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Effect of Fasciola Hepatica Invasion on Cow Productivity

Igor Gennadievich Glamazdin, Ilya Nikolaevich Medvedev*, Ilnura Ibragimovna Fayzullina, Natalya Yuryevna Sysoeva, Marina Mikhailovna Goryacheva, Galina Mikhailovna Kryukovskaya, Tatyana Olegovna Maryushina

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Abstract

In the study, the level of antibodies to fasciolas in the milk of cows in the Moscow region of Russia was assessed and the relationship between their number and the productive characteristics of these animals was traced. In October 2021, a milk sample was taken from a dairy farm in the Moscow region of Russia, and the number of antibodies to Fasciola hepatica (ODRf) and Ostertagia ostertagi (ODRo) was assessed. Their level was determined using multivariate linear regression models and their relationship with economically significant indicators was traced: the amount of protein in milk, the percentage of fat in milk, and the interval between past calving. An increase in the ODRf quartile from 25% (0.412) to 75% (0.976) was associated with a decrease in the volume of milk produced per year by 0.8 kg (day of the lactation process) (p=0.002), with a decrease in fat content in milk by 0.07 % (p<0.001), with an increase in the interval between sections by 4.6 days (p=0.04) in the absence of a significant relationship with the level of protein in milk. In the case of simultaneous infection with both parasites, the effect of ODRf and ODRo on the volume of milk produced was additional, rather than synergistic.

Keywords: Cows, Parasitism, Fasciola hepatica, Ostertagia ostertagi, Milk production, ELISA

Introduction

Modern science continues to search for approaches to improve health (Karpov *et al*., 2021; Zavalishina *et al*., 2022) and increase the viability of the mammalian organism (Zavalishina, 2018b; Kočović *et al*., 2021). For this purpose, direct observations are carried out (Zavalishina, 2018c; Zavalishina *et al*., 2021), and various schemes of experimental work are set up (Vorobyeva *et al*., 2018; Kulikov *et al*., 2020). At the same time, despite all the efforts made by science and practice, the Fasciola hepatica parasite is still common and very common in cattle. The situation is complicated by the fact that, as a rule, fascioliasis in cattle is mostly asymptomatic (Zavalishina, 2018d; Yıldız *et al*., 2021), but it causes serious physiological changes that lead to significant economic losses (Torgerson & Claxton, 1999). Despite this fact, few studies related to the impact of the disease on productivity levels have been performed on this parasitic disease (Vercruysse & Claerebout, 2001; Drljača *et al*., 2020). At the same time, in some cases, there were questions about the scheme or design of the study (Burden *et al*., 1978; Dargie, 1987).

Igor Gennadievich Glamazdin, Ilnura Ibragimovna Fayzullina, Natalya Yuryevna Sysoeva, Marina Mikhailovna Goryacheva, Galina Mikhailovna Kryukovskaya, Tatyana Olegovna Maryushina
Department of Veterinary Medicine, Moscow State University of Food Production, 125080, Moscow, Russia.

Ilya Nikolaevich Medvedev*
Faculty of Medicine, Russian State Social University, 129226, Moscow, Russia.

***E-mail:** ilmedv1@yandex.ru

The presence of F. hepatica in the body, as a rule, is determined using scatological methods. At the same time, in recent years, specific tests have been increasingly used to determine its presence, which is gradually replacing scatological research methods. Newer tests are associated with the detection of specific fasciola antigens in blood and feces (Leclipteux *et al*., 1998; Mezo *et al*., 2004) or the detection of the presence of specific antibodies to fasciola in the blood (Farrell *et al*., 1981; Salimi-Bejestani *et al*., 2005b; Korobov & Glamazdin, 2010) and in milk (Boulard *et al*., 1985; Reichel *et al*., 2005; Salimi-Bejestani *et al*., 2005a). The available reagent kits for antibodies to fasciolae have already been sufficiently developed and allow detecting the presence of the parasite with an unmistakable accuracy, which makes it possible to judge the degree of its spread. Given the impossibility of the complete eradication of F. hepatica, it is important to direct diagnostic control to reduce its frequency of occurrence and prevalence, in which the invasion would have a minimal impact on the overall lactation capacity of the dairy herd of cows (Vercruysse & Claerebout, 2001; Mai *et al*., 2022).

Previously, the prevalence of invasion in the amount of 30 different flukes with a prevalence in the herd of 25% of the total population of the herd was supposed to be considered as threshold values for optimum productivity (Vercruysse & Claerebout, 2001; Roest Henk *et al*., 2021). However, these figures either cannot be determined prior to slaughter or are based on questionable information. Recording the amount of highly specific antibodies to the parasite present in marketed milk is now considered a valid method of tracking the overall level of parasite infestation in a dairy herd. Also, this method is recognized as reliable for the diagnosis of parasites in the herd, where the level of infestation of cows causes a decrease in productivity (Sanchez & Dohoo, 2002; Charlier *et al*., 2005a). Scientists have elucidated the relationship between the content of antibodies specific to fasciola in milk and the number of antibodies in the blood of lactating cows (Salimi-Bejestani *et al*., 2005a; Sloan *et al*., 2021).

The purpose of the study: is to find out the relationship between the number of antibodies specific to fasciola in milk and indicators reflecting the productivity of dairy cows in the Moscow region of Russia to assess possible economic losses.

Materials and Methods

The study was conducted in dairy farms of the Moscow region of Russia with a total number of livestock of more than 2000 dairy cows. Milk samples were taken from the milk collection containers. All taken milk samples were delivered to the laboratory of the Department of Veterinary Medicine of the Moscow State University of Food Production in Moscow within 6 hours after milking. After delivery to the laboratory, all milk samples were subjected to centrifugation (16000 g for 5 minutes). Fat was removed from them, the supernatant was obtained and frozen at -20°C until the scheduled analyzes were performed. All selected milk samples were tested using F. hepatica ELISA and O. ostertagi ELISA.

Cows that gave milk, samples of which were taken for analysis, were kept in farms in the Moscow region of Russia. They were purebred for the Black-and-White breed or had different bloodlines for the Black-and-White and Holstein breeds. In general, the cows that gave milk for sampling had 2-4 lactations. The study was completed in March 2021.

After defrosting all milk samples, they were examined using 2 indirect ELISAs to detect the content of specific antibodies capable of reacting with Fasciola hepatica and Ostertagia ostertagi antigens.

The amount of antibodies capable of reacting with F. hepatica was recorded by the traditional method by Salimi Bejestani *et al.* (2005b). For this, 96-well microplates with a flat bottom were used. They were loaded per well with 0.5 mg/ml excretory and secretory (ES) antigen in 0.05 M carbonate-bicarbonate buffer (pH 9.6). An overnight incubation followed by washing the well six times with phosphate-buffered saline containing 0.05% tween 80 (PBST). Then blocking was done with 100 ml of 2% skimmed milk powder (Marvel, Premier Beverages, Stafford, UK) in PBST (SMP/PBST) and the incubation process was carried out for 1 hour at 37.0°C. The plates used in the work were traditionally washed and undiluted samples of the studied milk (100 ml) were added to their wells. Evaluated serum samples, considered as negative and positive controls, were diluted at 1:800 in SMP/PBST and added (100 ml/well) to six wells of each plate. Tablets are traditionally incubated and washed.

Rabbit anti-bovine IgG conjugated with horseradish peroxidase (Jackson Immunoresearch Laboratories) was used. It was diluted 1/6000 in SMP/PBST and added to wells per 100 ml. The substrate solution consisted of 0.1% OPD (orthophenylenediamine) in citric acid/phosphate buffer (pH 5.0) with 1/2000 H_2O_2 and was applied to wells at a rate of 100 ml. The plates thus prepared were incubated at room temperature in the absence of light for 10 min. Blocking of the reaction was carried out by introducing 50 ml of 2.5 M HCl and then the optical density was evaluated at a wavelength of 492 nm. The amount of antibodies was expressed as the ratio of the detected optical density (ODRf) according to the formula ODRf = (OD NC)/(PC NC) where OD is the value of the optical density of the assessed image, and NC and PC OD are negative and positive levels. The sensitivity of this enzyme immunoassay makes it possible to detect infection in milk in a herd of more than 25% of cows with an accuracy of 95% (95% CI 89–100%) and 80% (95% CI 66–94%), respectively.

It has been noted that the ES antigen is unable to bind to O. ostertagi (Salimi-Bejestani *et al*., 2005a; Salimi-Bejestani *et al*., 2005b). Anti-O. ostertagi antibodies were quantified using a known ELISA method (Charlier *et al*., 2005b). The ELISA used crude antigen from mature O. ostertagi. The result of this test is reported as an optical density ratio (ODRo). The expected 95% scatter difference between two ODRo values of a particular sample using separate plates and on different days reaches 0.16 (Charlier *et al*., 2005b) The adult worm crude antigen interacts very well with other infections of the gastrointestinal tract, including Cooperia spp. (Keus *et al*., 1981) and with other worms, including F. hepatica (Eysker & Ploeger, 2000).

Differences in ODR values were noted between dairy farms for which production situation data were and were not obtained by applying a two-tailed Mann-Whitney U-test (a = 0.05) for ODRf and a two-tailed t-test (a = 0.05) for ODRo. The level of Spearman's rank correlation between ODRf and ODRo was determined.

The initial relationship of ODRf with the number of economically significant indicators (the amount of milk produced, the percentage of protein in milk, the fat content of milk, and the time between the onset of calving) was estimated using linear regression models. The dairy herd was considered as a whole, and the significance of the parameters was assessed using two-tailed F-tests (a=0.05). The average indicators of the amount of milk received, the protein content in milk, and the level of the fat content of milk over the past year before taking milk samples for the study were taken into account and their relationship with ODRf was traced. Several covariates were introduced into the model as independent variables because they were economically important factors: mean number of lactating cows, mean number of lactations, mean duration of lactation, mean logarithm [(number of somatic cells/1000)/mL], and ratio miscarriages to the number of calving for the whole year. Since a negative correlation is known between the value of the average annual milk yield and % of its fat content (apparently due to the effect of dilution of milk), milk yield was considered as a covariant in the course of studying the relationship of ODRf with % of milk fat. The effect of simultaneous parasitism in the body of the cow F. hepatica and gastrointestinal nematodes on the amount of milk received was considered an independent factor in the model that evaluates the relationship between ODRf and the volume of milk received and reveals the relationship between ODRf and ODRo. The relationship between the number of ODRf and the time between calving was determined using a model that included covariates in the form of the distribution of calving, the bulk of cows in the herd, the average annual number of productive animals in the herd, and the number of lactations in cows. The adequacy of the models was determined by evaluating the quadratic terms for any independent variable. The normality of the individual considered models was determined using standard plots of residuals and plots of probability compared to the predicted values.

Results and Discussion

The results of the assessment of antibody levels in relation to ODRf and ODRo are shown in **Table 1**. The values of the ODRf content were shifted to the right side, while the distribution of ODRo values showed a normal character **(Figure 1)**. The numbers of ODRf and ODRo were lower in holdings reporting performance data than in herds for which such information was available. The differences found were not high, but statistically significant (ODRf: p<0.001; ODRo: p=0.003). A positive correlation was found between the level of ODRf and the amount of ODRo (R=0.35; p<0.001).

Table 1. General information on the levels of antibodies to F. hepatica (ODRf) and O. ostertagi (ODRo) in milk samples taken from tanks, in October 2021 in the Moscow region of Russia

Characteristics	Minimum	Quantile value	Maximum	25%	50%	75%
ODRf						
Information received from the farm	52	0.248	0.386	0.510	0.931	1.756
Farm data not received	36	0.165	0.437	0.632	1.012	2.117
General level	88	0.165	0.412	0.559	0.976	2.117
ODRo						
Production information received	54	0.063	0.305	0.418	0.508	0.962
Production data not received	31	0.085	0.327	0.436	0.542	1.157
General level	85	0.085	0.315	0.422	0.530	1.157

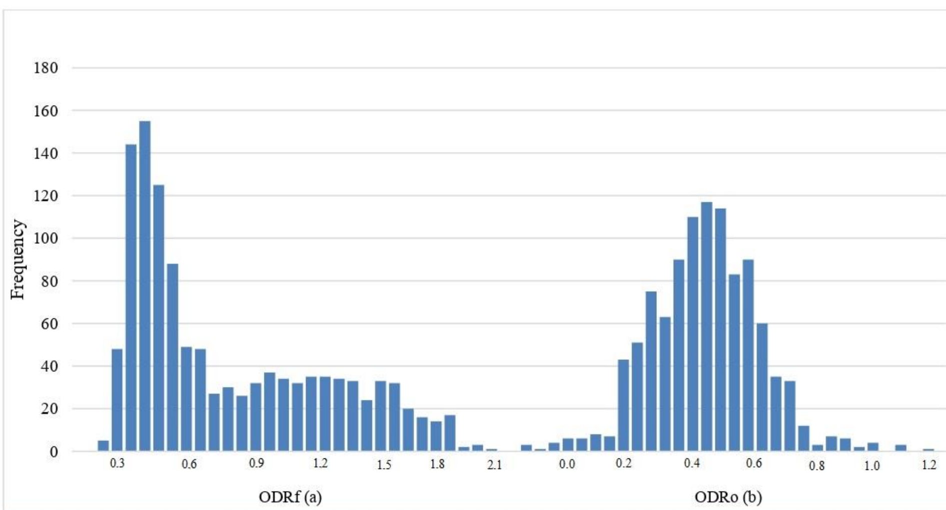

Figure 1. Frequency distribution of levels of antibodies to F. hepatica (ODRf) and to O. ostertagi (ODRo) in milk from reservoirs in the Moscow Region collected in March 2021

The overall performance statistics collected in the year prior to sampling are summarized in **Table 2**.

Table 2. Performance characteristics at the herd level from April 2020 to March 2021

Index	**Minimum**	**quartiles**			Maximum
		25%	**50%**	**75%**	
The volume of milk received, kg / cow day Median ODRf	11.2	21.3	23.6	25.4	32.8

Median ODRf	12.7	18.5	21.1	23.6	30.5
Lactation days Median ODRf	72	183	194	205	293
Median ODRf	155	183	195	210	480
Milk fat % Median ODRf	3.4	4.2	4.4	4.6	5.2
Median ODRf	3.4	4.2	4.4	4.5	5.0
Protein content in milk, % Median ODRf	3.0	3.2	3.3	3.4	3.7
Median ODRf	3.0	3.2	3.3	3.4	3.5
Number of lactation Median ODRf	1.4	2.3	2.5	2.7	4.1
Median ODRf	1.9	2.3	2.5	2.8	3.9
Number of breeding animals Median ODRf	4	31	40	51	123
Median ODRf	15	28	37	49	127
The ratio of miscarriages and annual calving Median ODRf	0.08	0.71	0.78	0.84	0.99
Median ODRf	0.01	0.60	0.76	0.82	0.91

A significant negative relationship was noted between the value of ODRf and the volume of average annual milk produced **(Table 3)**. The increase in ODRf within the interquartile interval was associated with a decrease in the volume of milk supplied during the year by 0.8 kg/(cow day) (p=0.002), which is associated with a decrease in the volume of milk supplied per year by 3%.

It was not possible to find a statistically significant relationship between the level of ODRf and the percentage of protein in milk [slope = 0.013; 95% CI = (0.035; 0.007); p = 0.24]. An increase in ODRf within the interquartile range was associated with a 0.07% drop in milk fat [slope = 0.095; 95% CI = (0.145; 0.046); p<0.001], and when the range between calvings increases by 4.6 days [slope = 7.6; 95% CI = (0.78; 14.3); p=0.04].

When looking for a relationship between ODRf and ODRo on the one hand, and with milk yield on the other hand, the negative relationship for ODRo [slope index = 4.47; 95% CI = (6.41; 2.61); p<0.001]. An unexpressed negative relationship was found for the ODRf level [slope index = 0.64; 95% CI = (1.32; 0.09), p=0.07]. At the same time, there was no significant relationship between the levels of ODRf and ODRo in relation to their influence on the level of milk yield (p=0.69).

Table 3. Linear regression model data to find the relationship between ODRf in milk and average annual milk yield

Variable	*б*	95% CI		*n*- meaning
		LL	UL	
Meaning ODRf	1.11	1.80	0.40	0.003
Average number of cows	0.025	0.007	0.010	0.002
Average number of lactated cows	5.5	0.5	10.6	0.028
Average number of lactations squared	1.2	1.9	0.3	0.018
Average duration of lactation	0.014	0.025	0.005	0.009

The existence of an organism is always associated with many biological interactions (Zavalishina, 2018a). This largely determines the success of the development of the entire ontogeny and the adaptation of the organism to the external environment (Zavalishina, 2020b). A very common interaction in wildlife is parasitism. It has a varying degree of impact on the animal's body, and many of its aspects still need to be clarified.

The milk samples analyzed in the study were taken in October 2021. The activity of the intermediate hosts of fasciola begins in April-May. It is known that antibodies against F. hepatica ES antigens are registered 2-4 weeks after the parasite enters the body (Salimi-Bejestani *et al*., 2005a) and can remain for a long time after its complete removal from the body (Boulard *et al*., 1995; Chauvin *et al*., 1997). In view of the fact that the presence of antibodies to

F. hepatica in body fluids is a consequence of past interactions between the parasite and the organism, it has been suggested that a negative impact on productivity is associated with migratory young flukes (Zavalishina, 2020a). This occurs, as a rule, during the stay of animals on the pasture, and by adult flukes, as a rule, during the winter maintenance. In the work, the available production characteristics of the year preceding the collection of milk samples were used, which made it possible to evaluate the relationship between ODRf and productivity.

The presence of a somewhat distorted distribution of the ODRf level and an approximately normal distribution of the ODRo level was quite expected, in view of the fact that the ODRf distribution includes non-infected and infected groups of animals, while for O. ostertagi the entire population should be considered as infected with it (Agneesssens *et al*., 2000).

ELISA data and farm worker data cannot be obtained from all randomly selected herds. At the same time, the differences in ODR levels between herds, in which the level of milk yield was taken into account or not, turned out to be fully statistically significant. In this regard, the conclusions obtained using models that traced the relationship between production parameters and parasitism cannot be considered representative of the entire sample of animals considered. Although several possible confounding factors have been considered, it should be recognized that the associations found may be partly due to the influence of other factors that can reduce the amount of milk produced, but are caused by parasitism in the body of F. hepatica. At the same time, the conducted study contains an assessment of economic losses traced in many livestock farms, and the patterns found for the simultaneous presence of several parasites will be true for various dairy herds kept in areas endemic to F. hepatica. Also, the obtained information can be applied in studies, when it turns out the cost or economic efficiency of individual measures to control F. hepatica. ELISA on F. hepatica is strictly species-specific, while ELISA on O. ostertagi can also bind to F. hepatica. In this regard, the impact of ODRf on productivity levels was assessed, without considering ODRo in the course of the study. ODRf and ODRo were used simultaneously as part of the model to determine the possibility of a synergistic effect of the presence of concomitant parasites.

There is concern that the presence of F. hepatica in the body of cows reduces the overall level of nutrients in milk (Black and Froyd, 1972). The results obtained in the work show that infection of the body of a cow with liver fluke does not affect the amount of protein present in milk, contributing to a decrease in the concentration of fat molecules in milk. This is in contrast to nematode infestations, which have little effect on milk fat content and milk protein levels (McPherson *et al*., 2001; Nødvedt *et al*., 2002; Charlier *et al*., 2005a).

It was previously noted that the presence of liver fluke in the body of a cow reduces the likelihood of conception and complicates the course of pregnancy (Dargie, 1987). The data obtained by the authors confirm this in view of the fact that farms with a low level of ODRf in cows showed the greatest intervals between calving. In this regard, in the course of assessing the damage from the invasion of F. hepatica, it is rational to take into account the upcoming reproductive pathology.

It was previously noted that the simultaneous invasion of F. hepatica and O. Ostertagi leads to more negative changes than either of them separately. The work performed by the authors allows us to assume the presence of a total, rather than mutually aggravating, the influence of each parasite. This is quite consistent with the data (Dargie, 1987), which did not reveal a relationship between Ostertagia and Fasciola invasions concerning the general condition of the animals or concerning changes in the body weight of the young born.

The negative relationship between the number of ODRf and economically important characteristics of animals makes it possible to determine the "economic threshold" that allows identifying herds with liver fluke invasion, which affects the level of milk secretion. Estimation of the level of antibodies at its cost is quite affordable and should be done to record their number, capable of predicting a positive response to the productivity of animals from interventions against Fasciola infestations. More observations are required to elucidate the dynamics of the level of productivity with the use of fasciolicide among dairy cows with different levels of ODRf and to determine the relationship between the number of ODRf and productive capacity in some dairy cows.

Conclusion

Invasion of the parasite Fasciola hepatica is still common in cattle throughout the world. Even though fasciolosis is often asymptomatic in cattle, it causes a significant decrease in the productivity of cows. In this regard, the broad diagnostics of this parasite in the livestock of cows is very relevant. The presence of F. hepatica in the body is traditionally determined using scatological methods. Nowadays, highly sensitive immunological tests are increasingly being used to detect it. Tests for antibodies to fasciola are well developed and can accurately detect the presence of the parasite. In modern farms, it is important to monitor the frequency of occurrence of this parasite, given that with its prevalence, a minimal decrease in the productivity of a herd of dairy cows up to 25% of their livestock is possible. In the work carried out in October 2021, the level of antibodies to fasciolas Fasciola hepatica (ODRf) and Ostertagia ostertagi (ODRo), which are in the composition of milk, which was obtained from healthy cows in the Moscow region of Russia, was assessed and a relationship was traced with the productive characteristics of dairy cows. The number of antibodies was estimated using multivariate linear regression models, tracing their relationship with protein content in milk, percentage of fat in milk, and intervals between births in cows. An increase in the ODRf level from the 25% quantile (0.412) to the 75% quantile (0.976) in the Moscow region of Russia was associated with a decrease in the amount of milk supplied during the year by 0.8 kg (lactation day) ($p=0.002$), with a decrease in the value the average amount of fat in the composition of milk by 0.07% ($p<0.001$), with an increase in the intervals between sections by 4.6 days ($p=0.04$) and in the absence of a significant relationship with the level of protein in milk. It turned out that the relationship between ODRf and ODRo was complementary rather than synergistic in terms of co-infection.

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