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Veterinary monitoring of gastrointestinal parasites in European bison, *Bison bonasus* designated for translocation: Comparison of two coprological methods

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ABSTRACT

It is important to monitor the level of parasitic loads in herds of European bison and to identify threats early enough to prevent their spread to other populations or species. The aim of the present study was to compare the detection sensitivity of two fecal flotation techniques, *viz.* the modified Willis method (WM) with centrifugation and modified McMaster flotation technique (MM), in the diagnostics of gastrointestinal parasites of European bison before the translocation of animals.

Out of 166 feces samples, *Eimeria* spp. oocysts (84.3% in WM and 71.1% in MM) and Trichostrongylidae eggs (82.5% in WM and 53.6% in MM) predominated. These were accompanied by eggs from *Capillaria* spp. (prevalence: 13.9% in WM and 3.61% in MM), *Nematodirus* spp. (prevalence: 18.1% in WM and 4.8% in MM) and *Trichuris* spp. (prevalence: 12.7% in WM and MM) were identified. The lowest prevalence was noted for cestode eggs of *Moniezia* spp. (5.4% in WM and 3.0% in MM).

The Willis method yielded a higher prevalence of eggs and oocysts than the modified McMaster method, and hence has a higher probability of detecting parasitic structures than the modified McMaster method, especially in cases of very low levels of invasion.

As the two methods yield consistent results, it is recommended to use the Willis method for diagnosis of internal parasite infection in European bison. This test offers more sensitive method than McMaster technique of detecting the presence of low levels of a variety of parasite eggs and oocysts in feces, while also being inexpensive and adaptable to field work.

1. Introduction

The European bison (*Bison bonasus*), the largest terrestrial mammal in Europe, has had a tumultuous history. At one point, in 1919, the species was extinct in the wild and had to be restored from only 12 founders (Wróblewski, 1927; Raczyński, 1978; Pucek, 1991; Krasińska and Krasiński, 2007). Due to this extreme bottleneck, the species experienced a dramatic drop in genetic variability (Olech and Perzanowski, 2002), resulting in its inclusion in the IUCN Red List of Threatend Species (IUCN, 2021). The status of the European bison has since risen from vulnerable to near threatened; however, the species still requires concerted conservation efforts (Klich et al., 2018; Olech et al., 2019).

The wildlife health is a dynamic state depending on characteristics of the individual and its environment. The comprehensive approach is crucial to determine health status, which depends on several interacting factors, among others: biologic, social and environmental (Stephen, 2014). Thus, the conservation projects conduct monitoring of the population in veterinary, genetic, spatial and ecological aspects to ensure the stability of the perspectives for the development of European bison population (Klich, 2017).

Due to the low genetic variability of the species, European bison populations face a number of biological and environmental threats, including infectious and invasive diseases (Kita and Anusz, 2006; Pyziel et al., 2014; Olech et al., 2019). The parasitic fauna of European bison has been well described and consists of 88 species of parasites. The most numerous groups of parasites are nematodes (43 species) and protozoa

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(22 species), followed by mites (7 species), trematodes (4 species), cestodes (4 species) and Ixodidae ticks (4 species). The least numerous groups are Hippoboscidae flies (2 species) and Anoplura (1 specie) (Karbowiak et al., 2014a).

Parasitism may serve as a tool of natural selection and it is important to distinguish if it is impacting the survival of endangered species as European bison. Parasitic invasions are common in European bison e.g. protozoans, gastrointestinal and lung nematodes and may cause severe symptoms including hemorrhagic diarrhea, cachexia and bronchitis, and can have a fatal outcome (Demiaszkiewicz et al., 2009b; Jolley and Bardsley, 2006; Kita and Anusz, 2006; Pyziel et al., 2018b). Severe damages threatening the reintroduction of E. bison may be caused by gastrointestinal parasites, e.g. blood-sucking abomasal nematode, *Ashworthius sidemi*, described as one of the most pathogenic among parasites found in European bison (Kołodziej-Sobocińska et al., 2018). *A. sidemi* invasion may cause deterioration of red blood cell parameters and increase in reticulocytes. In severe infections reticulocytosis may be insufficient to compensate significant loss in red blood cells. As a result it affects the animal's condition and increases its vulnerability to other pathogens (Kołodziej-Sobocińska et al., 2016).

Another major concern is the occurrence of parasitic diseases which can cross the host-species barrier, and thus be transmitted between wildlife and livestock (Karbowiak et al., 2014a, 2014b). Most of the identified species of parasites can be found in other wild ungulates or cattle (Kita and Anusz, 2006; Krasińska and Krasiński, 2007). The most parasite species are shared with cattle (*Bos taurus*), deer (*Cervus* spp.) and other Bovidae, however groups of parasites are also shared with moose (*Alces alces*), Caprinae and Canidae. (Karbowiak et al., 2014b). Such cross-species transmission was observed for *Ashworthius sidemi*, which is believed to have been transmitted to Polish European bison by sika deer (*Cervus nippon*) from Ukraine and Slovakia (Dróżdż et al., 1998, 2003); over the years, the species colonized various local wild ruminant populations, such as European bison, red deer, roe deer and moose (Dróżdż et al., 1998, 2003). Subsequently *A. sidemi* was introduced to the Czech Republic along with European bison individuals translocated from Poland (Vadlejch et al., 2017).

Moreover, as illustrated by the case of *A. sidemi*, the translocation of animals carries a high risk of spreading pathogenic agents into native wildlife populations (Pyziel et al., 2018a; Demiaszkiewicz et al., 2009a, 2013; Vadlejch et al., 2017) and to domestic livestock (Moskwa et al., 2015). This threat highlights the need for effective parasitological supervision of European bison with the use of properly-chosen diagnostic methods (Karbowiak et al., 2014; Pyziel et al., 2019, 2020) and appropriate conservation efforts.

The preservation of genetic diversity plays the leading role in management of European bison population and it is achieved by capturing and transferring animals between enclosure conditions and free-living herds. Prior to translocation every individual have to be quarantined and undergo required health tests, which are listed by veterinarians at the place of destination (Hławiczka, 2008; Kaczmarek-Okrój et al., 2016).

This paper evaluates two fecal flotation techniques that can be used for coprological examination of individual European bison in quarantine and before translocation. Ideally, they should yield quick results, and be easy to use and adaptable in the field.

To this end, the aim of this study was to compare two popular coprological methods, the modified Willis method (Willis, 1921; Ziomko and Cencek, 1999) and the modified McMaster technique (Gordon and Whitlock, 1939), with regard to their sensitivity in the diagnosis of gastrointestinal parasites of European bison.

2. Material and methods

2.1. Material collected and area of the study

A total of 166 individual fecal samples were collected from captive and free-living herds of European bison in Poland in the years 2008 and 2011. Samples were taken from Borecka Forest (54.1222° N, 22.1036° E), Białowieża Forest (52.7229° N, 23.6556° E), Knyszyn Forest (53.2167° N, 23.2500° E), the West Pomeranian Voivodeship (53.4658° N, 15.1823° E), as well as in enclosures located in Gołuchów (51.8487° N, 17.9307° E), Pszczyna (49.9858° N, 18.9477° E), Smardzewice (51.4728° N, 20.0262° E), Niepołomice (50.0407° N, 20.2225° E) and Białowieża (52.7007° N, 23.8677° E). The samples were placed in 30-ml labeled plastic tubes and stored at 4 °C for laboratory investigation. The samples were examined immediately after delivery to the laboratory.

2.1.1. Coprological investigation

Each sample was mixed with a spoon, weighed and examined simultaneously with two coprological methods: the modified Willis method with centrifugation and the modified McMaster flotation technique. Both were performed in sucrose solution (SG = 1.27) using 3 g of feces per method (MAFF, 1986; Dryden et al., 2005; Blagburn et al., 2006; Pyziel et al., 2019). The samples were examined using an Olympus BX50 light microscope (Olympus, Japan) at × 100–400 magnification.

The eggs of gastrointestinal helminths were detected at genus or family level according to Taylor et al. (2007). Eimerians were detected at the species level according to Pyziel et al. (2014).

The direct flotation method, as modified by Willis (WM) (1921), was performed with the use of a centrifuge according to Ziomko and Cencek (1999), in sucrose solution according to Blagburn and Butler (2006). In each case, 3 g of feces was passed through a sieve in 10 ml of flotation solution, poured into test tubes and centrifuged for 2 min at 2000 rpm. The tubes were then filled with flotation solution until a convex meniscus was obtained. The meniscus was covered with a coverslip with dimensions of 24 mm × 24 mm for 20 min, allowing any floating oocysts and eggs to adhere to the glass. After this time, the coverslip was transferred to a microscope slide and examined with the use of light microscope at × 100–400 magnification (Olympus BX50, Olympus, Japan).

Although the Willis method is a qualitative and not a quantitative method, parasite eggs and oocysts were counted in every examined sample during microscope evaluation and an estimate of parasite eggs or oocysts per 3 g of feces was calculated based on the weight of the samples and volume of flotation solution (Jacobs et al., 2016).

The modified McMaster method (MM) was used to quantitatively evaluate the numbers of EPGs and OPGs (i.e. eggs and oocysts per gram of feces) in the feces samples (Jacobs et al., 2016) according to Taylor et al. (2007). Briefly, 3 g samples of faces were precisely pounded in 42 ml of water until the suspension appeared homogeneous, and the mixture was wiped through a sieve. The filtrate was transferred to a 15 ml test tube and centrifuged for 2 min at 2000 rpm. The supernatant was decanted and the tube was filled with sucrose solution. The sediment and the flotation solution were thoroughly mixed by mechanical agitation and the suspension was carefully pipetted into both chambers of a McMaster counting slide, ensuring no air bubbles remain. After three to 5 min, the slide was examined under the microscope at × 100–400 magnification (Olympus BX50, Olympus, Japan) according to Roepstorff and Nansen (1998).

EPG and OPG values were calculated by multiplying the total numbers of eggs and oocysts observed in both chambers by 50 (Foreyt, 2013). This approach was used instead of the proposed technique of counting the number in one chamber and multiplying the value by 100 (Taylor et al., 2007), which has been found to be less accurate (Pereckiene et al., 2007).

2.2. Statistical analysis

The prevalence of the identified egg and oocyst taxa, as well as *Eimeria* oocysts, were compared between the Willis and modified McMaster methods with the McNemar test. For taxa that presented higher prevalence, the efficiency of parasite detection by the modified

McMaster method was determined using logistic regression models created for each taxon separately. In each model, the dependent variable was the presence of the given taxon in the sample, as detected by the modified McMaster method, and the independent variable was the number of eggs/oocysts of that taxon detected by the Willis method. The presence of a taxon in the sample was always marked as 1 and its absence was always marked as 0.

Three linear regression models were also run to verify any relationship between the results of the modified McMaster and Willis method for each given taxon or *Eimeria* species: a) eggs/oocysts, b) *Eimeria* species, c) eggs/oocysts and *Eimeria* species in one model. The prevalence of a taxon in the modified McMaster method was used as the dependent variable, and the prevalence of that taxon according to the Willis method was the independent variable. All statistics were performed using SPPS software (version 24.0, IBM Corporation, Armonk, NY, USA).

3. Results

The results of coprological investigation, obtained by both methods, are presented in Table 1. The examined European bison shed nematode eggs of Trichostrongylidae, *Capillaria* spp., *Nematodirus* spp. and *Trichuris* spp., as well as cestode eggs of *Moniezia* spp. in their feces (Tables 1 and 2).

Additionally, oocysts of eleven species of *Eimeria* were identified (Tables 1 and 2). These oocysts demonstrated the highest prevalence among all the identified parasite structures (prevalence: 84.3% in WM and 71.1% in MM, respectively). Trichostrongylidae eggs were seen in 82.5% of samples in WM and 53.6% samples in MM, whereas the prevalence of *Trichuris* spp., *Capillaria* spp. and *Nematodirus* spp. eggs ranged from 13.9 to 18.1% in WM, and 3.61–12.7% in MM. Eggs of *Moniezia* spp. were least frequently observed, being found in 5.4% of samples in WM and 3% of samples in MM (Table 1).

According to the Willis method, the highest mean and median amounts of eggs/oocysts per 3g were observed for *Moniezia* spp. (x = 210; *Me* = 12), *Trichuris* spp. (x = 164.1; *Me* = 85) and Trichostrongylidae (x = 69.5; *Me* = 28). The lowest mean and median were noted for *Capillaria* spp. (Table 1).

According to the modified McMaster method, the highest mean and median EPG/OPG values were observed for *Trichuris* spp. (x = 692.9; *Me* = 350), *Moniezia* spp. (x = 560; *Me* = 350), *Eimeria* spp. (x = 463; *Me* = 150) and Trichostrongylidae (x = 239.2; *Me* = 150). The lowest mean values were observed for *Nematodirus* spp. and *Capillaria* spp., and the lowest median for *Nematodirus* spp. (Table 1).

Among the eimerians, the most prevalent oocysts were those of *E. bovis* (74.7% in WM, 63.9% in MM), *E. zuernii* (39.8% in WM, 24.7% in MM) and *E. ellipsoidalis* (29.5% in WM, 12.6% in MM). The rarest were from *E. subspherica* (1.2% in WM, 2.4% in MM) and *E. brasiliensis* (1.8% in WM, 1.2% in MM) (Table 2).

Based on the modified Willis method, the highest mean numbers of oocysts were obtained for *E. bovis* (x = 29.2), *E. ellipsoidalis* (x = 10.7) and *E. alabamensis* (x = 10.5), whereas the lowest means were observed for *E. bukidnonensis* (x = 1.8) and *E. cylindrica* (x = 3.8). Additionally, the highest median values were observed for *E. bovis* (*Me* = 6), *E. alabamensis* (*Me* = 4) and *E. subspherica* (*Me* = 4), while the lowest were found for *E. bukidnonensis* (*Me* = 1) and *E. canadensis* (*Me* = 1.5) (Table 2).

Based on the modified McMaster method, the highest mean OPGs were noted for *E. bovis* (x = 322.6), *E. auburnensis* (x = 321) and *E. subspherica* (x = 260), and the lowest for *E. bukidnonensis* (x = 50). The highest median value (*Me* = 100) was obtained by four species: *E. alabamensis, E. bovis, E. bukidnonensis* and *E. subspherica.* The lowest median (*Me* = 50) was noted in all other species except *E. pellita* (*Me* = 75) (Table 2).

Statistically significant differences in prevalence of eggs/oocysts were observed between the two methods for all taxa apart from *Moniezia* spp., *Trichuris* spp., *E. brasiliensis, E. canadensis, E. cylindrica* and *E. subspherica*, which were rarely found (Tables 1 and 2).

The results suggest that the detection of eggs/oocysts with the use of modified McMaster technique depended on the taxon of studied parasite. To have a probability of detecting eggs or oocysts higher than 0.5 with the use of MM, one oocyst had to be detected by WM in the case of *Eimeria* spp. (Fig. 1), while 17 eggs had to be detected by WM in the case of *Trichuris* spp., and 23 in the case of Trichostrongylidae (Figs. 2 and 3).

Additionally, significant relationships were observed between the two methods regarding the prevalence of detected eggs/oocysts on the taxa/genus level (R^2 = 0.95, F = 73.25, p = 0.001; Fig. 4) Consequently, both methods tended to yield similar values for the prevalence of eggs/oocysts (Fig. 4). Despite this, the regression coefficient (*B* = 0.793) indicated that the prevalence obtained with the use of MM was lower than that obtained with WM. Moreover, the coefficient for the intercept (*B0* = −3.769) indicated a very low prevalence of significant eggs/oocysts obtained with the use of WM, which may mean that they would not be detected with the use of MM (Fig. 4).

Additionally, a strong correlation was found between both analyzed methods regarding *Eimeria* spp. prevalence (Fig. 5). The linear regression model was also highly significant, and presented a high value of coefficient of determination (R^2 = 0.93, F = 124.08, p = 0.001). Also in this case, the relationship between prevalence values was positive, and the slope indicated that the MM method yielded lower prevalence values than the WM method (*B* = 0.810). Similarly, eimerians of a very low prevalence which were detected with the use of WM method may be not detected with MM (*B0* = −3.408).

In one regression model, including data from the above regressions for both the taxa and eimerians, all the included taxa presented similar results (R^2 = 0.945, F = 257.79, p = 0.000); this allowed their prevalence to be compared between methods. To predict the prevalence for the modified McMaster method based on the results of the Willis method, the following formula should be used:

$$y = 0.796x - 3.392$$

where: y - predicted prevalence in the modified McMaster method, and x - real prevalence in the Willis method, with the standard errors (*SE* =

Table 1
Comparison of coprological findings in European bison (n = 166) between Willis (WM) and McMaster (MM) method, and statistical comparison with Mc Nemar test, *statistically significant difference.

Parasite	Prevalence				Statistical difference (Mc Nemar)	Eggs/Oocysts per 3 g (WM)			EPG/OPG (MM)		
	%		95% CI			Mean	Median	Range	Mean	Median	Range
	WM	MM	WM	MM	*p*						
Capillaria spp.	13.9	3.61	5.3	2.8	0.002*	4.7	3	1–14	83.3	100	50–100
Eimeria spp.	84.3	71.7	5.5	6.9	0.004*	38.7	7	1–658	463	150	50-6500
Moniezia spp.	5.4	3	3.4	2.6	0.500	210	12	1-1368	560	350	100-1050
Nematodiurus spp.	18.1	4.8	5.9	3.3	<0.001*	51.3	7.5	1–521	81.3	50	50–300
Trichostrongyloidea	82.5	53.6	5. 8	7.6	<0.001*	69.5	28	1-1316	239.2	150	50-2950
Trichuris spp.	12.7	12.7	5.1	5.1	1.000	164.1	85	2-1339	692.9	350	50-3100

Prevalence (% and 95% confidence interval), EPG/OPG: eggs/oocyst count per gram (average, *X*, and range).

Table 2
Comparison of *Eimeria* oocysts findings in European bison ($n = 166$) between Willis (WM) and McMaster (MM) method and statistical comparison with Mc Nemar test, *statistically significant difference.

Species	Prevalence				Statistical difference (Mc Nemar)	Eggs/Oocysts per 3 g (WM)			EPG/OPG (MM)		
	%		95% CI			Mean	Median	Range	Mean	Median	Range
	WM	MM	WM	MM	p						
E. alabamensis	12	4.8	4.94	3.25	0.004*	10.5	4	1–105	231.2	100	50-1200
E. auburnensis	25.3	11.5	6.61	4.85	<0.001*	8.7	2.5	1–77	321	50	50-4050
E. bovis	74.7	63.9	6.61	7.31	0.012*	29.2	6	1–530	322.6	100	50-6150
E. brasiliensis	1.8	1.2	2.02	1.66	1.000	7.7	4	1–18	100	100	50–150
E. bukidnonensis	6.6	1.2	3.78	1.66	0.012*	1.8	1	1–6	50	50	50
E. canadensis	10.8	10.2	4.72	4.60	1.000	10.1	1.5	1–105	81	50	50–450
E. cylindrica	6.6	4.2	3.78	3.05	0.508	3.8	2	1–12	100	50	50–250
E. ellipsoidalis	29.5	12.6	6.94	5.05	0.001*	10.7	2	1–345	195.2	50	50–1800
E. pellita	12.7	4.8	5.07	3.25	0.004*	4.9	2	1–23	81.3	75	50–150
E. subspherica	1.2	2.4	1.66	2.33	0.625	4	4	1–7	260	100	100–550
E. zuernii	39.8	24.7	7.45	6.56	0.001*	6.5	2	1–80	100	50	50–950

Prevalence (% and 95% confidence interval), EPG/OPG: eggs/oocyst count per gram (average, *X*, and range).

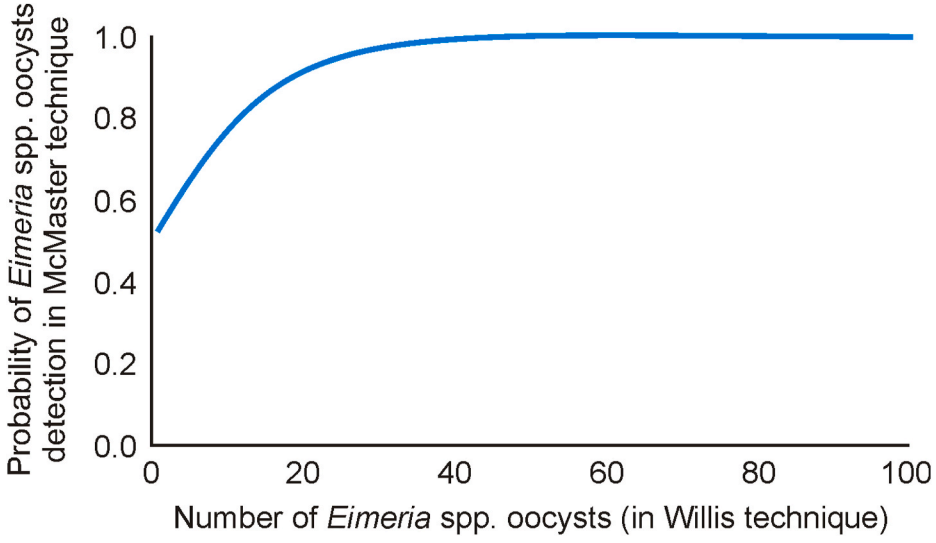

Fig. 1. Probability of detection of *Eimeria* spp. oocysts with the modified McMaster technique based on the number of oocysts detected using the Willis technique.

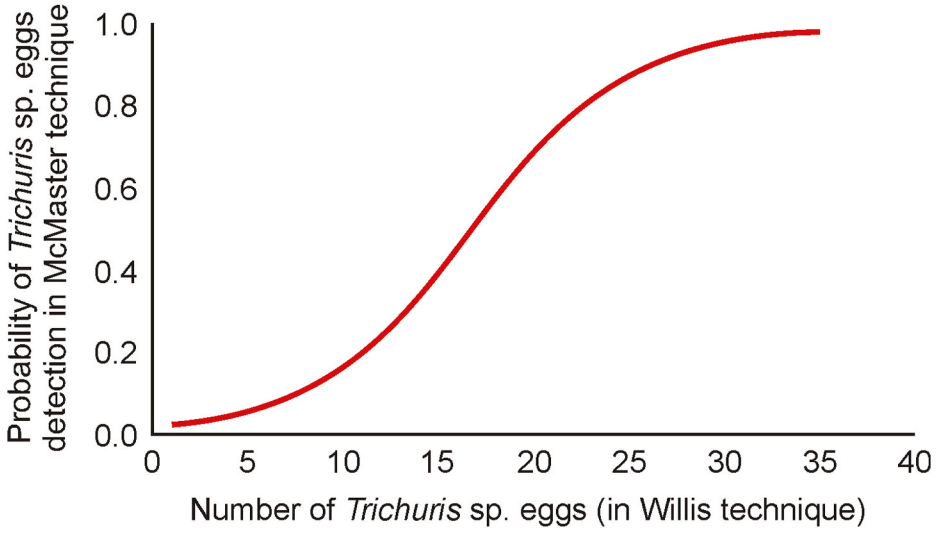

Fig. 2. Probability of detection of *Trichuris* sp. eggs with the modified McMaster technique based on the number of eggs detected using the Willis technique.

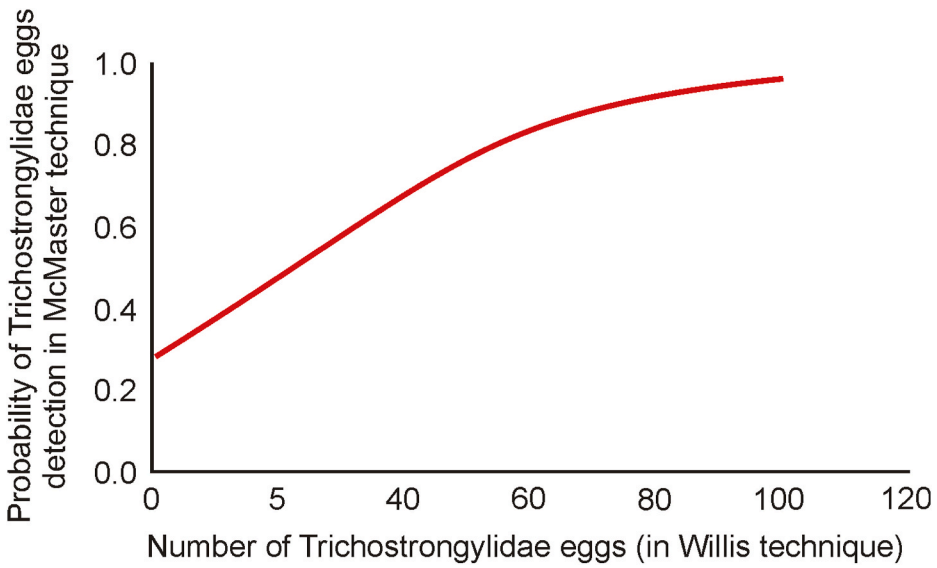

Fig. 3. Probability of detection of Trichostrongylidae eggs with the modified McMaster technique based on the number of eggs detected using the Willis technique.

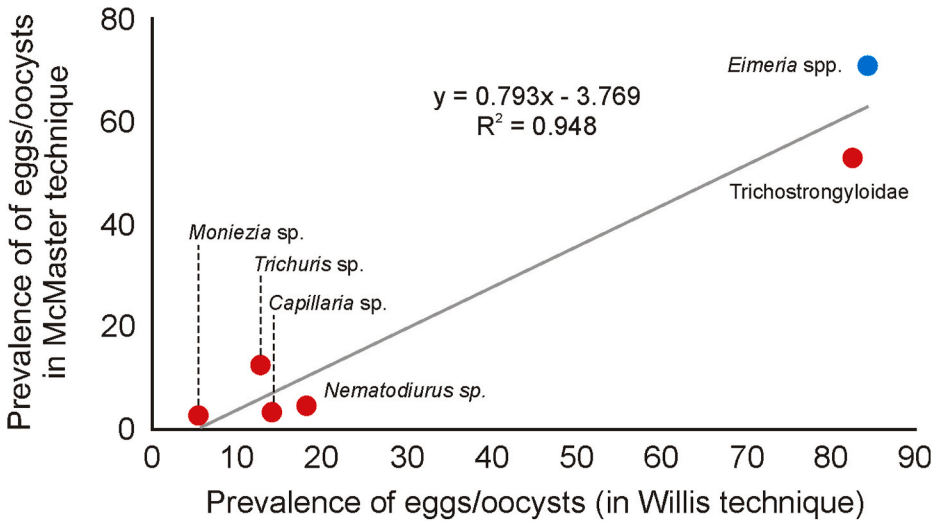

Fig. 4. The relationship between the prevalence of various taxa eggs/oocysts in European bison feces measured by the Willis and modified McMaster techniques (each point represents an individual taxon/genus, blue points stand for oocysts and red point for eggs). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

0.05, $SE = 1.855$, respectively).

Conversely the prevalence for the Willis method can be predicted based on the results of the modified McMaster method using the formula:

$$y = 1.178x + 5.443$$

where: y - predicted prevalence in Willis method, and x - real prevalence in the modified McMaster method, with the standard errors ($SE = 0.074$, $SE = 2.074$, respectively).

4. Discussion

Translocation of animals is essential element of conservation efforts focused on preservation of genetic diversity in endangered species. In case of European bison it involves relocation of individuals from both captive and free-living herds, which is connected with fulfilling certain requirements. All animals, kept in enclosure and captured from the free-living population, are obliged to be kept under quarantine conditions for at least 30 days and need to be tested for the presence of diseases listed in the EU TRACES-document No. 92–65 and required by veterinarians at the place of destination. One of the recommended procedures prior the translocation of European bison is coprological evaluation and prophylactic anthelmintic treatment (Treboganova, 2011; Kaczmarek-Okrój et al., 2016; Hławiczka, 2008).

Veterinary practice has at its disposal a variety of coprological techniques for the diagnosis of internal parasitic infections; one such tool is the flotation method, which is simple, quick, inexpensive and can be easy applied in the field (Pouillevet et al., 2017; Jolles et al., 2008; Sousa et al., 2016). However, this wide variety of methods limits the possibility for comparison of coprological results between different scenarios. Hence, one goal of our present study is to partially overcome these methodological barriers by attempting to unify the results of two widely-used approaches: the Willis and modified McMaster methods.

Both methods are flotation techniques, with the difference that the modified Willis method is a qualitative and the modified McMaster method is a quantitative technique. The modified Willis method is a direct flotation method helpful in detecting most gastrointestinal

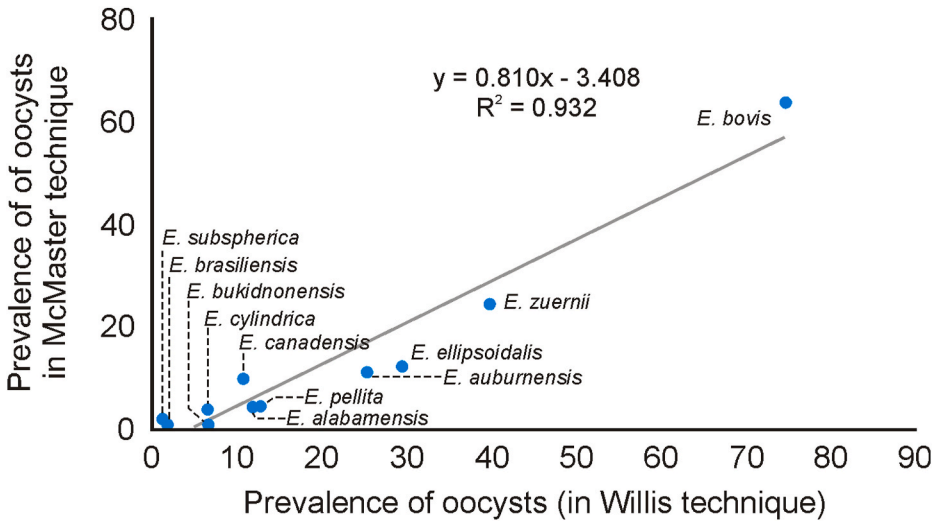

Fig. 5. The relationship between the prevalence of *Eimeria* spp. oocysts in European bison feces measured by the Willis and modified McMaster techniques (each point represents an individual parasite species).

parasites including helminth eggs and protozoan cysts in the feces, which is often used in preliminary surveys. It can be satisfactorily applied by the veterinarian in the field, because it is inexpensive, easy and fast to perform and doesn't require any specialized equipment (Foreyt, 1989; Carvalho et al., 2012; Sousa et al., 2016; Mesquita et al., 2017). The inefficiency may be observed while the number of eggs or oocyst is high in the sample and the precision in the egg counting procedure may be decreased due to lack of the grid on the coverslip.

The modified McMaster method enables to determine fecal egg count and is the most universally applied fecal egg count technique in veterinary parasitology. It is considered a standard reference method for detecting internal parasites, and is recommended by World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of anthelmintic drugs in ruminants (Wood, 1995) and for detecting anthelmintic resistance (Coles, 2006; Cringoli, 2010). Performing a modified McMaster method requires a McMaster chamber for counting parasite eggs and oocysts, whereas the analytic sensitivity of the method is established at 50 EPG. A significant disadvantage is that the height of the counting chamber may prevent the possibility to perform the examination under greater magnification than at × 200, which may interfere with the identification of parasite structures (Kochanowski et al., 2013).

The aim of the study was to compare detection sensitivity of two different methods, qualitative and quantitative, modified Willis and modified McMaster methods in order to offer recommendation for technique applied for coprological examination of European bison prior to the translocation. In this aspect the most important information was the presence or absence of the parasites, identification of parasite species and the prevalence of invasion. As the parasite egg number in feces does not directly show infection intensity, it was impossible to validate the infection intensity because the difference of characteristics of compared methods prevented from analyzing EPG/OPG for both methods. Willis method is a qualitative technique which allows to obtain the values of eggs/oocysts in 3 g of feces and it cannot be converted to EPG/OPG because the score would not be reliable. The weight of the fecal sample was the same for both methods, which allowed to align the values and compare the prevalence of applicated methods.

As predicted, the Willis and modified McMaster methods yielded different qualitative and quantitative findings. Significant differences in prevalence were noted for four out of the six identified genera, including *Capillaria* spp., *Nematodirus* spp. and the Trichostrongyloidea. Among *Eimeria* spp., noteworthy differences were observed for seven out of eleven identified species, including *E. alabamensis*, *E. auburnensis* and *E. bovis*. The presence of a significant difference in the prevalence of *Eimeria* spp. between the two techniques seems to be more related to the level of parasitism than to the species.

However, while the two methods yielded similar prevalence for some taxa, this alone does not imply that their findings are comparable. Similarly, the results of the quantitative analysis (mean, median and range) cannot be compared due to discrepancies between the units: WM values are given in eggs/oocysts per 3g, while those for MM are in EPG/OPG (Tables 1 and 2). This is understandable, considering that WM is a qualitative direct flotation method used in detecting most gastrointestinal parasites, including helminth eggs and protozoan cysts in the fecal samples, and so is often used in preliminary surveys (Foreyt, 1989; Carvalho et al., 2012; Sousa et al., 2016; Mesquita et al., 2017), while MM is quantitative fecal egg count technique, which uses a McMaster counting chamber for counting helminth eggs and coccidian oocysts in 3g of feces (Gawor, 2017). Therefore, to allow some degree of comparison between the two methods, the results of the WM were based on the quantity of eggs or oocysts per 3g of feces by determining the sample weight and flotation solution volume (Jacobs et al., 2016).

Although the two methods were fundamentally different, the results of this calculation allowed a quantitative comparison of the WM and MM tests. Linear regression showing the relationship between prevalence in both methods, i.e. in which the prevalence values are convergent (Figs. 4 and 5) can be used to make a direct comparison of the prevalence in the same location, or the same herd in different periods.

For MM, prevalence can be predicted using the regression $y = 0.796x - 3.392$ (where: y - predicted prevalence in modified McMaster method, and x - real prevalence in the Willis method) and for WM, it can be predicted using $y = 1.178x + 5.443$ (where: y - predicted prevalence in Willis method, and x - real prevalence in the modified McMaster method). Both conversion methods are simple and easy applicable. Although the values used for linear regressions were based on a particular genus or species, the methods demonstrated a high percentage of mutual explanation ($R2 = 0.945$). As such, we propose that these formulas should be used for comparisons of tests performed with the two methods.

Our findings demonstrate the detection sensitivity of the two methods. They also confirm the comparative sensitivity of WM and MM, and highlight their value in the diagnosis of gastrointestinal parasites in European bison in the field. WM appears to be more sensitive to the presence of eggs and oocysts than MM. Thus, performing a coprological examination with WM provides higher probability of detecting parasitic structures which may not be found with MM, especially in case of

parasitic invasions of very low prevalence. Low sensitivity of MM have been observed by Levecke et al. (2011, 2012) in the case of low baseline fecal egg counts; in addition, Cringoli et al. (2010) regard the modified MM method as inadequate for rigorous parasitological diagnosis.

Based on our present findings, we recommend the use of WM for the parasitological supervision of European bison prior to the translocation due to its higher sensitivity than MM. The use of WM instead of MM does not present a limitation as our findings confirm that the results obtained by the two methods are comparable.

We suggest that as a precaution, European bison intended for transfer should be placed in quarantine before translocation and that coprological examination should be performed based on the Willis method. Should a positive parasitological diagnosis be obtained, effective antiparasitic treatment can be implemented.

5. Conclusion

It is recommended that the Willis method be used for the parasitological diagnosis before translocation of European bison, as the test offers more sensitive method than McMaster technique of detecting the presence of low levels of a variety of parasite eggs and oocysts in feces.

is easy to use, inexpensive and adaptable for the field work. Alternatively, it is possible to predict the prevalence of parasite eggs and oocysts measured by the Willis methods based on the modified McMaster method and vice versa; this could be of value when reviewing coprological examinations obtained in previous studies.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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