

Multinomial logistic regression based on neural networks reveals inherent differences among dairy farms depending on the differential exposure to *Fasciola hepatica* and *Ostertagia ostertagi*



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ABSTRACT

Fasciola hepatica and *Ostertagia ostertagi* are cattle parasites with worldwide relevance for economic outcome as well as animal health and welfare. The on-farm exposure of cattle to both parasites is a function of host-associated, intrinsic, as well as environmental and farm-specific, extrinsic, factors. Even though knowledge on the biology of both parasites exists, sophisticated and innovative modelling approaches can help to deepen our understanding of key aspects fostering the exposure of dairy cows to these pathogens. In the present study, multiple multinomial logistic regression models were fitted via neural networks to describe the differences among farms where cattle were not exposed to either *F. hepatica* or *O. ostertagi*, to one parasite, or to both, respectively. Farm-specific production and management characteristics were used as covariates to portray these differences. This elucidated inherent farm characteristics associated with parasite exposure. In both studied regions, pasture access for cows, farm-level milk yield, and lameness prevalence were identified as relevant factors. In region 'South', adherence to organic farming principles was a further covariate of importance. In region 'North', the prevalence of cows with a low body condition score, herd size, hock lesion prevalence, farm-level somatic cell count, and study year appeared to be of relevance. The present study broadens our understanding of the complex epidemiological scenarios that could predict differential farm-level parasite status. The analyses have revealed the importance of awareness of dissimilarities between farms in regard to the differential exposure to *F. hepatica* and *O. ostertagi*. This provides solid evidence that dynamics and relevant factors differ depending on whether or not cows are exposed to *F. hepatica*, *O. ostertagi*, or to both.

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1. Introduction

Parasitic diseases have a tremendous impact on global health and economy (Hotez et al., 2007; Palmieri et al., 2011). In ruminant livestock production, helminth infections are widespread and largely impinge upon production efficiency, animal health, and economic outcome (Vercruyse et al., 2018; Charlier et al., 2020a). *Ostertagia ostertagi* and *Fasciola hepatica* represent the main helminths of concern in cattle (Bloemhoff et al., 2015; Jones et al., 2022; Jonsson et al., 2022). *Fasciola hepatica* has been associated

with estimated costs of € 635 million in Europe alone, attributable to decreased milk production, impaired reproductive performance, reduced growth, increased mortality, as well as due to preventive and therapeutic interventions, and a higher carbon footprint of cattle production (Schweizer et al., 2005; Charlier et al., 2020b; Jonsson et al., 2022). Similar constraints on productivity have been identified for *O. ostertagi* with predominantly subclinical infections interfering with milk yield, weight gain, and fertility (Charlier et al., 2009; Höglund et al., 2010). Per farm per year, Fanke et al. (2017) estimated median costs of € 721.38 for gastrointestinal nematode

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infections. In this very context, the emergence of anthelmintic resistance in both gastrointestinal nematodes (Geurden et al., 2015) and *F. hepatica* (Castro-Hermida et al., 2021) makes the need for a more profound understanding of relevant factors associated with the presence of these pathogens more urgent. A comprehensive knowledge of their epidemiology is one of the key aspects required to effectively design targeted and sustainable intervention strategies and to implement integrated control measures shifting the focus from reliance on anthelmintic compounds towards preventive veterinary medicine and applied epidemiology (Mas-Coma et al., 2019; Howell and Williams, 2020; Navarre, 2020). Mathematical models of infectious diseases have an outstanding potential to comprehend complex epidemiological situations and to estimate relevant key parameters (Grassly and Fraser, 2008; Woolhouse, 2011). Machine learning approaches in particular represent powerful tools to review complex, multi-dimensional data and to discover underlying trends (Goodswen et al., 2021). Cluster analysis is an unsupervised machine learning technique that is able to delineate subgroups in a data set solely based on the presence of discrete differences and to identify inherent patterns without a-priori labelling or classification (Dalmajer et al., 2022). Hence, the algorithm determines structure without human intervention and allows conclusions to be made that otherwise might not have appeared evident (Guha et al., 2003; Botyarov and Miller, 2022). This creates an attractive prerequisite for a deeper understanding of complex infectious disease epidemiology (Goodswen et al., 2021; Ledien et al., 2022; Zafar et al., 2022). Several lines of evidence have indicated that the level of parasitism is substantially dependent on management factors and that dairy farms may differ to a biologically relevant extent when assessing parasite presence (Gutián et al., 1999; Bennema et al., 2009; Villa-Mancera et al., 2018). Predictive models of parasite occurrence on farms enable us to more precisely understand how parasite epidemiology is influenced by local farm-level factors and management characteristics. Based on the hypothesis that inherent differences exist among dairy farms on whether exposure to *F. hepatica* and *O. ostertagi* is present (only *F. hepatica* or *O. ostertagi* or both) or absent on farms (Oehm et al., 2022b), we decided to look deeper into the matter and characterise the differences among these farms based on covariates of farm-level features, management procedures, and animal health indicators. Farms were grouped depending on whether or not cows were exposed to *F. hepatica*, *O. ostertagi*, or both. Subsequently, multiple multinomial logistic regression models were fitted via neural networks in order to understand how the farms differed amongst each other based on parasite exposure and how we can predict different scenarios at a farm level. This is particularly useful, since poly-parasitism in cattle is common and necessitates appropriate diagnostic approaches and decisions for control to be effective (Charlier et al., 2023). Furthermore, even though they may share some common epidemiological elements, *O. ostertagi* and *F. hepatica* differ in some aspects which may translate into different epidemiological scenarios associated with their occurrence. Since simultaneous monitoring of both *O. ostertagi* and *F. hepatica* is possible, knowledge of local epidemiology and specific farm conditions are crucial for sustainable intervention approaches (Charlier et al., 2023). Exploring the interplay of these factors in a setting of poly-parasitism using a mathematical modelling approach, this study contributes to the progress in this area by providing insights into the complex epidemiological settings of parasitic infections in the dairy sector (Bellet et al., 2016; Oehm et al., 2022b, Charlier et al., 2023). As long as the interplay of relevant factors in a setting of co-exposure to *F. hepatica* and *O. ostertagi* remains partly understood, decision algorithms will be less useful in the identification of farm-specific control strategies (Charlier et al., 2015, 2023; Bellet et al., 2018).

2. Materials and methods

2.1. Study population and farm selection

Study population, sample size calculation and farm selection processes are elaborated in Abele et al. (2022) and Oehm et al. (2022a, b). In brief, data on animal husbandry, management procedures, and animal health were collected on German dairy farms in an extensive, cross-sectional study. The potential housing and management practices prevalent in dairy farming were covered by the inclusion of three structurally and geographically separate regions: region 'North': federal states of Lower Saxony and Schleswig-Holstein; region 'East': federal states of Thuringia, Saxony-Anhalt, Brandenburg, and Mecklenburg-Western Pomerania; region 'South': federal state of Bavaria. This led to the assessment of a total amount of 86,304 dairy cows (North: 24,980 cows; East: 49,936 cows; South: 11,388 cows) on 765 farms (North: 253; East: 252; South: 260). Farm selection was based on the estimation of 250 farms to be visited within each of the study regions. Sampling was stratified by administrative district and herd size (number of cows) within the federal states and study regions. Sampling information was obtained from the national animal information data base (HIT) as well as farm data from the Milchprüfing Bayern e.V. and an automated approach was implemented to yield farms that were to be contacted. Farms received a letter providing information about the study as well as an invitation to participate, and were asked to voluntarily make contact with the study team in the respective study region. Farms were visited once between January 2017 and August 2019. Farm visits included retrieval of individual farm data which was obtained with written consent from the farm manager. All personal and farm-specific data was treated according to the German and European data protection legislation.

2.2. On-farm data collection

During the farm visits, data were recorded on paper-based questionnaires and data entry forms. During an interview with the farm manager, farm characteristics (e.g. farming type, pasture access, presence of outdoor exercise pen for cows, feeding of hay to cows) were documented. All cows present on farm on the day of the visit were subjected to individual scoring for lameness (Sprecher et al., 1997; Leach et al., 2009), body condition score (BCS) (Edmonson et al., 1989), leg hygiene (Cook, N.B., Reinemann, D.J., 2007. A tool box for assessing cow, udder and teat hygiene, 46th Annual Meeting of the National Mastitis Council. San Antonio, Texas, USA), udder cleanliness (Cook and Reinemann, 2007), presence of hock lesions (Regula et al., 2004; Kielland et al., 2009), and presence of tail alterations, i.e. deviations, bulges or amputations (Abele et al., 2022). Data on yearly milk yield (in kg), milk fat content (in kg), and milk protein content (in kg) were available at a farm level for the 3 years prior to the farm visit. Data on breed, calving intervals (in days) and parity were available at an individual cow level. Data on somatic cell count (SCC) were retrieved at a cow level for up to 12 months prior to the farm visit.

Production level and cow-related information was retrieved from the national animal information data base and from national milk recording system (DHI).

2.3. ELISA to detect antibodies against *F. hepatica* and *O. ostertagi* in bulk tank milk (BTM) (Springer et al., 2021)

Farm managers were asked to draw a milk sample from the central bulk tank towards the end of the grazing season (August – November) in order to increase comparability across farms. Milk samples were centrifuged (2000g, 15 min) upon arrival at the lab-

oratory and skimmed milk was stored at -20°C until further processing. The commercial IDEXX Fasciolosis Verification test kit (IDEXX GmbH, Germany) was used according to the manufacturer's instructions to detect antibodies against the f2 antigen of *F. hepatica*. Results of a sample/positive (S/P) rate $>30\%$ were interpreted as positive as recommended. Antibodies against *O. ostertagi* were detected via the commercial SVANOVIR® *O. ostertagi* Ab ELISA (Boehringer Ingelheim, Germany) for the assessment of *O. ostertagi* status and possible impact on performance (Forbes et al., 2008). Results, expressed as the optical density ratio (ODR) at 405 nm, were interpreted as indicative for herds likely to suffer from production losses due to ostertagiosis (Forbes et al., 2008; Springer et al., 2021) for ODR values ≥ 0.5 . ELISA results were transformed into a binary variable (*Fasciola/Ostertagia* seropositive/seronegative) for analysis based on the aforementioned thresholds of the ELISAs.

2.4. Data processing

Data collected on farm were manually transferred from the paper-based forms into a central data base which allowed for automated plausibility checks based on a-priori determined threshold values. Plausibility was further checked by three of the co-authors in order to detect potential implausible values during data export or data transcription. Lameness in an individual animal was determined to be a Sprecher score of ≥ 3 (Oehm et al., 2019) in loose housing systems or if ≥ 2 out of the four indices of the Stall Lameness Score were observed during a 90 s period of observation in tie-stall facilities (Leach et al., 2009; Gibbons et al., 2014; Palacio et al., 2017). BCS was categorised into “undercondition”, “optimal condition”, and “overcondition” depending on the stage of lactation and breed as elaborated on in previous work (Abele et al., 2022; Oehm et al., 2022a). Cleanliness of the lower legs and of the udder were dichotomised into “no/slight contamination” (scores 1 and 2) versus “more severe contamination” (scores 3 and 4). As for hock lesions, animals scored 6 (too dirty to assess) were excluded and the variable was dichotomised into “no lesions/hairless patches” versus “more severe alterations” (wound and/or swelling). Alterations of the tail were transformed to “no changes” (score 1) and “visible alterations” (scores 2 and 3). All animal level information was raised to farm level by calculating the prevalence of overconditioned, optimally conditioned, and underconditioned cows, of lameness, dirty legs and udders, hock lesions, and tail changes at the farm level for each farm. A Bayesian, non-parametric bootstrap with 1,000 resamples with replacement was implemented to condense individual animal-level information on parity and calving intervals, and to produce a single Bayesian median reflecting the farm level situation for these variables (Rubin, 1981). This allowed for a close reflection of the individual farm in regard to these variables. As for SCC, cow level data were available for up to 12 months. Hence, the Bayesian bootstrap was applied to obtain a median value reflecting the individual animal in a first step before a second round of bootstrapping raised the yielded information to farm level. Farms were assigned to be of the German Holstein, German Simmental or Brown Swiss breed, if at least 90% of the cows housed at the date of the farm visit belonged to one of the respective breeds. Otherwise, a farm was assigned to the “mixed breed” category.

2.5. Statistical analyses

All analyses were carried out in R Software for Statistical Computing version 4.2.0 (R Core Team, 2022). Throughout the analyses, statistical significance was set at $P \leq 0.05$. Continuous variables were checked for normality, creating quantile–quantile plots. Furthermore, the Shapiro-Wilk test was applied (Shapiro and Wilk,

1965). Regarding farm parasite status (*F. hepatica/O. ostertagia* seropositive/-negative for both or only one) as the categorical target, univariable multinomial models were built for both regions, taking each farm being negative for both parasites as the reference category. These were complemented by the Kruskal-Wallis test to detect differences among farms in regard to the available covariates (Kruskal and Wallis, 1952). Due to the problem of multiple comparisons, P values were adjusted via the Holm-Bonferroni method (Holm, 1979). Categorical variables and their relationship with the different farms were further evaluated using Pearson's Chi-squared test (Pearson, 1900).

2.5.1. Partitioning around medoids clustering

Cluster analyses were performed separately for both study regions. Results from the BTM-ELISAs entered the clustering procedure as binary, categorical variables *Fasciola/Ostertagia* positive/negative. A k-medoids algorithm based on partitioning around medoids was implemented. Silhouette width around 1 determined four to be the optimal number of clusters for both study regions and indicated a distinct structure and very strong evidence for clustering within the data (Sander and Lubbe, 2018; Dalmaijer et al., 2022). Accordingly, an excellent alignment of the observations and their assigned cluster could be anticipated. In contrast to the common k-means algorithm, k-medoids clustering, a non-parametric approach not making assumptions about data distribution is a powerful machine learning technique capable of handling categorical input variables. Representatives of a cluster, i.e. medoids, are identified and all other data points (=farms) are subsequently assigned to a cluster based on their similarity/dissimilarity to the cluster representatives (Kaufman and Rousseeuw, 1990; Chaturvedi et al., 2001).

2.5.2. Multinomial logistic regression to characterise clusters and predict farm level parasite status

Multivariate multinomial log-linear models were fitted via neural networks with farm level parasite status as the target variable. The multinom() function from the R package nnet was applied to predict a multinomial target using the power of neural networks. Hence the multinomial logistic regression is calculated, implementing a neural network algorithm (Venables and Ripley, 2002).

Parasite exposure (only *F. hepatica* or *O. ostertagi*, or both) or no exposure, respectively, was predicted based on a set of covariates not used for clustering. A backward elimination procedure was pursued to obtain the candidate model with the best fit given the data. Starting with a model including all available covariates (i.e. 20 covariates in region North with 15 being continuous and five being categorical, and 22 covariates in region South, of which 15 were continuous and seven categorical) within the data set, the independent variable with the highest P value was removed before the model was run again. Moreover, all predictor variables were added again or removed throughout the model building process in order to further increase model fit. Candidate models were compared using models' Akaike's information criterion (AIC) values (Burnham and Anderson, 2004). The model with the lowest AIC value was regarded as having the best predictive power. Multicollinearity among predictors was assessed by creating variance inflation factors (VIFs). Since the variables milk yield, milk fat, and milk protein appeared to be multicollinear due to VIFs >5 (Hair et al., 2018), the one variable that yielded the best model fit based on the aforementioned criteria was selected to enter the model. A confusion matrix of the final model was produced to visualise predictive accuracy of the multi-class classification, i.e. the percentage of all correctly classified observations. Due to class imbalance present in the data set, the harmonic mean, i.e. the F1 score, was calculated to account for the distribution of the data (Sokolova et al., 2006; Gu et al., 2009; Bekkar et al., 2013).

Throughout the analyses, statistical significance was set at $P \geq 0.05$.

2.6. Data accessibility

The data analysed in this study, a complete list of implemented R packages as well as R script of the analyses including comments are available in Mendeley Data at <https://doi.org/10.17632/gvbt4byrcs.1>.

3. Results

In the current study, only data from study regions North and South were analysed, since *F. hepatica* was present on only two farms in study region East. Parts of the descriptive results (Table 1) were described previously, since the analyses were based on subsets of the same data (Springer et al., 2021; Oehm et al., 2022b).

3.1. Region North

In region North, parasitological data were available for 200 out of 253 farms. After removal of 12 farms which did not participate in DHI, the final data set for analysis consisted of 188 farms housing 17,806 cows. The mean herd size was 69 ± 64 cows. The predominant breed was German Holstein with 183 operations (97.3%) being assigned to this category. The remaining farms were four mixed breed farms (2.1%) and one German Simmental farm (0.5%). Free stall housing was the predominant housing system with 161 farms (85.6%) being in this category. Since pasture-based systems, i.e. systems where pasture was an integral part of animal husbandry with cows spending a substantial amount of time on pasture as opposed to hourly pasture access, were relatively common ($n_{\text{farms}} = 14$, 7.4%), this was created as a category for analysis and tie-stall facilities and others were sorted into the category “other” ($n_{\text{farms}} = 13$; 6.9%). Organic farming principles were adhered to on seven farms (3.7%) and dairy farming was the main source of income on 186 operations (98.9%). Cows had access to pasture grounds and an outdoor exercise pen on 150 farms (79.8%) and 52 farms (27.7%), respectively. Hay was fed on two farms (1.1%) being produced by other ways than ground-

drying or automated ventilation. In the first year of the study, 64 farms (34.0%) were visited compared with 74 farms (39.4%) in the second, and 50 farms (26.6%) in the third year. *Fasciola hepatica* was present on 28 operations (15.0%) and 94 farms (50%) were seropositive for *O. ostertagi*. Table 1 provides a descriptive overview of the continuous variables within the final data set.

The four clusters in study region North incorporated separation based on parasite status (Fig. 1): Cluster 1 ($n_{\text{farms}} = 90$) consists of farms negative for both parasites. Farms only positive for *O. ostertagi* ($n_{\text{farms}} = 70$) were grouped into cluster 2, whereas farms positive only for *F. hepatica* were assigned to cluster 3 ($n_{\text{farms}} = 4$). Cluster 4 contained all farms positive for both parasites ($n_{\text{farms}} = 24$).

Descriptive cluster statistics are displayed in Tables 2 (continuous variables) and 3 (categorical variables). Results of the univariable tests are provided in Supplementary Table S1 and Supplementary Figs. S1–S4.

The final multivariate multinomial model incorporated eight covariates characterising the different groups of farms and predicting farm-level parasite status (Table 4). Farms negative for both parasites were regarded as the reference category. The predictive accuracy of the model was 70.7% and the F_1 score was 82.5%. High odds ratios (ORs) indicate a very strong association with certain farms and a distinct separation among farms. The percentage of underconditioned cows in a herd appeared to be relevant for all farms. Whereas the variable was positively associated with farms positive only for *O. ostertagi*, ($\beta = 0.9$, OR 2.4, $P < 0.001$) and farms positive for both parasites ($\beta = 5.5$, OR 248.9, $P < 0.001$), a higher percentage of underconditioned animals in a herd decreased the odds to group into farms positive only for *F. hepatica* [$\beta = -18.2$, OR $1.2e-8$, $P < 0.001$] versus parasite-free operations. Compared with parasite-negative farms, a higher prevalence of severe hock changes entailed a decreased chance of being positive for *O. ostertagi* ($\beta = -7.3$, OR $6.9e-4$, $P < 0.001$), *F. hepatica* ($\beta = -9.4$, OR $7.9e-5$, $P < 0.001$), or for both parasites ($\beta = -8.8$, OR $1.5e-4$, $P < 0.001$). A similar result was observed for farm-level milk yield: the higher the milk yield, the lower the chance of exposure to *O. ostertagi* ($\beta = -0.0004$, OR 0.9996, $P < 0.001$), *F. hepatica* ($\beta = -0.002$, OR 0.998, $P < 0.001$), or both parasites ($\beta = -0.001$, OR 0.999, $P < 0.001$) on farm. As would be expected, pasture access increased

Table 1

Descriptive overview of all continuous variables from dairy farms in Germany within the data set (study region North: $n_{\text{farms}} = 188$, $n_{\text{cows}} = 17,806$; study region South: $n_{\text{farms}} = 212$, $n_{\text{cows}} = 9,847$).

Variable	Study region North			Study region South		
	Mean \pm S.D.	Median	IQR	Mean \pm S.D.	Median	IQR
Lameness ^a	25.9 \pm 16.1	22.6	21.1	24.7 \pm 14.5	23.1	18.9
Overconditioned ^a	11.0 \pm 12.7	7.7	10.0	17.7 \pm 13.0	15.7	17.2
Optimally conditioned ^a	69.0 \pm 11.4	69.7	10.1	71.5 \pm 12.7	71.7	18.2
Underconditioned ^a	20.0 \pm 10.8	19.9	15.2	10.8 \pm 10.3	8.3	10.3
Leg cleanliness ^a	41.6 \pm 18.7	38.9	30.9	32.4 \pm 18.5	29.8	24.2
Udder cleanliness ^a	22.7 \pm 13.5	18.9	17.5	21.4 \pm 14.2	19.3	19.8
Hock alterations ^a	14.2 \pm 9.4	11.4	12.9	15.9 \pm 12.9	12.5	17.6
Tail changes ^a	11.7 \pm 7.4	10.1	8.0	5.8 \pm 5.2	4.9	6.3
Milk yield ^{b, c}	9,047 \pm 1,224.6	9,170.0	1,736.2	7,538.0 \pm 1,158.5	7,600.0	1,444.5
Milk fat content ^{b, c}	369.9 \pm 45.4	374.7	58.7	312.4 \pm 49.1	316.2	58.8
Milk protein content ^{b, c}	307.5 \pm 42.0	314.9	55.9	263.6 \pm 43.7	267.4	53.8
SCC ^{b, d, e}	218.6 \pm 58.3	211.8	54.0	205.0 \pm 51.0	197.6	64.4
Parity ^b	2.5 \pm 0.3	2.4	0.4	2.5 \pm 0.4	2.5	0.4
Herd size ^f	94.7 \pm 68.8	79.0	64.3	46.5 \pm 29.7	40.5	32.0
Calving interval ^b	413.8 \pm 25.5	409.4	23.0	396.0 \pm 25.3	392.1	35.1

^a Farm level prevalence in %.

^b Bayesian median value per farm.

^c in kg.

^d $\times 1,000$.

^e in number of cells/ml.

^f number of lactating and dry cows.

SCC, somatic cell count; IQR, interquartile range.

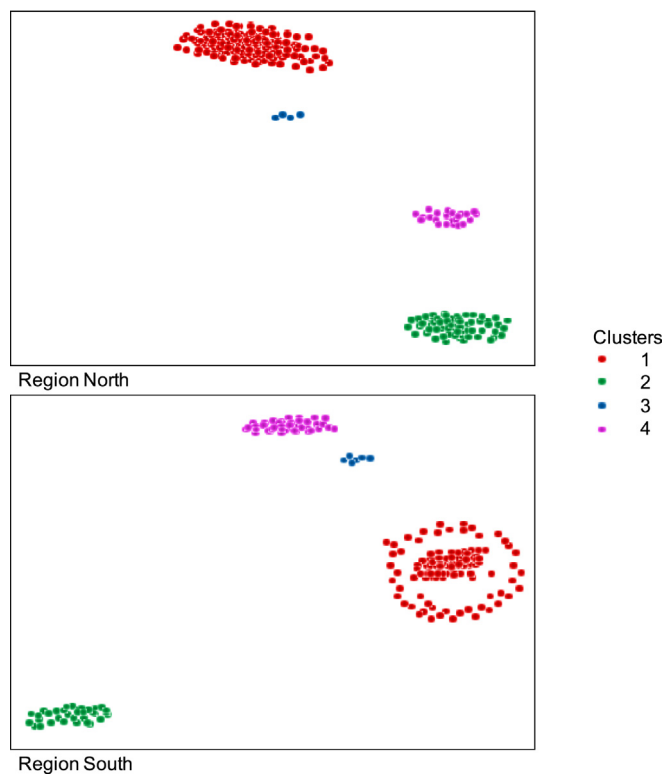


Fig. 1. Cluster plot of the partitioning medoids clustering process. In study region North ($n_{\text{farms}} = 188$, $n_{\text{cows}} = 17,806$) four distinct clusters aggregated naturally with 90 dairy farms in cluster 1 (red, negative for both *Fasciola hepatica* and *Ostertagia ostertagi*), 70 farms in cluster 2 (green, farms only positive for *Ostertagia ostertagi*), four farms in cluster 3 (blue, farms only positive for *Fasciola hepatica*), and 24 farms in cluster 4 (lilac, farms positive for both parasites). Region South ($n_{\text{farms}} = 188$; $n_{\text{cows}} = 9,847$) farms were allocated to four clusters with 128 farms in cluster 1 (red, negative for both *Fasciola hepatica* and *Ostertagia ostertagi*), 34 farms in cluster 2 (green, farms only positive for *Ostertagia ostertagi*), six farms in cluster 3 (blue, farms only positive for *Fasciola hepatica*), and 44 farms in cluster 4 (lilac, farms positive for both parasites).

the chance of exposure to *O. ostertagi* ($\beta = 1.3$, OR 3.6, $P < 0.001$), *F. hepatica* ($\beta = 22.5$; OR $5.8e + 9$, $P < 0.001$), or both ($\beta = 11.6$, OR $1.1e + 5$, $P < 0.001$). Increasing herd size was associated with the presence of *F. hepatica* ($\beta = 0.1$, OR 1.1, $P = 0.003$), yet decreased the chance for *O. ostertagi* ($\beta = -0.01$, OR 0.99, $P = 0.01$). Similarly, a higher lameness prevalence was associated with higher odds for the presence of *F. hepatica* ($\beta = 0.2$, OR 1.2, $P < 0.001$), yet with lower odds for *O. ostertagi* ($\beta = -0.02$, OR 0.98, $P = 0.04$). Compared with parasite-negative farms, a higher SCC increased the chance of exposure to *O. ostertagi* ($\beta = 0.01$, OR 1.01, $P < 0.001$). Study year 2 was associated with positivity for *O. ostertagi* ($\beta = 1.0$, OR 1.01, $P < 0.001$), *F. hepatica* ($\beta = 0.5$, OR 1.7, $P < 0.001$), and both ($\beta = 0.4$, OR 1.6, $P < 0.001$). In study year 3, farms had a higher chance of being positive for *O. ostertagi* ($\beta = 1.6$, OR 4.9, $P < 0.001$) or both parasites ($\beta = 0.8$, OR 2.3, $P < 0.001$), yet lower odds of positivity for *F. hepatica* ($\beta = -0.6$, OR 0.6, $P < 0.001$) compared with study year 1.

3.2. Region South

In study region South, 240 out of the 265 visited farms participated in the parasitological examination of BTM samples. Out of the 240 farms, 28 farms were not enrolled with DHI and hence excluded from further analyses. The final data set in region South included a total of 212 dairy farms housing 9,847 cows with a mean herd size of 47 ± 30 cows. In study year 1, 83 farms

(39.2%) were visited compared with 84 farms (39.6%) in year 2, and 45 farms (21.2%) in study year 3. Thirty-three farms (15.6%) were organic operations and 72 farms (34.0%) provided cows with pasture access, whereas an outdoor exercise pen was available on 50 farms (23.6%). Hay was fed to cows on 44 farms (20.9%) with ground-dried hay representing the principal (65.9%) means of hay production ($n_{\text{farms}} = 29$) followed by a combination of ground-drying and automated ventilation systems ($n_{\text{farms}} = 7$; 15.9%), solely automated ventilation systems ($n_{\text{farms}} = 5$; 11.4%), and other methods ($n_{\text{farms}} = 3$; 6.85). Dairy farming represented a main source of income on 179 farms (84.4%) and a supplementary income on 33 (15.6%) farms. Tie-stall housing was present on 54 operations (25.5%) compared with free stall housing on 158 farms (74.5%). German Simmental was the predominant breed in region South with 164 Simmental farms (77.4%), followed by 25 mixed breed operations (11.8%), 18 Brown Swiss farms (8.5%), and five German Holstein farms (2.4%). *Fasciola hepatica* was present on 50 farms (23.6%) and *O. ostertagi* on 78 farms (36.8%).

The four clusters of farms in region South (Fig. 1) appeared to be distinct in regard to parasite status with cluster 1 ($n_{\text{farms}} = 128$) negative for both *F. hepatica* and *O. ostertagi*, cluster 2 ($n_{\text{farms}} = 34$) positive only for *O. ostertagi*, and cluster 3 ($n_{\text{farms}} = 6$) only positive for *F. hepatica*. Farms in cluster 4 ($n_{\text{farms}} = 44$) were positive for both parasites. Descriptive statistics for all clusters based on the covariates within the data set are compiled in Tables 5 (continuous variables) and 6 (categorical variables). Results of the univariable tests are provided in Supplementary Table S1 and Supplementary Figs. S1–S4.

Table 7 displays the results of the final multivariate multinomial model for study region South. A total number of four covariates entered the model with a prediction accuracy of 76.4% and an F_1 score of 86.2%. Farms negative for both parasites were regarded as the reference category. Separation among farms based on external covariates was distinct, reflected by high ORs. Similar to study region North, pasture access for cows entailed higher odds for the presence of *F. hepatica* ($\beta = 10.1$, OR $2.4e + 4$, $P < 0.001$), *O. ostertagi* ($\beta = 2.3$, OR 9.5, $P < 0.001$), or both ($\beta = 4.6$, OR 97.6, $P < 0.001$). This was also true for organic farming which was associated with *F. hepatica* ($\beta = 2.0$, OR 7.1, $P < 0.001$), *O. ostertagi* ($\beta = 1.5$, OR 4.3, $P < 0.001$), or both ($\beta = 1.7$, OR 5.6, $P < 0.001$). The higher the farm-level prevalence of lameness, the lower the odds for farms to be positive for *F. hepatica* ($\beta = -0.2$, OR 0.9, $P = 0.02$), or both parasites ($\beta = -0.1$, OR 0.9, $P = 0.002$). Increasing milk yield at a farm level was inversely associated with exposure to *O. ostertagi* ($\beta = -0.0002$, OR 0.9998, $P < 0.001$) or both ($\beta = -0.0003$, OR 0.997, $P < 0.001$). Conversely, a higher milk yield increased a farm's risk of being positive for *F. hepatica* ($\beta = 0.0004$, OR 1.0004, $P = 0.001$).

4. Discussion

Parasitic infections in dairy cows represent complex disease settings which not only exert a plethora of effects on the host species but which are also dependent on a variety of both extrinsic and intrinsic factors. The implications of helminth infections for dairy cow health and welfare, as well as for the economic outcome of cattle production, are considerable and parasitic diseases represent major concerns globally (Hotez et al., 2007; Palmieri et al., 2011; Fanke et al., 2017). Due to the epidemiology of bovine helminth parasites such as *F. hepatica* and *O. ostertagi*, sophisticated mathematical approaches are very valuable to enable inference and to enhance the in-depth understanding of risk indicators and key elements linked to differential exposures to these parasites (Grassly and Fraser, 2008; Woolhouse, 2011). Given the situation that cattle often have multiple infections with endoparasites, knowledge of

Table 2

Descriptive results per cluster (representing dairy farms based on farm-level parasite status for *Fasciola hepatica* and *Ostertagia ostertagi*) of continuous variables from German dairy farms in study region North (n_{farms} = 188, n_{cows} = 17,806).

Variable	Parasite-free n _{farms} = 90			Only <i>Ostertagia ostertagi</i> n _{farms} = 70			Only <i>Fasciola hepatica</i> n _{farms} = 4			Both parasites n _{farms} = 24		
	Mean ± S.D.	Median	IQR	Mean ± S.D.	Median	IQR	Mean ± S.D.	Median	IQR	Mean ± S.D.	Median	IQR
Lameness ^a	28.5 ± 14.2	28.2	22.1	22.9 ± 17.4	19.6	18.8	36.6 ± 20.1	37.3	27.3	23.2 ± 17.2	21.3	21.3
Overconditioned ^a	10.9 ± 8.0	10.1	8.9	11.3 ± 14.6	7.1	9.7	35.6 ± 43.7	18.9	34.0	6.2 ± 6.7	4.9	5.0
Optimally conditioned ^a	70.0 ± 8.4	71.0	8.4	69.0 ± 12.7	69.0	10.7	50.2 ± 33.5	66.3	19.9	68.3 ± 9.3	69.3	11.3
Underconditioned ^a	18.8 ± 9.4	16.5	12.0	20.2 ± 11.7	20.3	15.0	14.2 ± 13.3	13.7	17.8	25.6 ± 11.5	26.0	16.0
Leg cleanliness ^a	39.0 ± 16.4	34.8	23.0	41.1 ± 20.3	38.9	34.9	52.5 ± 11.9	51.7	17.1	50.5 ± 20.6	49.4	30.3
Udder cleanliness ^a	21.7 ± 12.6	18.8	16.4	22.6 ± 13.8	18.8	18.2	28.6 ± 11.0	31.0	14.0	25.6 ± 15.9	20.6	25.1
Hock alterations ^a	16.6 ± 10.0	14.7	17.1	12.2 ± 8.5	9.7	10.1	11.7 ± 8.1	9.8	10.6	11.8 ± 8.0	11.0	8.5
Tail changes	12.7 ± 6.8	11.7	8.6	9.9 ± 6.8	8.5	7.7	12.1 ± 2.1	12.4	2.3	12.8 ± 10.6	8.9	6.1
Milk yield ^{b, c}	9,357.0 ± 1,000.0	9,603.0	1,430.0	8,956.0 ± 1,370	9,094.0	1930.0	8,955.0 ± 752.0	8,526.0	817.0	8,234.0 ± 814.0	8,238.0	1,100.0
Milk fat content ^{b, c}	380.7 ± 39.4	385.4	48.9	365.9 ± 52.2	375.5	70.2	354.5 ± 23.1	351.4	18.9	343.7 ± 35.9	357.8	38.8
Milk protein content ^{b, c}	317.7 ± 36.5	327.0	48.6	304.5 ± 48.3	314.9	69.1	295.7 ± 16.9	288.4	13.1	279.4 ± 30.2	278.4	29.1
SCC ^{b, d, e}	210.0 ± 48.6	208.8	49.9	231.9 ± 72.4	217.1	63.9	212.0 ± 30.5	210.5	41.0	213.2 ± 41.8	212.4	34.2
Parity ^b	2.4 ± 0.2	2.4	0.3	2.5 ± 0.4	2.4	0.5	2.5 ± 0.1	2.5	0.1	2.5 ± 0.3	2.4	0.3
Herd size ^f	112.4 ± 84.6	100.5	78.8	71.7 ± 41.4	63.0	41.2	158.2 ± 44.5	157.5	45.3	85.1 ± 42.1	76.0	45.0
Calving interval ^b	411.6 ± 24.3	408.0	26.2	416.2 ± 23.2	410.6	20.5	409.4 ± 18.9	405.4	21.4	416.0 ± 36.0	409.3	16.7

^a Farm level prevalence in %.

^a Bayesian median value per farm.

^c in kg.

^d × 1,000.

^e in number of cells/ml.

^f number of lactating and dry cows.

SCC, somatic cell count; IQR, interquartile range.

Table 3

Descriptive cluster (representing dairy farms based on farm-level parasite status for *Fasciola hepatica* and *Ostertagia ostertagi*) results of categorical variables from German dairy farms in study region North (n_{farms} = 188, n_{cows} = 17,806).

Variable	Category	Parasite-free	Only <i>Ostertagia ostertagi</i>	Only <i>Fasciola hepatica</i>	Both parasites
		n _{farms} = 90 Counts [%]	n _{farms} = 70 Counts [%]	n _{farms} = 4 Counts [%]	n _{farms} = 24 Counts [%]
<i>F. hepatica</i>	Seronegative	90 [100]	70 [100]	0 [0]	0 [0]
	Seropositive	0 [0]	0 [0]	4 [100]	24 [100]
<i>O. ostertagi</i>	Seronegative	90 [100]	0 [0]	4 [100]	0 [0]
	Seropositive	0 [0]	70 [100]	0 [0]	24 [100]
Farming type	Conventional	90 [100]	66 [94.3]	4 [100]	21 [87.5]
	Organic	0 [0]	4 [5.7]	0 [0]	3 [12.5]
Pasture access	Absent	31 [34.4]	7 [10]	0 [0]	0 [0]
	Present	59 [65.6]	63 [90]	4 [100]	24 [100]
Exercise pen	Absent	64 [71.1]	53 [75.7]	2 [50]	17 [70.8]
	Present	26 [28.9]	17 [24.3]	2 [50]	7 [29.3]
Housing type	Free stall housing	85 [94.4]	51 [72.9]	4 [100]	21 [87.5]
	Pasture based system	4 [4.4]	8 [11.4]	0 [0]	1 [4.2]
	Other	1 [1.1]	11 [15.7]	0 [0]	2 [8.3]
Income type	Main income	88 [97.8]	70 [100]	4 [100]	24 [100]
	Supplementary income	2 [2.2]	0 [0]	0 [0]	0 [0]
Breed	Brown Swiss	0 [0]	0 [0]	0 [0]	0 [0]
	German Holstein	89 [98.9]	66 [94.3]	4 [100]	24 [100]
	German Simmental	0 [0]	1 [1.4]	0 [0]	0 [0]
	Mixed	1 [1.1]	3 [4.3]	0 [0]	0 [0]
Study Year	1	36 [40]	19 [27.1]	1 [25]	8 [33.3]
	2	35 [38.9]	28 [40]	2 [50]	9 [37.5]
	3	19 [21.1]	23 [33]	1 [25]	7 [29.3]

aspects describing the differential exposure to *F. hepatica* and *O. ostertagi* is crucial in designing suitable interventions (Charlier et al., 2023). Investigating the interrelationships of relevant factors in poly-parasitism using a powerful machine learning approach contributes to the development in this field, especially since decision algorithms are challenged to determine farm-specific intervention strategies with only partial knowledge of a poly-parasitic epidemiological scenario (Charlier et al., 2015, 2023; Bellet et al., 2018).

In the present study, we were able to demonstrate distinct differences among farms depending on whether or not cows were

exposed to either *F. hepatica* or *O. ostertagi*, or both, on German dairy operations. Our hypothesis that farms are distinct depending on the exposure to *F. hepatica* and/or *O. ostertagi* was confirmed by cluster analysis. Subsequently, in order to elucidate dissimilarities as well as characteristics of the farms, a set of external covariates was evaluated. Multivariate, multinomial logistic regression models were fitted via neural networks to understand the associations of covariates with farms in a multivariate setting and to predict farm level parasite status.

The potential of this study is two-fold: firstly, the k medoids algorithm using PAM autonomously identifies underlying patterns

Table 4

Results of the multiple multinomial regression model based on neural networks to describe clustered dairy farms in region North ($n_{\text{farms}} = 188$, $n_{\text{cows}} = 17,806$). All covariates were assessed regarding their association with the target variable, i.e. farm-level parasite status of German dairy farms in study region North for *Fasciola hepatica* or *Ostertagia ostertagi*. Farms negative for both parasites were considered as the reference category.

Predictor	Category	Only <i>Ostertagia ostertagi</i>			Only <i>Fasciola hepatica</i>			Both parasites		
		Estimate	OR	P	Estimate	OR	P	Estimate	OR	P
Underconditioned ^a	Continuous ^b	0.9	2.4	<0.001	−18.2	1.2e-8	<0.001	5.5	248.9	<0.001
Hocks ^a	Continuous ^b	−7.3	6.9e-4	<0.001	−9.4	7.9e-5	<0.001	−8.8	1.5e-4	<0.001
Pasture access	Present	1.3	3.6	<0.001	22.5	5.8e + 9	<0.001	11.6	1.1e + 5	<0.001
Herd size ^a	Continuous ^b	−0.01	0.99	0.01	0.1	1.1	0.003	0.003	1.0	0.7
Lameness	Continuous ^b	−0.02	0.98	0.04	0.2	1.2	<0.001	−0.01	0.99	0.4
Milk yield ^c	Continuous ^b	−0.0004	0.9996	<0.001	−0.002	0.998	<0.001	−0.001	0.999	<0.001
SCC ^c	Continuous ^b	0.01	1.01	<0.001	0.005	1.0	0.8	0.01	1.0	0.1
Study year	2	1.0	2.6	<0.001	0.5	1.7	<0.001	0.4	1.6	<0.001
	3	1.6	4.9	<0.001	−0.6	0.6	<0.001	0.8	2.3	<0.001

^a Farm level prevalence in %.

^b OR of >1 indicates that a 1-unit increase in the predictor variables increases the odds, whereas an OR of <1 indicates that a 1-unit increase in the predictor variables decreases the odds.

^c Bayesian median value per farm.

SCC, somatic cell count; OR, odds ratio.

Table 5

Descriptive cluster (representing dairy farms based on farm-level parasite status for *Fasciola hepatica* and *Ostertagia ostertagi*) results of continuous covariates from German dairy farms in study region South ($n_{\text{farms}} = 212$, $n_{\text{cows}} = 9,847$).

Variable	Parasite-free $n_{\text{farms}} = 128$			Only <i>Ostertagia ostertagi</i> $n_{\text{farms}} = 34$			Only <i>Fasciola hepatica</i> $n_{\text{farms}} = 6$			Both parasites $n_{\text{farms}} = 44$		
	Mean ± S.D.	Median	IQR	Mean ± S.D.	Median	IQR	Mean ± S.D.	Median	IQR	Mean ± S.D.	Median	IQR
Lameness ^a	28.3 ± 14.5	27.3	14.5	24.1 ± 13.7	22.4	18.0	10.2 ± 6.6	11.3	7.3	16.8 ± 11.1	15.5	15.4
Overconditioned ^a	20.3 ± 13.0	17.9	16.3	14.3 ± 10.8	12.5	13.6	21.3 ± 18.5	17.1	19.4	12.5 ± 12.1	8.6	16.4
Optimally conditioned ^a	69.9 ± 11.7	70.3	18.3	73.0 ± 12.2	75.9	16.2	73.4 ± 19.8	76.8	26.8	74.9 ± 14.4	75.2	20.2
Underconditioned ^a	9.9 ± 8.5	7.7	9.0	12.7 ± 12.9	9.9	9.3	5.1 ± 5.1	4.6	6.0	12.7 ± 12.8	9.2	12.5
Leg cleanliness ^a	30.3 ± 15.7	27.8	21.2	39.1 ± 22.0	36.9	29.3	44.9 ± 33.3	53.0	51.5	31.7 ± 19.4	29.8	25.1
Udder cleanliness ^a	20.9 ± 13.1	18.9	17.8	22.0 ± 16.8	19.9	18.7	28.4 ± 18.6	33.6	26.7	21.4 ± 14.6	19.9	21.7
Hock alterations ^a	17.6 ± 12.7	14.9	16.9	15.7 ± 15.0	12.7	15.4	7.4 ± 5.5	5.5	9.0	12.1 ± 11.2	8.7	16.1
Tail changes ^a	6.3 ± 5.6	5.3	5.9	6.4 ± 4.9	4.5	5.5	6.4 ± 3.7	7.3	4.3	3.7 ± 4.2	2.8	6.2
Milk yield ^{b, c}	7,759 ± 1,000	7,785	1,260	7,323.0 ± 1,330.0	7,421.0	2,380	7,781.0 ± 491	7,576	347	7,029.0 ± 1,330	6,910	1,480.0
Milk fat content ^{b, c}	324.3 ± 41.2	324.9	49.0	302.0 ± 53.4	306.0	86.9	331.6 ± 26.6	324.7	9.34	283.2 ± 55.8	282.1	58.1
Milk protein content ^{b, c}	272.3 ± 36.8	275.5	47.4	256.4 ± 51.5	256.0	90.9	281.4 ± 14.5	278.7	9.5	241.3 ± 49.9	236.7	52.4
SCC ^{b, d, e}	205.3 ± 52.5	193.1	73.4	197.2 ± 197.0	194.3	24.3	186.9 ± 40.2	195.5	40.4	212.7 ± 53.3	206.8	52.3
Parity ^b	2.5 ± 0.3	2.4	0.4	2.5 ± 0.3	2.5	0.4	2.7 ± 0.3	2.7	0.3	2.7 ± 0.4	2.7	0.5
Herd size ^f	51.2 ± 30.8	45.5	31.0	50.9 ± 33.8	44.0	31.5	28.7 ± 15.4	26.5	16.0	31.7 ± 16.1	27.5	20.5
Calving interval ^b	393.8 ± 23.6	390.4	30.7	394.6 ± 27.9	387.0	24.3	410.0 ± 13.6	410.1	6.0	401.6 ± 28.3	398.2	4.3

^a Farm level prevalence in %.

^b Bayesian median value per farm.

^c in kg.

^d × 1,000.

^e in number of cells/ml.

^f number of lactating and dry cows.

SCC, somatic cell count; IQR, interquartile range.

depending on the differential exposure to the two parasites. This was carried out without supervision and a priori labelling of farms. Hence, we demonstrate that not only do biologically and epidemiologically relevant differences exist between parasite-negative and -positive farms, but also among farms that are negative, single-positive or double-positive. This is of particular relevance, since studies assessing poly-infections have been rare (Bellet et al., 2016). A high accuracy of the multinomial predictions corroborates the reliability of our results as well as of the inference drawn. Secondly, this study included a large number of animals and farms in two geographically and structurally different dairy regions in Germany. This is especially interesting given the fact that the relevance of covariates varies across study regions and even the association of the same factor can be divergent based on the study environment. This study has underscored the need to stratify data collection and analysis in order to identify relevant patterns and to understand complex disease settings. Moreover, differential results in regard to covariates may complement each other and contribute to our overall understanding of disease epidemiology.

In study region North, a higher farm-level milk yield was associated with lower odds for farms to be positive for *F. hepatica*, *O. ostertagi*, or both. Yet, in region South, higher milk yield increased the odds for *F. hepatica* positivity. The finding that both parasites negatively affect milk production levels in dairy cows concurs with previous results. Schweizer et al. (2005) showed that annual losses in milk production represent the main source of economic damage due to bovine fasciolosis. Milk losses have been estimated to be approximately 0.5 kg/cow/day (Villa-Mancera et al., 2018) for *O. ostertagi* and 0.5–1.6 per parasitised cow in cases of *F. hepatica* (Villa-Mancera and Reynoso-Palomar, 2019; May et al., 2020). It is plausible to assume that co-exposure to both parasites exerts an additive detrimental effect on productivity. Poly-infections with *F. hepatica*, *Ostertagia* spp. and rumen flukes adversely impacted cold carcass weight in cattle at slaughter (Bellet et al., 2016). Liver flukes and gastrointestinal nematodes may interfere with host performance through different mechanisms (Loyacano et al., 2002; Bellet et al., 2016). The true nature of these effects on host performance still requires elucidation and might be part of an explana-

Table 6

Descriptive cluster (representing dairy farms based on farm-level parasite status for *Fasciola hepatica* and *Ostertagia ostertagi*) results of categorical variables from German dairy farms in study region South (n_{farms} = 212, n_{cows} = 9,847).

Variable	Category	Parasite-free	Only <i>Ostertagia ostertagi</i>	Only <i>Fasciola hepatica</i>	Both parasites
		n _{farms} = 128 Counts [%]	n _{farms} = 34 Counts [%]	n _{farms} = 6 Counts [%]	n _{farms} = 44 Counts [%]
<i>F. hepatica</i>	Seronegative	128 [100.0%]	34 [100.0%]	0 [0.0%]	0 [0.0%]
	Seropositive	0 [0.0%]	0 [0.0%]	6 [100.0%]	44 [100.0%]
<i>O. ostertagi</i>	Seronegative	128 [100.0%]	0 [0.0%]	[100.0%]	0 [0.0%]
	Seropositive	0 [0.0%]	34 [100.0%]	0 [0.0%]	44 [100.0%]
Farming type	Conventional	124 [96.9]	26 [76.5]	4 [66.6]	25 [56.8]
	Organic	4 [3.1]	8 [23.5]	2 [33.3]	19 [43.2]
Pasture access	Absent	118 [92.2]	18 [52.9]	0 [0.0]	4 [9.1]
	Present	10 [7.8]	16 [47.1]	6 [100]	40 [90.9]
Exercise pen	Absent	105 [82.0]	23 [67.6]	4 [66.6]	30 [68.2]
	Present	23 [18.0]	11 [32.4]	2 [33.3]	14 [31.8]
Housing type	Tied housing	27 [21.1]	6 [17.6]	1 [16.7]	20 [45.5]
	Free stall housing	101 [78.9]	28 [82.4]	5 [83.3]	24 [54.5]
Income type	Main income	116 [90.6]	27 [79.4]	4 [66.7]	32 [72.7]
	Supplementary income	12 [9.4]	7 [20.6]	2 [33.3]	12 [27.3]
Breed	Brown Swiss	3 [2.3]	2 [5.9]	5 [83.3]	8 [18.2]
	German Holstein	3 [2.3]	2 [5.9]	0 [0]	0 [0.0]
	German Simmental	110 [85.9]	23 [67.6]	0 [0]	31 [70.5]
	Mixed	12 [9.4]	7 [20.6]	1 [16.7]	5 [11.4]
Study Year	1	48 [37.5]	15 [44.1]	4 [66.7]	16 [36.4]
	2	50 [39.1]	14 [41.2]	1 [16.7]	19 [43.2]
	3	30 [23.4]	5 [14.7]	1 [16.7]	9 [20.5]

Table 7

Results of the multiple multinomial regression model based on neural networks to describe clustered dairy farms in region South (n_{farms} = 212, n_{cows} = 9,847). All covariates were assessed regarding their association with the target variable, i.e. farm-level parasite status of German dairy farms in study region South for *Fasciola hepatica* or *Ostertagia ostertagi*. Farms negative for both parasites were considered as the reference category.

Predictor	Category	Only <i>Ostertagia ostertagi</i>			Only <i>Fasciola hepatica</i>			Both parasites		
		Estimate	OR	P	Estimate	OR	P	Estimate	OR	P
Pasture access	Present	2.3	9.5	<0.001	10.1	2.4e+4	<0.001	4.6	97.6	<0.001
Milk yield ^c	Continuous ^b	-0.0002	0.9998	<0.001	0.0004	1.0004	0.001	-0.0003	0.997	<0.001
Lameness ^a	Continuous ^b	-0.02	0.98	0.1	-0.2	0.9	0.02	-0.1	0.9	0.002
Farming type	Organic	1.5	4.3	<0.001	2.0	7.1	<0.001	1.7	5.6	<0.001

^a Farm level prevalence in %.

^b OR of >1 indicates that a 1-unit increase in the predictor variables increases the odds, whereas an OR of <1 indicates that a 1-unit increase in the predictor variables decreases the odds.

^c Bayesian median value per farm OR, odds ratio.

tion why the outcome for *Fasciola* positivity was different in study region South. Furthermore, it should be taken into consideration that this outcome might be an artefact from modelling. Regardless, this particular aspect necessitates further investigation.

As expected, pasture access for cows was a key factor characterising farms with single or dual exposure to *F. hepatica* and *O. ostertagi* in both study regions. Considering the epidemiology of liver flukes and gastrointestinal nematodes, transmission requires the ingestion of infective stages via fresh or cut grass, or ground-dried hay (Enigk et al., 1964; Enigk and Hildebrand, 1964; Knubben-Schweizer et al., 2010). Organic farming principles were associated with exposure to *F. hepatica*, *O. ostertagi*, or both in region South. A fundamental concept of organic farming is the maintenance of animal health and welfare via proactive rather than reactive methods (Rutherford et al., 2009). This includes a distinct focus on animal welfare, less intensive production, smaller herds, strict regulations for feed provenance and usage of feed, as well as restrictions for the use of therapeutic drugs (Sato et al., 2005; Pol and Ruegg, 2007; Rutherford et al., 2009). Moreover, the organic mindset is inseparably associated with pasturing dairy cows which as aforementioned creates an optimal environment for *F. hepatica* and *O. ostertagi* to prosper.

In study year 2, farms had a higher chance to be positive for *F. hepatica*, *O. ostertagi*, or both in region North compared with study year 1. This was similar for study year 3 with the exception of decreased odds for *F. hepatica* positivity. The impact of meteorolog-

ical determinants on the transmission risk and spatial presence of bovine internal parasites has been well-known in epidemiology (Ollershaw, 1966; Villa-Mancera et al., 2018; Roessler et al., 2022). Interestingly, the chance of being *F. hepatica*-positive appeared to be reduced in study year 3. The summer of study year 2 was exceptionally hot, dry, and sunny, characterised by local arid conditions and drought (Deutscher Wetterdienst, 2018. Erste Bilanz des Deutschen Wetterdienstes zum Jahr 2018 in Deutschland, Offenbach, Germany. Last accessed 18th March, 2023. https://www.dwd.de/DE/presse/pressemitteilungen/DE/2018/20181220_jahr2018_rekor_news.html;jsessionid=2D01A3C0A203924A32B278F1A84ED0DE.live21062; Drouard et al., 2019). This was pronounced in Northern Germany and could have translated into the survival of intermediate hosts of *F. hepatica* which are dependent on humid conditions and wet areas (Roessler et al., 2022). The habitats of potential intermediate hosts may have dried out during this year, which then resulted in an impact on *F. hepatica* transmission in the following year, i.e. study year 3. Since *F. hepatica* uses an intermediate host to complete its life cycle, it might be more affected by the meteorologically induced unavailability of gastropods due to extreme climatic conditions compared with *O. ostertagi* which does not rely on intermediate hosts and whose larval stages on pasture are very resistant to harsh environmental conditions (Charlier et al., 2020a).

The indirect life cycle may also explain why increasing herd size was associated with higher odds of being *F. hepatica*-positive, but

lower odds for *O. ostertagi* positivity. Farms with more cows may be more intensively managed and access to pasture or fresh grass may be limited or absent. *Fasciola hepatica* metacercariae can also be transmitted via hay or silage, especially via ground-dried hay or in cases when a proper silaging process is not taking place (Enigk et al., 1964; Persson, 1974a, 1974b; John et al., 2019). Yet, whereas metacercariae of *F. hepatica* are reliably killed during the process of hay production in cases where automated ventilation systems ensure temperatures above 30 °C are used, ground-dried hay could represent a possible infection source for fasciolosis, but not for infective stages of *O. ostertagi* which perish quickly also in ground-dried hay (Enigk et al., 1964; Enigk and Hildebrand, 1964). Since ground-dried hay is the main means of hay production in region South, the outcome of the present study points towards a potential role of hay in *Fasciola* transmission and underscores the regional differences regarding transmission routes and parasite epidemiology. Due to the limited number of farms feeding hay, this hypothesis could not be followed up any further and is an aspect that is yet to be investigated.

An increasing prevalence of hock lesions in a herd entailed lower odds of being parasite-positive in region North. This association is probably mediated by other variables such as pasture access which has been shown to be very beneficial in regard to the presence of hock lesions, since cows on pasture are able to move more freely and are less intensely exposed to potentially harmful effects of the housing environment (Rutherford et al., 2008; Potterton et al., 2011). Similarly, the reduced chance for poly-exposure or presence of *F. hepatica* in region South and decreased odds for *O. ostertagi* positivity in region North may be explained by increasing lameness prevalence in the herd. Lameness dynamics in a herd are beneficially influenced by pasture access (Rutherford et al., 2009; Oehm et al., 2022a). Hence, lameness prevalence may likely act as a proxy, indicating farms with a lower number of lame cows offer beneficial housing or management conditions that may translate into a higher chance of parasite positivity. Conversely, a higher lameness prevalence in region North was associated with *F. hepatica* positivity. Considering that higher lameness prevalence may be reflecting a housing environment where pasture access is absent, it appears counterintuitive that this may result in a higher chance for *F. hepatica* positivity. As discussed earlier, *F. hepatica* metacercariae can remain infective in hay and silage. Thus, even on farms without pasture access, exposure to the parasite could occur. When interpreting this result, it is important to be aware of the fact that four farms in region North were solely positive for *F. hepatica*. Even though univariate analyses support the outcome of the multivariate model of an increased lameness prevalence in *F. hepatica*-positive herds, and despite the good predictive performance of the multiple multinomial model, the outcome might be attributable to the model. In this regard, more thorough investigation in similar settings to this study region should aim at disentangling the underlying relationships with a larger number of farms only positive for *F. hepatica*.

A similar artefact from the model may arise when looking at the result for the covariate undercondition. Previous work has demonstrated that parasitic infections are associated with loss of body condition and decreased weight gain in cattle (Kagenda and Angwech, 2018). Therefore, the increased odds for exposure to *O. ostertagi* and both *O. ostertagi* and *F. hepatica* seem intuitive. On the contrary, a higher percentage of underconditioned cows were associated with a lower chance of *F. hepatica* positivity which is in sharp contrast to the extant literature indicating a negative impact of this parasite on body condition (Schweizer et al., 2005; da Costa et al., 2019). This could, as previously hypothesised (Loyacano et al., 2002; Bellet et al., 2016), be the result of varying mechanisms exerted on host performance by flukes and gastrointestinal nematodes. Moreover, a higher prevalence of undercondi-

tioned cows in a herd may suggest that the animals are very intensively managed without pasture access, but simultaneously with exposure to *F. hepatica* via conserved feed as outlined above. However, we must be aware that this result could be spurious as a consequence of only a low number of farms positive for *F. hepatica* only. Consequently, further work specifically addressing this topic is required.

A higher SCC was associated with exposure to *O. ostertagi*. This could be attributable to technical features of the ELISA. Charlier et al. (2006) have stated that mastitis can cause an increase in *O. ostertagi* ODR values as a result of both specific and unspecific serum antibodies entering the milk. Furthermore, it is well known that intestinal helminths are able to induce a potent immune response (Claerebout and Vercruysse, 2000; Grecnis, 2015; Cortés et al., 2017). This may be associated with an overall increased immunological alertness of the host organism which then translates into a higher SCC which is mainly composed of leukocytes (Schwarz et al., 2011). Moreover, since SCC has been routinely used as an indicator of udder health of a herd (Barkema et al., 1999), an overall increased farm-level SCC may also indicate a setting of sub-optimal housing and management which translates into compromised animal health and promotes the presence of parasitic infections. Claerebout and Vercruysse (2000) reviewed the antibody response to gastrointestinal helminths being a function of antigenic stimulation. Considering that worm burdens in *F. hepatica* infection are remarkably lower and often below 100 specimens per host (Radfar et al., 2015; Garcia-Campos et al., 2019) compared with several thousand specimens of gastrointestinal nematodes (Rehbein et al., 2013; Teixeira et al., 2021), the latter may evoke a more pronounced immune response in the udder and hence cause a higher SCC.

In a cross-sectional study such as the present one, we can only detect associations and not conclude causal relationships among variables (Setia, 2016). Moreover, observer bias may enter during the data collection process since predictors and outcomes are simultaneously recorded. A precise and rigorous study protocol including standard operating procedures was developed prior to the onset of data collection in order to combat this issue. Moreover, observers were jointly trained prior to, and three times during, the data collection period. Therefore we are optimistic of a minimal introduction of observer bias, to a level that is negligible.

Farmers' participation was voluntary. This might have entailed a certain degree of selection bias, especially given the fact that the response rate was low initially and a second drawing of samples had to be conducted. One might argue that proactive, extroverted farmers are more inclined to participate. Furthermore, these farm managers may be more interested in optimising animal health and management on their farms, which could have translated into an overly positive animal health situation on their operations compared with the entirety of the sampled population. On the other hand, the invitation to participate may specifically have reached out to farmers with health problems on their dairies who requested external consultation. We cannot rule out sampling bias in regard to one of the aforementioned scenarios, but are convinced that it was minor, due to the randomisation process and since the reported prevalences and outcomes are in alignment with previous work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpara.2023.05.006>.

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