Helminth infections on organic dairy farms in Spain

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Summary

The aim of this study was to assess the prevalence of the major helminth infections affecting organic dairy cattle in northern Spain. Milk and faecal samples were obtained from 443 milking cows. *Ostertagia ostertargi* and *Fasciola hepatica* exposure was assessed by detection of specific antibodies in milk samples and *F. hepatica* infection was diagnosed by the detection of coproantigens in faecal samples. *Dictyocaulus viviparus* and *Calicophoron daubneyi* infections were diagnosed by conventional coprological techniques. The prevalence of infections caused by *F. hepatica* was considerable low, but similar to data reported from conventional farming in the same area. The prevalence rate of *C. daubneyi* infection was higher than previous data mirroring an increase of the prevalence that was also reported in other European countries in recent years. Specific antibodies against *O. ostertargi* were detected in all herds and the median levels of antibodies, determined by ELISA, exceeded thresholds indicating milk production losses. The prevalence of *D. viviparus* was almost negligible. For each parasite, an ordinal logistic-regression analysis was used to assess the risk of infection by taking into account the administration of effective anthelmintics and the number of lactations. Treatment of cows with fasciolicides decreased the risk of *F. hepatica* infection in multiparous cows, whereas treatment with oxiclozanide or albendazol did not decrease the risk of *C. daubneyi* infection or *O. ostertargi* exposure, respectively. The study findings demonstrate that helminth infection in organic dairy farming is similar or even lower than previous data reported from conventional farming. Special attention should be paid to the impact of these infections on milk production.

Key words: Cattle, Grazing, Organic milk production, Helminth infections

Introduction

Helminthoses transmitted by pasture are among the most common causes of detrimental effects on the health and welfare of grazing livestock. Even when subclinical, as usually observed in adult cows, these types of infection may cause significant production losses (Mezo et al., 2011). In Europe, the following species are considered the most pathogenic: the stomach worm *Ostertagia ostertagi* (Forbes et al., 2008), the lungworm *Dictyocaulus viviparus* (González-Warleta et al., 2015) and the liver fluke *Fasciola hepatica* (Mezo et al., 2008).

Control of helminthic parasites in livestock is typically based on the use of parasiticides. However, there are few anthelmintics authorized for the treatment of lactating cows and most of them can only be administered during the dry period. This period does not always coincide with the optimal time for disrupting the life cycle of the various helminth species, and re-infection therefore can occur immediately. The situation is aggravated in organic farming systems, in which livestock is reared outdoors and the use of anthelmintics is legally restricted (Council Regulation, 2007), thus increasing the risk of parasitic infections (Ellis et al., 2011). However, other authors argue that tolerance to parasites may be higher in livestock reared on organically managed farms. Thus, the host-parasite relationship may be modified by exposure of the livestock to parasite challenge from an early age, which may lead to improved resilience (Keatinge et al., 2002). Nevertheless, proper control of parasite infections is essential to achieve the underlying goals of organic milk production (i.e. improving both animal welfare and product quality). Previous knowledge of the infection status of herds is necessary for this purpose. The aim of this study was to assess for the first time the prevalence of the major helminths affecting dairy cattle on organic farming in Spain. Four helminth species were considered: *O. ostertargi, D. viviparus* and *F. hepatica*, regarded as the most prevalent and pathogenic helminths in Europe, and *C. daubneyi*, an increasingly prevalent trematode whose pathogenicity has so far not been assessed.

Material and Methods

The study was conducted on dairy cattle farms in four regions in northern Spain (Galicia, Asturias, Cantabria and the Basque Country). All owners of organic farms registered in the official dairy control (ODC) system in northern Spain were invited to participate in the study, and the farm was included when a positive reply was received. A total of 22 farms, representing 79% of the organic farms included in ODC in northern Spain (n=28), were included in the study. All farms operated a rotational system which lasted throughout the year, the normal practice in grazing farms in this area.

During October – November of 2011, all farms were visited to record data on animal husbandry and to obtain faecal and milk samples. The following data were recorded: number of cows in lactation, lactation number for each cow, feeding system/pasture management and anthelmintic administration for the last two years. Only 27% of the farms have used anthelmintics: oxiclozanide (n=2), triclabendazol (n=1), albedazol (n=2) and nitroxinil (n=1). All treatments were applied during the dry period and two farms used also homeopathy in spring and summer. Milking cows (n=443) were randomly selected (25% of each herd, ranging from 4 to 30 cows per farm using WinEpiscope® software) for faecal (50 g) and milk (20 ml) sampling. All samples were immediately refrigerated and were transported to the laboratory within 4 hours of collection. The milk fat was separated by centrifugation at 2000 x *g* for 10 minutes and the skimmed portion was stored at -20°C until analysis.

The presence of specific antibodies against *O. ostertargi* in milk samples were determined by the commercial test *O. ostertagi*-Ab SVANOVIR® by following the manufacturer's instructions. Results were expressed as ODR (optical density ratio), and cows were considered positive when the value of the negative control provided by the manufacturer was exceeded (ODR>0.4). The detection of *F. hepatica* was performed using two different techniques in order to detect both exposure and active infection. *Fasciola hepatica* antibodies in milk samples were analyzed by MM3-SERO ELISA, an in-house antibody capture immunoassay that detects antibodies against the highly specific antigens recognized by the monoclonal antibody (Mab) MM3 (Mezo et al., 2010). Results were expressed as OD (optical density) values and cows with OD > 0.100 were classified as positive. *F. hepatica* coproantigens were detected by using MM3-COPRO ELISA test (Mezo et al., 2004). Results were expressed as antigen concentration (ng/ml) and samples with concentrations higher than 0.6 ng/ml were classified as positive. For all techniques, positive and negative controls were analyzed in each plate to check the test validity.

For the diagnosis of *D. viviparus* and *C. daubneyi* infections, the faeces (10 g) were analysed using the Baermann and sedimentation techniques. Results were expressed as larvae per gram of faeces (LPG), in the case of *D. viviparus*, and eggs per gram of faeces (EPG), in the case of *C. daubneyi*. The ODR was considered a proxy of exposure whereas antigen concentration, LPG and EPG were considered proxies for intensity of infection.

The percentages of prevalence were expressed as the median value and the interquartile range.

An ordinal logistic-regression analysis was used to evaluate the association between the presence of each parasite infection and the following covariables: number of lactation in course (first or beyond, as an indicator of grazing experience) and treatment (yes or no) with specific drugs against the parasite/s detected. To *F. hepatica*, only data from coproantigen detection were included in the logistic regression analysis. For each parasite, animals were divided based on the median value for positive samples, so that three status were established: negative (0), slightly positive (1) and highly positive (2). Robust estimates of variance were applied to make adjustments within herd and animal cluster effects. In the analyses the following odds were modelled: 1. Status 0 vs. 1 and 2; 2. Status 0 and 1 vs. 2. The test of parallel lines was used to determine the hypothesis of proportionality; that indicates whether the estimated parameters for predictor variables are equivalent across the two comparisons made for the parasite infection. All analyses were conducted using SPSS® (V.20.0) and STATA (V 11.1) for Windows, and differences were considered significant at $p<0.05$.

Results and discussion

In this study, we analyzed faecal and milk samples from organic dairy cows reared on farms in the top milk producing regions in Spain, where helminthoses are endemic (Forbes et al., 2008; González-Warleta et al., 2013). The data on herd prevalence, individual prevalence and within-herd prevalence of each helminth infection and the intensity of each parasite infection or exposure are summarized in Table 1.

Fasciola hepatica

The percentage of herds exposed to *F. hepatica* was 72.7% whereas individual prevalence rate was 40.2%. Median of within-herd prevalence was 46.6% and on a quarter of farms 93.3% of their animals were exposed. The median of anti-*F. hepatica* antibody levels were 1.567, which exceed the threshold indicating potential milk production losses (Mezo et al., 2011). In fact, 58.8% of positive farms presented median of antibody levels higher than the cut value established. When active infection was investigated, the herd prevalence rate was 54.5% whereas only 13.3% of animals studied were infected with *F. hepatica*. In each farm, a median of 4.5% of animals excreted antigens of *F. hepatica* and the median of coproantigen concentrations were 5.36 ng/ml. Both the individual prevalence and coproantigen concentrations reported in the present study were considerably low, but similar to that reported in a previous study in conventional farms from the same area (16% and 6.7 ng/ml respectively; Mezo et al., 2008). However, the high proportion of exposed herds (72.7%) demonstrate that fasciolosis is widespread in organic farms; mainly because of climatic conditions (high humidity, frequent rainfall and mild temperatures) provide a suitable environment for the development of its intermediate host, *Galba truncatula* (Mezo et al., 2008).

Calicophoron daubneyi

The herd prevalence of *C. daubneyi* infection was 59.1% and the individual prevalence of infection was 33.9%. A median of 21.3% of animals on each farm were positive to *C. daubneyi* and on a quarter of farms, all animals were excreting *C. daubneyi* eggs in their faeces. We observed that the individual prevalence rate exceeded previously reported levels for conventionally reared cows from Spain (González-Warleta et al., 2013; Ferreras et al., 2014). Egg counts were 68.0 EPG and 25% of animals excreted more than 173 eggs per gram of faeces. This high faecal egg counts indicates the existence of larger parasite burdens, judging by the observed close correlation between both parameters (González-Warleta et al., 2013; Sargison et al., 2016). Our findings may mirror an increase in the prevalence of this parasite, which is consistent with that observed in different European countries in recent years (Toolan et al., 2015).

Ostertagia ostertagi

Specific antibodies against *O. ostertagi* were detected in all herds. Nevertheless, individual prevalence rate was 75.2% and the within-herd prevalence was 82.1%. As expected, a high exposure to this helminth was detected on all farms given the known ubiquity of this parasite in temperate climates. Similar findings were reported in organic farms from Sweden (Höglund et al., 2010) and conventional grazing farms from Spain (Pablos-Tanarro et al., 2013). The median of ODR levels was 0.675, lower than previous data published in organic farming (Höglund et al., 2010). However, these levels in all farms exceeded the threshold indicating milk production losses (Forbes et al., 2008),

Dictyocaulus viviparus

The herd prevalence of *D. viviparus* was 9.1%. However, the individual prevalence rates (0.7%) and faecal larvae counts (1.4 LPG) were extremely low. In northern Spain, there are no studies about the prevalence of lungworms in cattle, but Ploeger et al. (2012), in a study from Netherlands, found prevalence rates of infection of 11.7 and 18.9% in conventionally reared cows and heifers, respectively. The low prevalence of infection found, may be explained because of adult cows can develop immunity to lungworms after suffering from the infection as calves (Ploeger et al., 2012) so that they do not develop patent infections. However, given the results obtained in other European countries in adult cows, northern Spain seems to be an area with low prevalence of lungworms.

Infection risk assessment

Anthelmintic treatments, lactation number and grazing management are among the most important factor affecting helminth infection. In this study, the effects of using specific anthelmintics and lactation number (first or multiparous) on the risk of infection varied for each helminth species considered (Table 2).

In the case of *F. hepatica* (active infection), none of two factors had a significant effect on the risk of infection when they were analyzed individually. However, there was a significant interaction between both factors indicating that the probability of cows suffering fasciolosis was lower when specific anthelmintics were applied in multiparous cows. Although fasciolicides were successfully used for control of fasciolosis in cattle (Mezo et al., 2008), primiparous cows had less opportunity to be treated than older cows, which could have been treated several times during the different dry periods.

For *C. daubneyi* infection, the probability of a positive diagnosis was 14.1 times higher when specific anthelmintics were used (95% CI: 4.3-30.8; $p<0.001$) and multiparous cows showed slightly more probability of being infected (OR: 1.2; 95%CI: 1.2-1.6; p<0.015). An interaction between both factors was detected, indicating that multiparous cows using anthelmintics had higher probability of being infected compared to primiparous heifers. This paradoxical situation may be explained because of infections occurred predominantly on those farms which also treated their animals with oxiclozanide. Moreover, this drug should only be administered during dry period, which probably is not sufficient to disrupt the life cycle of the trematodes and prevent rapid re-infection (Mezo et al., 2008). On the other hand, older cows are more likely to be infected because of repeated exposure to this parasite does not confer protection against reinfection (Ferreras et al. 2014).

The risk of having a positive diagnose to *O. ostertargi* was 1.5 times higher in cows treated with specific anthelmintics (95% CI: 1.5-1.6; $p<0.001$) and multiparous cows showed lower probability being positive (OR: 0.7; 95% CI: 0.7-0.8; $p<0.001$). However, the risk of being positive was higher in multiparous cows treated with anthelmintics, as the interaction between both terms showed. It should be considered that albendazole (the antinematode used) only can be administered in dry period and the treatment with antinematodes only reduce the *O. ostertagi* antibody levels during the stabling period (Charlier et al., 2007). When cattle remain on pasture throughout the year, as in organic farming, it may occurs continuous reinfections, so that the no (or lower) effect of anthelmintics was expectable. Moreover, regulations controlling organic production (Council Regulation, 2007) restrict the administration of prophylactic treatments, as clearly reflected in less use in this study. Probably, older cows were treated only when clinical signs of disease appeared, which explains the interactions between both terms.

Organic farming systems are frequently associated with an increased risk of helminth infection because the prophylactic use of chemotherapy is prohibited and animals are kept on pasture for prolonged periods of time. Our results showed similar, or even lower, prevalence ratios than previous data reported from conventional and organic farming in the same area or other European countries. Nevertheless, data about helminth prevalence on organic farming is sparse, and most of them were not comparable with our results due to they tested helminth exposure instead of patent infection. An appropriate helminth control is essential in all farming systems, but in organic farming we face with the term integrated control, which implies a rational use of biological, biotechnological and chemical control measures, farming practices and breeding strategies in order to reduce the use of chemical control agents to a minimum. In addition to the classical control methods used in the farms studied (grazing management and anthelmintic treatment), in the longer term, they can be supplemented by biological control or the use of specialized crops which reduce infection levels (Thamsborg et al., 2010). In fact, both pasture management and anthelmintic treatment is not sufficient to prevent the outburst of infections in all situations especially in animals reared in pasture for long periods of time.

In conclusion**,** the study findings clearly demonstrate that helminth infection in organic dairy farming is similar or even lower than previous data reported from conventional farming. Although organic farms were highly exposed to *F. hepatica*, only a small proportion of animals presented active infection. The prevalence of *C. daubneyi* was higher compared with previous data, but consistent with the increasing prevalence observed in other countries in the last years. All farms studied were exposed to *O. ostertagia* although exposure levels were lower than previous data. The prevalence of *D. viviparus* was almost negligible. Special attention should be paid to the impact of helminth infections on milk production.

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References

Charlier, J., Duchateau, L., Claerebout, E., Vercruysse, J., 2007. Predicting milk-production responses after an autumn treatment of pastured dairy herds with eprinomectin. Vet. Parasitol. 143, 197–390.

Council Regulation, 2007. Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. Off. J. Eur. Commisions 834/2007.

Ellis, K. A., Jackson, A., Bexiga, R., Matthews, J., McGoldrick, J., Gilleard, J., Forbes, B., 2011. Use of diagnostic markers to monitor fasciolosis and gastrointestinal nematodes on an organic dairy farm. Vet. Rec. 169, 524-531.

Ferreras, M.C., González-Lanza, C., Pérez, V., Fuertes, M., Benavides, J., Mezo, M., González-Warleta, M., Giráldez, J., Martínez-Ibeas, A.M., Delgado, L., Fernández, M., Manga-González, M.Y., 2014. *Calicophoron daubneyi* (*Paramphistomidae*) in slaughtered cattle in Castilla y León (Spain). Vet. Parasitol. 199, 268–271.

Forbes, A.B., J.Vercruysse, Charlier, J., 2008. A survey of the exposure to *Ostertagia ostertagi* in dairy cow herds in Europe through the measurement of antibodies in milk samples from the bulk tank. Vet. Parasitol. 157, 100–107.

González-Warleta, M., Lladosa, S., Castro-Hermida, J.A., Martínez-Ibeas, A.M., Conesa, D., Muñoz, F., López-Quílez, A., Manga-González, Y., Mezo, M., 2013. Bovine paramphistomosis in Galicia (Spain): Prevalence, intensity, aetiology and geospatial distribution of the infection. Vet. Parasitol. 191, 252–263.

Höglund, J., Dahlström, F., Engström, A., Hessle, A., Jakubek, E.-B., Schnieder, T., Strube, C., Sollenberg, S., 2010. Antibodies to major pasture borne helminth infections in bulk-tank milk samples from organic and nearby conventional dairy herds in south-central Sweden. Vet. Parasitol. 171, 293–9.

Keatinge, R., Jackson, F., Kyriazakis, I., Cork, S.A., 2002. Developing parasite control strategies in organic systems. In: Powell, Jane and et al., (Eds.) Proceedings of the UK Organic Research 2002 Conference, Organic Centre Wales, Institute of Rural Studies, University of Wales Aberystwyth, pp. 341-346. URL: http://orgprints.org/8454

Mezo, M., González-Warleta, M., Carro, C, Ubeira, F.M., 2014. An Ultrasensitive Capture

ELISA for Detection of Fasciola Hepatica Coproantigens in Sheep and Cattle Using a New Monoclonal Antibody (MM3). J Parasitol. 90, 845-852.

Mezo, M., González-Warleta, M., Castro-Hermida, J.A., Muíño, L., Ubeira, F.M., 2010. Field evaluation of the MM3-SERO ELISA for detection of anti-*Fasciola* IgG antibiodies in milk samples for individual cows and bulk milk tanks. Parasitol Int. 59, 610-615.

Mezo, M., González-Warleta, M., Castro-Hermida, J.A., Muiño, L., Ubeira, F.M., 2011. Association between anti-*F. hepatica* antibody levels in milk and production losses in dairy cows. Vet. Parasitol. 180, 237–242.

Mezo, M., González-Warleta, M., Castro-Hermida, J.A., Ubeira, F.M., 2008. Evaluation of the flukicide treatment policy for dairy cattle in Galicia (NW Spain). Vet. Parasitol. 157, 235–243.

Pablos-Tanarro, A, Pérez-Cabal, M.A., Ortega-Mora, L.M., Ferre, I. 2013. Presence of *Ostertagia ostertagi* antibodies in bulk tank milk from cattle herds in northern Spain. Vet. Parasitol. 197, 388-392.

Ploeger, H.W., Verbeek, P.C., Dekkers, C.W.H., Strube, C., Van Engelen, E., Uiterwijk, M., Lam, T.J.G.M., Holzhauer, M., 2012. The value of a bulk-tank milk ELISA and individual serological and faecal examination for diagnosing (sub)clinical *Dictyocaulus viviparus* infection in dairy cows. Vet. Parasitol. 184, 168–179.

Sargison, N., Francis, E., Davison, C., Bronsvoort, B.M. deC., Handel, I., Mazeri, S., 2016. Observations on the biology, epidemiology and economic relevance of rumen flukes (Paramphistomidae) in cattle kept in a temperate environment. Vet. Parasitol. 219, 7–16.

Thamsborg, S.M., Roepstorff, A., Nejsum, P., Mejer, H. 2010. Alternative approaches to control of parasites in livestock: Nordic and Baltic perspectives. Acta. Vet. Scand. 52 (1), 1–27.

Toolan, D.P., Mitchell, G., Searle, K., Sheehan, M., Skuce, P.J., Zadoks, R.N., 2015. Bovine and ovine rumen fluke in Ireland—Prevalence, risk factors and species identity based on passive veterinary surveillance and abattoir findings. Vet. Parasitol. 212, 168–174.

Table 1. Herd prevalence, individual prevalence, within herd prevalence and intensity of infection or exposure of the helminthes studied in 22 organic farms.

Helminth	Herd prevalence	Individual prevalence	Within herd prevalence Median (Q_{25}-Q_{75})	Intensity of infection or exposure Median (Q_{25}-Q_{75})
*Fasciola hepatica Ag**	54.5	13.3	4.5 (0-14.8)	5.36 (2.57-12.43)
*Fasciola hepatica Ab*ˠ	72.7	40.2	46.6 (20.0-93.3)	1.567 (0.597-1.841)
Calicophoron daubneyi†	59.1	33.9	21.3 (0.0-100.0)	68.0 (16.0-173.5)
Ostertagia ostertagi‡	100	75.2	82.1 (53.5-92.0)	0.675 (0.560-0.820)
*Dictyocaulus viviparus*Ᵽ	9.1	0.7	-	1.4 (1.0-2.0)

Intensity of infection
*ng/ml (coproantigens), ˠ OD value (optical density; exposure), †epg (eggs per gram of faeces), ‡ ODR (optical density ratio; exposure), Ᵽlpg (larvae per gram of faeces)

Table 2. Risk factors associated with infection of *Fasciola hepatica*, *Calicophoron daubneyi* and *Ostertagia ostertagi* in 443 cows from 22 farms in northern Spain. Infections were diagnosed by milk antibody-ELISA (*O. ostertagi)* by coproantigen-ELISA (*F. hepatica*) and coprology (*C. daubneyi*).

Parasite	Factor	Class	Infection rate (%)	OR	95% CI	*p*
F. hepatica	Anthelmintic*	No	56/404 (13.9)	Reference		
		Yes	2/36 (5.5)	0.7	0.4-1.2	0.254
	Lactations	Primiparous	16/101 (15.8)	Reference		
		Multiparous	41/339 (12.1)	0.7	0.3-1.8	0.505
	Interaction			0.3	0.1-0.8	0.014
C. daubneyi	Anthelmintic*	No	105/395 (26.6)	Reference		
		Yes	42/42 (100)	14.1	6.5-30.7	<0.001
	Lactations	Primiparous	34/101 (33.7)	Reference		
		Multiparous	113/337(33.5)	1.2	1-1.6	0.015
	Interaction			8.1	3.7-18	<0.001
O. ostertagi	Anthelmintic*	No	300/408 (73.5)	Reference		
		Yes	30/33 (90.9)	1.5	1.5-1.6	<0.001
	Lactations	Primiparous	79/101 (78.2)	Reference		
		Multiparous	251/340 (73.8)	0.7	0.7-0.8	<0.001
	Interaction			1.8	1.8-1.9	<0.001

* For each helmithosis, an animal was considered as treated only if was given drugs of proved effectiveness against the corresponding aetiological agent.
Infection rate (%): number of animals with positive diagnose/total number of examined animals; OR: odds ratio; CI: confidence interval