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Short communication / Kurzmitteilung

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Presence of antibodies against *Ostertagia ostertagi* in bulk tank milk from cattle herds in the northeastern Baltic countries of Europe

Vorkommen von Antikörpern gegen Ostertagia ostertagi in Tankmilchproben aus Rinderherden in den europäischen Ländern des nordöstlichen Ostseeraumes

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Summary

Ostertagia (O.) ostertagi is a common cattle parasite that can be found on all cattle farms where animals have access to pastures. To evaluate the presence of anti-*O. ostertagi* antibodies in cattle herds, bulk tank milk (BTM) samples were evaluated using a commercial ELISA test (SVANOVIR® *O. ostertagi* Ab ELISA, Svanova, Sweden). Samples (n=830) were collected from dairy cattle herds in Estonia, Latvia, Lithuania, and Poland. The average BTM optical density ratio (ODR) values in Poland and Estonia were 0.400 and 0.493, respectively. Higher BTM ODRs were obtained in samples collected in Lithuania (0.695) and in Latvia (0.703), which may correlate with the reduction in milk yield by 0.75–1.2 kg of milk per cow per day in Lithuania and Latvia. The first investigations on ostertagiosis using BTM samples were conducted in Poland in 2011. To our knowledge, this is the first report to use BTM ELISA ODRs to evaluate ostertagiosis in cattle in Estonia, Latvia, and Lithuania.

Keywords: Baltic Sea, ostertagiosis, ODR, Estonia, Latvia, Lithuania

Zusammenfassung

Ostertagia (O.) ostertagi ist einer der häufigsten Parasiten beim Rind. *Ostertagia* wurde daher in allen Milchviehställen, in denen die Tiere Zugang zu Weiden haben, gefunden. Um das Vorkommen von *Ostertagia ostertagi* zu bewerten, wurden Tankmilchproben (BTM) gesammelt und mithilfe eines ELISA untersucht (SVANOVIR® *O. ostertagi* Ab ELISA, Svanova, Sweden). Die Proben (n = 830) wurden in den Milchviehbeständen aus Estland, Lettland, Litauen und Polen genommen. Der Mittelwert für BTM-Dichteverhältnisse (ODR) betrug 0,400 und 0,493 in Polen und Estland. Höhere ODR-Werte wurden in den Proben aus Litauen (0,695) und Lettland (0,703) nachgewiesen, was mit der Abnahme der Milchleistung um rund 0,75–1,2 kg Milch/Kuh/Tag in Litauen und Lettland korrelieren könnte. Erste Untersuchungen zur Ostertagiose mit den BTM-Proben wurden im Polen in 2011 durchgeführt. Nach unserem Wissen ist dies die erstmalige Beschreibung der Anwendung des ELISA ODR für die Evaluation der Ostertagiose in Estland, Lettland und Litauen.

Schlüsselwörter: Ostsee, Ostertagiose, ODR, Estland, Lettland, Litauen

Introduction, materials and methods

Ostertagia (O.) ostertagi is one of the most common gastrointestinal parasites in grazing cattle. In 2008, Forbes et al. reported on the exposure of dairy cow herds to *O. ostertagii* in several European countries, including Denmark, Germany, Ireland, the Netherlands, Portugal, Spain, and the United Kingdom. ELISA optical density ratio (ODR) profiles of bulk tank milk (BTM) from infected herds showed that they segregated into two groups: one group had intermediate ODRs of 0.45–0.48 and included herds from Germany, the Netherlands, and Denmark; and the other group had higher ODRs of 0.53–0.61 and included herds from Portugal, Spain, and the United Kingdom. The ODR of 0.31 from Italy was substantially lower than adopted ranges (Forbes et al. 2008). Although there have been several new reports from Switzerland (Frey et al. 2018), Germany (Fanke et al. 2017), and Mexico (Villa-Mancera et al. 2018) that used ELISA to measure antibodies against *O. ostertagi* in BTM, no comparable screening studies have been performed in northeastern Baltic countries. The aim of this study was to investigate *O. ostertagi* antibody levels in BTM from dairy cow herds in the northeastern Baltic countries of Europe, including Poland, Estonia, Latvia, and Lithuania.

Location of sampling, sample size and BTM collection

To determine the percentage of infected herds in the examined population, we used available lists of the numbers of dairy herds from the national databases of Estonia, Latvia, Lithuania, and Poland, which were 23,868, 25,740, 91,136, and 266,944, respectively. The number of farms to be evaluated to determine the presence of *O. ostertagi* infections with a confidence level of 0.99 was determined based on the following formula (1) (Thrusfield, 2007):

$$n = \frac{1{,}96^2 \times P_{exp} \times (1 - P_{exp})}{d^2} \quad (1)$$

n represents the required sample size, P_{exp} represents the expected prevalence, and *d* represents the accuracy of the measurement.

Due to the lack of data on the prevalence of *O. ostertagi* in dairy cattle populations in the northeastern Baltic region of Europe, the expected prevalence should be arbitrarily set at 50%, where at the accuracy of the measurement set at 10% (d=0.1) at least 97 herds from each country should be tested (Thrusfield, 2007). Never before farmers in Estonia, Latvia, and Lithuania have carried out or commisioned proposed the BTM ELISA test and therefore they were not convinced for its reliability. In such situation, due to the limited cooperation with farmers, expected prevalence was set at 10% (at the same level of the accuracy of the measurement), where the calculated sample size necessary to evaluate the presence of ostertagiosis was lower and amounted minimum 35 cattle herds.

Finally BTM samples were collected from 50 dairy farms in Estonia, 67 in Latvia, 101 in Lithuania, and 612 in Poland. Each herd was visited once between January 2016 and May 2017, but none of the herds were visited during the off-grass period from October to April. Except access to pasture, no specific criteria were imposed on farms for inclusion in the survey. The BTM samples were collected by local veterinarians, were stored in 100 ml containers without preservatives, and were transported at 4ºC directly to the Diagnostic Laboratory at the Faculty of Veterinary Medicine, Wroclaw. Immediately after receiving the samples, they were centrifuged at 16,000 *g* for 4 min at 4ºC and the fat fraction was removed. The skimmed milk was placed into 1 ml tubes and stored at –20ºC until further testing. The study complied with the quality management system (ISO/IEC 17025:2005 + API:2007 + AC:2007).

ELISA

Anti-*O. ostertagi* antibody levels were determined with a commercial, semi-quantitative indirect ELISA kit (SVANOVIR® *O. ostertagi* Ab ELISA, Svanova, Sweden), which was performed with the absorbance at 492 nm according to the manufacturer's instructions. Each test result was expressed as ODR calculated as follows:

$ODR = (OD_{BTM\ sample} - OD_{negative\ control}) / (OD_{positive\ control} - OD_{negative\ control})$

To compare our BTM ODR results with other studies, we used a unified ODR cut-off level of 0.5 to identify herds that may incur losses in milk yield. Bloemhoff et al. (2015) and Kowalczyk et al. (2018) similarly optimized the cut-off titer according to the ELISA manufacturer's manuals.

Statistical analysis

Statistical analysis was performed in STATISTICA v. 10 (StataSoft) and EXCEL (Microsoft) software. Kruskal-Wallis tests were conducted, supported by multiple comparisons of the average ranks for all samples and χ^2 calculations (http://turner.faculty.swau.edu/mathematics/math241/materials/contablecalc/).

Results and discussion

This analysis was performed on a total of 830 dairy cattle farms. A detailed description of the results, including mean, minimum and maximum, median, and interquartile range (Q_1 and Q_3) ODR values, as well as the distribution of cattle herds with an ODR >0.5 and <0.5 is presented in Table 1. The percentage of farms that have the potential to incur losses in milk yield caused by *O. ostertagi* was 47% (n=390). The countries with the highest number of herds with ODR >0.5 were Latvia and Lithuania (χ^2=148.345, df 3, p<0.001).

The lowest quartile BTM ODR values (Q_1) in Latvia (0.65) and Lithuania (0.57) were similar to the upper third quartile values (Q_3) in Estonia (0.607) and Poland (0.585). Poland had the lowest median BTM ODR value, but the widest BTM ODR range (0.63–1.28). Differences were found between the median BTM ODR values for the examined country pairs: Estonia vs. Latvia and Estonia vs. Lithuania; Latvia vs. Poland and Lithuania vs. Poland ($p \leq 0.001$) .

By comparing our BTM ODR results with the data from Forbes et al. (2008), we determined that the *O. ostertagi* statuses of cattle herds in Poland are highly variable, and this variability appears to be dependent on geographical location. In our previous study, we tested milk samples from the Lower Silesia region of Poland for the presence of anti-*O. ostertagi* antibodies by ELISA (Płonec-

TABLE 1: *Results of examination of dairy cattle herds in Estonia, Latvia, Lithuania and Poland with respect to the average value of BTM ODR in the surveyed countries. Statistically significant differences were estimated within countries, taking into account number of herds with ODR value >0,5 and <0,5.*

Country	No of examined herds	Mean ODR	Min/Max	Me (Q1; Q3)	ODR <0,5			ODR >0,5		
					No of herds	% of herds	Range of values	No of herds	% of herds	Range of values
Estonia	50	0.493	0.205 + 0.800	0.491 (0.379; 0.607)	28	56	0.205–0.492	22	44	0.5–0.801
Latvia*	67	0.703	0.200 + 1.040	0.730 (0.570; 0.820)	7	10,4	–0.15–0.48	60	89,6	0.5–1.06
Lithuania*	101	0.695	–0.150 + 1.060	0.730 (0.650; 0.830)	12	11,9	0.2–0.49	89	88,1	0.5–1.04
Poland	612	0.400	–0.630 + 1.280	0.379 (0.219; 0.585)	393	64,2	–0.638–0.49	219	35,8	0.5–1.281
Total	830	0.573			440	53	–0.638–0,492	390	47	0.5–1.281

* statistically significant values, p<0,001

zka-Janeczko et al. 2011), and determined that the mean BTM ODR was 0.53 (range 0.43–0.63). In a prior study involving small and medium size farms in southern Poland (Lower Silesia and Lesser Poland), Piekarska et al. (2013) calculated a lower mean BTM ODR value for all of the examined herds of 0.345 (range 0.07–0.778). A more recent study determined that the mean *Ostertagia* ELISA ODR was 0.505 (range 0.01–1.000) for herds in the central provinces of Poland and 0.725 (range 0.01–1.080) for herds in the northeastern provinces of Poland (Kowalczyk et al. 2018). An analysis and assessment of risk factors, such as grazing policy, grazing period duration, and proportion of diet composed of grazing grass, shed light on aspects of ostertagiosis beyond the use of anti-worm products (Kowalczyk et al. 2018). The factors that correlated positively with higher BTM ODRs were location (northeastern province of Poland), a longer grazing period, and a higher proportion of grazing grass in the diet (>50% of all roughage). These same factors could explain the variability in this study in which the maximum BTM ODR was 1.28, but the mean BTM ODR was only 0.40.

Two limitations of our study were that detailed information on the management of the examined herds was not analysed and that serological cross-reactivity with organisms in the Trichostrongylidae or Paramphistomidae families may occur with anti-*O. ostertagi* antibodies (Keus et al. 1981, Kowalczyk et al. 2018). Additionally, it was difficult to interpret the results obtained from Estonia, Latvia, and Lithuania because this was the first time (to the best of our knowledge) that serological monitoring of dairy cattle herds by BTM ODR ELISA was performed in these countries. Ostertagiosis research in Estonia, Latvia, and Lithuania has focused on small ruminants infections (Keidáne and Birgele 2007).

The simplest explanation of the higher BTM ODR results in the herds in northeastern Baltic countries is the influence of geographical location. Estonia, Lithuania, and Latvia are the northernmost regions analysed in this study and climatic differences may impact the behaviour of larvae in the pasture and thus, may result in an increased risk of infection and animal parasite burden (Heckler and Borges 2016). In support of this hypothesis, the BTM ODR values were much lower in the southern European countries of Italy (ODR 0.31) and Spain (ODR 0.33) (Forbes et al. 2008; Pablos-Tanarro et al. 2013). However, data from Sweden and Estonia, which are situated further north, do not support this hypothesis because the mean BTM ODR in cattle in Sweden was 0.52 (Bennema et al. 2010), and we found that the mean BMT ODR of cattle in Estonia was 0.493. Until multivariable analysis of detailed parameters, such as climatic conditions, duration of the pasture season, proportion of pasture in the diet, herd structure, herd size, and turnover of animals within herds, are performed, any conclusions regarding ostertagiosis in Lithuania, Latvia, and Estonia remain tentative.

This study provides the background for further in-depth analyses of ostertagiosis in dairy cattle herds in Lithuania, Latvia, Estonia, and Poland. For future studies, we recommend estimating the expected prevalence as 50% and the precision of the measurement as 5%, and based on these estimates, we recommend testing 379, 380, and 384 herds from Estonia, Latvia, and Lithuania, respectively.

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Conflict of interest

The authors declare that there is no conflict of interests. The authors confirm to have no protected, financial, occupational or other personal interests in a product, service and/or a company which could influence the content or options presented in the manuscript.

Ethical approval

The Ethical Committee for Animal Experiments, Wrocław, Poland approved this study, and all owners provided informed consent prior to initiation of the study.

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Authors contribution

KPJ, KR: concept of the work, serological testing, data analysis, preparation of the manuscript; JP: data analysis, preparation of the manuscript; MS, AM: BTM samples collection and participation in the serological analysis; TN: preparation of the database and analysis; AG: statistical analysis.

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