Sept), the breed (Orobic or cosmopolite) and the number of births (primiparous or pluriparous) were introduced into the models as categorical independent variables. To have a larger group, Alpine and Saanen goats, both highly selected for milk production, were considered together in statistical analysis. The identity of each goat was included as a random intercept effect. The interactions between the introduced independent variables and all their possible combinations were also considered, and the models that better explained EPG excretions were chosen by Akaike's information criterion (AIC). Statistical analyses were performed using SPSS version 20.0 (IBM, USA).

Results

At the beginning of the study (the June sampling), strongylida were detected in 100% of goats ($n = 55$), and mean egg excretion was 1,498.87 EPG (standard deviation (SD) 1,925.3). *Nematodirus* sp. and *Strongyloides papillosus* - infected goats made up 54% and 34%, respectively, and their mean excretions were 5 EPG (SD 6.31) and 11.09 EPG (SD 25.7), respectively. *Skrjabinema* sp. (18%), *Moniezia benedeni* (28%), and *Eimeria* sp. (100%) were also detected. No lungworm first-stage larvae or trematode eggs were found. Differences in EPG counts between the two goat breeds

were observed, particularly for strongylida: the cosmopolite goats showed EPG counts from 2.1 to 3.9 times higher than Orobic goats comparing the same sampling. Also, Orobic goats presented higher *Nematodirus* sp. EPG than cosmopolite ones (Table 1). The mean EPG of strongylida, *Nematodirus* sp., and *S. papillosus* observed in the four subsequent samplings (July, Aug1, Aug2, and Sept) showed different trends.

A GLMM that better explained the strongylida EPG was constructed introducing all three categorical variables (sampling, breed, and number of births) and their interaction, because all the other possible combinations of independent variables and their interactions were less able to explain the strongylida egg excretion, as demonstrated by the higher AIC values (Supplementary Table 1). Considering the *Nematodirus* sp. EPG, the smallest AIC value was obtained by constructing a GLMM with all three categorical variables and the interaction between the breed and the number of births. In the model that better explained the EPG of *S. papillosus*, only the sampling, the breed and their interactions were introduced as independent variables (Supplementary Table 1). The sampling was the variable that better explained the variation of the strongylida EPG (Table 1). Pairwise comparisons showed that the estimated strongylida EPG of the June sampling was significantly higher ($p < 0.001$) than all the other samplings (Fig. 2). Strongylida EPG was also explained by the interaction between sampling, breed, and number of births. Both Orobic and cosmopolite pluriparous goats presented a higher strongylida EPG

count than their primiparous flockmates in the June sampling when no animals had been treated yet with anthelmintic drugs. In the subsequent four samplings, the strongylida EPG differed between Orobic and cosmopolite goats. In Orobic goats, reinfection after treatment led to a higher strongylida EPG count in primiparous than pluriparous animals, while the opposite trend emerged in cosmopolite goats (Fig. 3).

Also for the *Nematodirus* sp. EPG, the sampling was the variable that better explained its variation (Table 2). Pairwise comparisons of the estimated *Nematodirus* sp. EPG of the June sampling with other samplings showed that its June value was significantly higher than that of the July sampling ($p < 0.001$). In contrast, the June EPG did not differ from the Aug1 EPG ($p > 0.05$) and was significantly lower than the Aug2 and Sept counts ($p < 0.001$; Fig. 4). Also, Orobic goats presented higher *Nematodirus* sp. EPG than cosmopolite ones (Fig. 5). The sampling and the interaction between sampling and breed were the significant explanatory variables of the *S. papillosus* EPG. Pairwise comparisons showed that the *S. papillosus* EPG of the June and July samplings did not differ. The EPG of the Aug1 and Aug2 samplings were significantly higher than those of June ($p < 0.01$ and < 0.001 , respectively), while the latter and the Sept sampling EPG did not differ from each other (Fig. 6). June, July, and Sept sampling EPG values followed the same trend in Orobic and cosmopolite goats, while higher EPG were observed in the Aug1 and Aug2 samplings of cosmopolite goats (Fig. 7).

Table 1. Mean EPG of strongylida, *Nematodirus* sp., and *S. papillosus*. in Orobic and cosmopolite (Alpine and Saanen) goats at each sampling point

Sampling	Breed	Strongylida mean EPG (\pm SD)	<i>Nematodirus</i> sp. mean EPG (\pm SD)	<i>S. papillosus</i> mean EPG (\pm SD)
June	Orobic	1228.4 (\pm 988.8)	5.8 (\pm 6.6)	11.4 (\pm 27.4)
	Cosmopolite	2614.75 (\pm 4197.2)	1.5 (\pm 2.9)	7.0 (\pm 15.5)
July	Orobic	35.1 (\pm 47.4)	2.1 (\pm 6.0)	12.9 (\pm 31.1)
	Cosmopolite	124.5 (\pm 206.3)	0.3 (\pm 0.7)	11.5 (\pm 20.6)
August 1	Orobic	170.2 (\pm 121.8)	8.2 (\pm 11.9)	11.7 (\pm 27.7)
	Cosmopolite	354.5 (\pm 371.4)	2.5 (\pm 2.1)	57.0 (\pm 140.9)
August 2	Orobic	67.2 (\pm 63.5)	17.2 (\pm 16.3)	10.6 (\pm 21.1)
	Cosmopolite	265.6 (\pm 432.84)	4.0 (\pm 4.9)	110.4 (\pm 193.5)
September	Orobic	52.2 (\pm 63.2)	17.4 (\pm 22.8)	4.11 (\pm 6.9)
	Cosmopolite	159.11 (\pm 228.1)	9.8 (\pm 6.0)	5.3 (\pm 11.3)

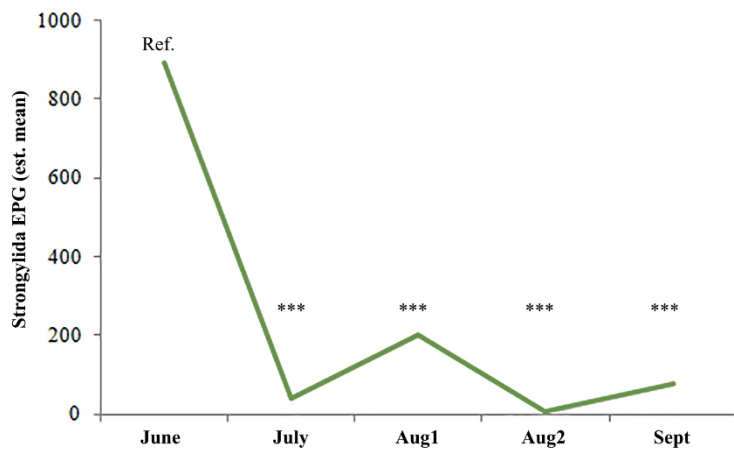


Fig. 2. Effect of sampling on strongylida EPG in both autochthonous and cosmopolite goats at summer mountain pasture in northern Italy

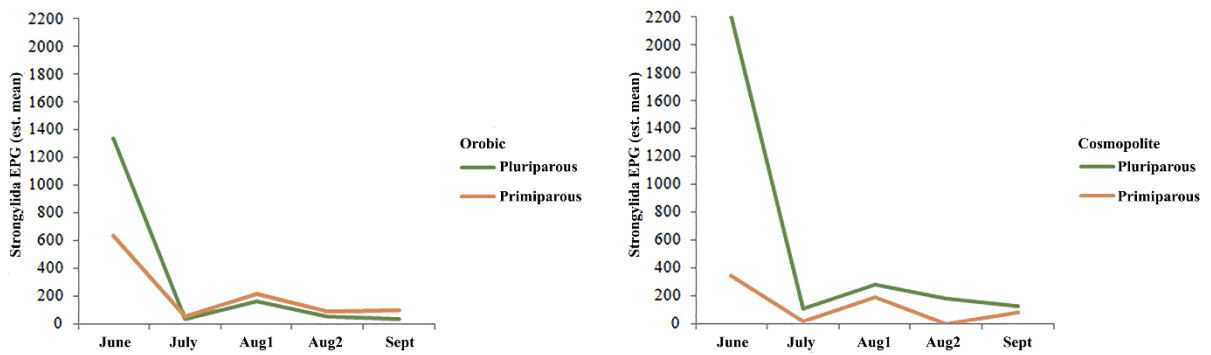


Fig. 3. Influence of sampling, births, and breed on strongylida EPG in goats at summer mountain pasture in northern Italy

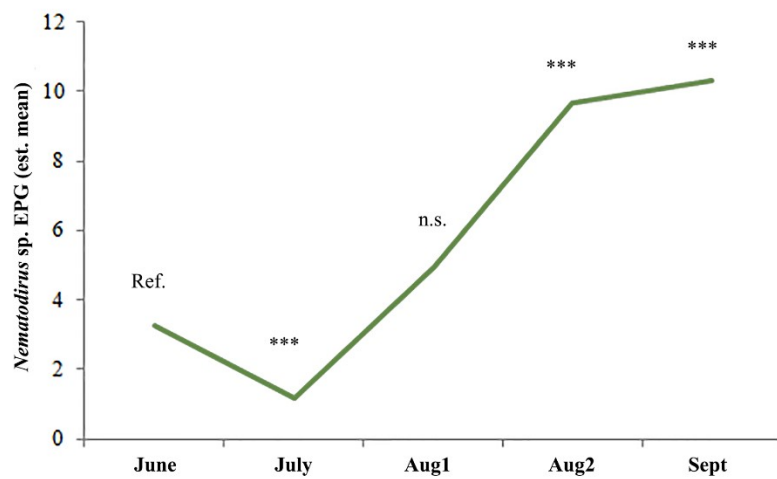


Fig. 4. Effect of sampling on *Nematodirus* sp. EPG in goats at summer mountain pasture in northern Italy
 Ref – reference; *** – P < 0.001; n.s. – not significant

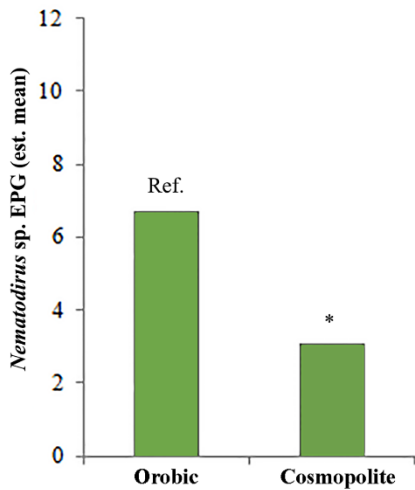


Fig. 5. Effect of breed on *Nematodirus* sp. EPG in goats at summer mountain pasture in northern Italy
 Ref. – reference; * – $P < 0.05$

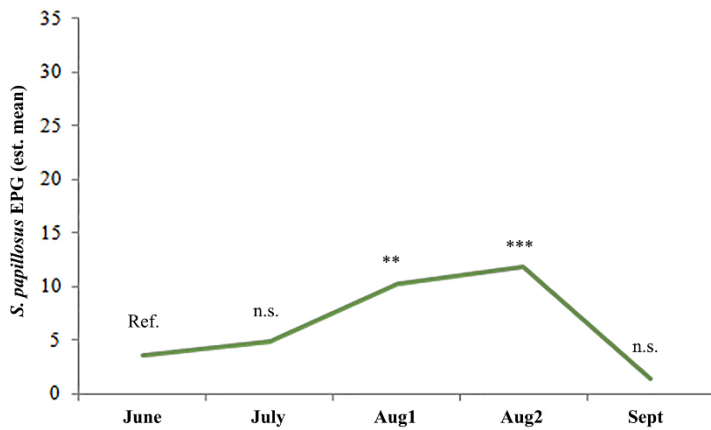


Fig. 6. Effect of sampling on *Strongyloides papillosus* EPG in goats at summer mountain pasture in northern Italy
 Ref. – reference; ** $P < 0.01$; *** – $P < 0.001$; n.s. – not significant

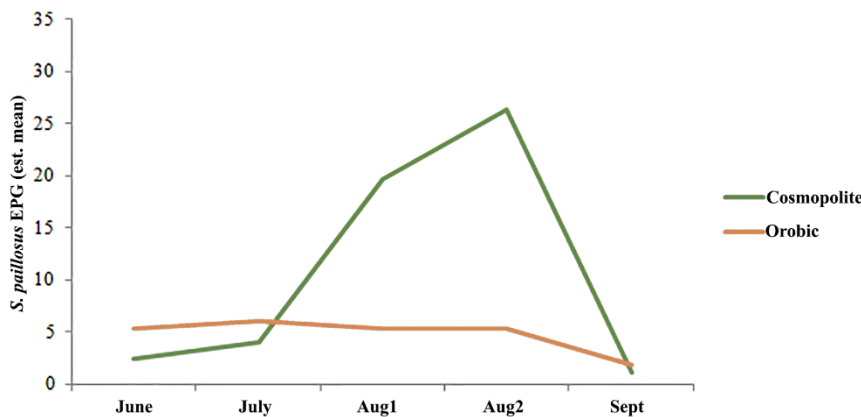


Fig. 7. Effect of sampling and breed on *S. papillosus* EPG in goats at summer mountain pasture in northern Italy

Discussion

Strongylida infection was the main parasitic infection observed in untreated goats that freely grazed on mountain pastures, and this finding was in accordance with previous parasitological surveys in

goats from northern Italy (11, 28); indeed, 100% of surveyed animals were infected at the beginning of the study, and high EPG values were found (mean EPG 1,498.87). The decrease in strongylida EPG recorded in the samplings subsequent to June was probably caused by the anthelmintic treatment. In particular, the lower

egg excretion observed in the July sampling, three weeks after treatment, was probably due to the immediate efficacy of the administered drug (9), whereas the prolonged lower excretion in the following samplings could also be due to other factors. Firstly, pour-on eprinomectin is known for its persistent efficacy, mainly in cattle (7, 12). Probably, as observed by Chartier and Pors (5), its persistence is shorter in goats, although pour-on eprinomectin at a dose of 1 mg/kg b.w. has been demonstrated to completely prevent *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* infections 7 days after administration and partially protect against *T. circumcincta* reinfection 21 days after. As observed by Genchi (16), a strategic anthelmintic treatment in late spring leads to lower contamination of pastures than treatments administered in early spring. As a consequence, a strategic prophylactic treatment in May or June, during the goat lactating period, has been observed to dramatically improve productivity in small ruminants (10, 33, 36). Some contribution to the observed persistent lower egg excretion by acquired immunity is possible but somewhat unlikely: in goats, constantly increasing egg excretion has been observed over the whole grazing season, probably because both the acquisition and the expression of immune responses against GIN are less efficient than in sheep (24). Moreover, goats seem to be unable to develop a response to nematode parasites after challenge infection (22).

Considering *Nematodirus* sp. infection, the EPG varied depending on samplings. As was also the case for strongylida, the EPG registered in the July sampling was significantly lower than that in its June antecedent, likely owing to the immediate efficacy of eprinomectin against *Nematodirus* sp. (18, 19). In the subsequent samplings, the egg excretion trend differed from that observed for strongylida; although the *Nematodirus* sp. EPG fell in July, it progressively rose to its maximum at the Sept sampling when it surpassed its pre-treatment level. During reinfection after treatment with macrocyclic lactones, differences between *Nematodirus* sp. and strongylida excretion were previously observed in cattle (31). In some studies, species belonging to the *Nematodirus* genus appeared to be dose-limiting for these drugs (4, 37); therefore, eprinomectin in goat at the dose used probably lacked persistent efficacy against the species belonging to this genus.

Finally, the trend of EPG of *S. papillosus* seemed utterly unaffected by anthelmintic treatment throughout the sampling period. This result was not unexpected because eprinomectin is not registered as effective against *S. papillosus*, and Hamel *et al.* (19) observed an efficacy of < 90% in goats treated with a dose of 1 mg/kg b.w. The rise in *S. papillosus* EPG in the August sampling was not definitively explainable, but possibly a substantial decrease in other GIN due to eprinomectin treatment could have reduced competition in its ecological niche. Also, other factors could have influenced excretion of *S. papillosus* in the study period, such as environmental and meteorological variables and reinfections.

Differences in the EPG of detected nematodes varied by other risk factors. At the beginning of the study period, the strongylida EPG was higher in pluriparous goats of both Orobic and cosmopolite breeds than primiparous. After anthelmintic treatment and the consequent reinfection, in contrast, the EPG differed in primiparous and pluriparous goats by breed. In cosmopolite goats, the strongylida EPG was characterised by higher values in pluriparous than in primiparous animals; according to Hoste *et al.* (23), this phenomenon is very likely to be a consequence of the low ability of goats to produce effective immunity against GIN. An opposite trend of reinfection was observed in Orobic goats, with higher EPG in primiparous than pluriparous individuals, possibly related to acquired immunity being stronger in the autochthonous than the cosmopolite breeds. Recently, low heritability of faecal egg counts was observed in the cosmopolite breeds (Alpine and Saanen), together with an unfavourable genetic correlation of faecal egg counts with milk yield (21). Heritability and genetic correlation could vary in autochthonous breeds. Indeed, GIN affected milk production in Nera di Verzasca goats (a local Italian breed) differently to cosmopolite breeds (2, 3), suggesting that autochthonous breeds could provide genetic resources for heritable resistance or resilience against strongylida.

The goat breed variable accounted for *Nematodirus* sp. EPG in that the autochthonous Orobic breed manifested higher EPG than cosmopolite goats. The Orobic goats have probably been selected locally in the Alpine and pre-Alpine environment for capacity to sustain the host-parasite relationship in the presence of abundant infective stages of *Nematodirus* sp. on pastures.

Finally, cosmopolite goats presented higher EPG values of *S. papillosus* than Orobic goats in August; it can be hypothesised that the Orobic variety become more resistant or resilient to *S. papillosus* infection when other GIN infections decrease. However, our attempt to explain the observed effect of goat breed on *Nematodirus* sp. and *S. papillosus* EPG counts can only be speculative, also for the lack of previous studies. Further investigations should be performed to verify these hypotheses.

In conclusion, acknowledging strongylida to be one of the major constraints on the productivity and health of grazing ruminants, the results of the present study showed that a strategic treatment in grazing lactating goats led to a prolonged effect on the strongylida EPG of excretion and thus contributed to efficient integrated control of GIN infection. It should also be considered that the use of a drug registered at a specific dose for goats and without a milk withdrawal period, such as eprinomectin, could support the farming of both cosmopolite and autochthonous breeds on marginal mountain pastures. Further studies on the genetic features of local autochthonous goat breeds, such as the Orobic, are needed, since they could present peculiar

features of susceptibility, resistance or resilience to GIN infection, providing genetic resources for selection.

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Animal Rights Statement: The experiments on animals were conducted in accordance with the local Ethical Committee laws and regulations as regards care and use of experimental animals.

Supplementary Table 1 comprises a separate pdf file viewable online at <http://content.sciendo.com/view/journals/jvetres/jvetres-overview.xml> and doi: 10.2478/jvetres-2019-0076

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