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Paper

Relationship between gastrointestinal nematode burden, blood parameters and hair cortisol in Italian local sheep not treated with anthelmintics

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Abstract

This study explored the interplay between gastrointestinal nematode burden, blood parameters, and hair cortisol in Zerasca sheep, a local breed of Northeastern Tuscany (Italy). Moreover, the correlation between BCS and Eggs per Gram (EPG) was analyzed to verify the possible parasite impact. The study involved twelve ewes sampled for faeces, blood and wool three times, in the morning, from August 2022 to March 2023. Complete blood count, alanina-aminotransferase, aspartate-aminotransferase, nitrogen ureic, beta-hydroxybutyric acid, non-esterified fatty acids, total proteins, creatinine, calcium, chloride, potassium, and oxygen free radicals were analyzed. Fresh faecal samples were collected and subjected to the Mac Master technique for EPG. Wool was collected to quantify hair cortisol. BCS was recorded using a 1-5 scale. The ANOVA test was performed to detect differences in metabolic and immunological parameters, as well as hair cortisol levels, in relation to EPG levels. Non parametric correlation between EPG and BCS was conducted. Only red blood cells, haematocrit, mean corpuscular haemoglobin concentration, and calcium were significantly associated with EPG levels. Some parameters were outside the normal range. The correlation between BCS and EPG resulted negative and statistically significant. These results showed an overall well-being of the tested animals within this specific context

Keywords

Sheep, Zerasca, Gastrointestinal parasite, Blood parameters

Introduction

In Italy, sheep farming is essentially based upon extensive systems characterized by several stressors such as inadequate housing conditions, improper procedures, treatments, vaccinations, shearing, hoof care, predators, and adverse weather conditions (Tüfekci & Sejian, 2023). In this type of farming system, nutrition is mainly provided by pastures and the grazing activity can introduce a further stressor due to the presence of gastrointestinal parasites (Fell et al., 1991). In fact, some parasites such as gastrointestinal nematodes complete their biological cycle in grass, becoming a threat to the health and welfare of sheep (Sotiraki et al., 2013), with potential negative consequences on many aspects such as production and reproduction (Dwyer & Bornett, 2004). Chemical anthelmintic drugs are widely used for gastrointestinal nematode (GIN) infections. However, their overuse without strategic control has led to resistance. To mitigate this occurrence, the effective parasite burden should be evaluated.

The repercussions of a high parasitic burden are multifaced, encompassing anemia, protein deficiency, and macroelement imbalances (Sotiraki et al., 2013). Indeed, hematochemical screenings are important tools for assessing the physiological and health status of farm animals, aiding the identification of possible imbalances (Rathwa et al., 2017). Moreover, biochemical investigation is useful for checking metabolic syndromes (Radkowska & Herbut, 2014) while minerals such as phosphorus, sodium, calcium, and potassium are essential for productive performances, with imbalances being common in ruminants grazing pastures (Masters, 2018). The immune status serves as an indicator of susceptibility to developing diseases conditioned by stressful events, and oxidative stress may contribute to pathological conditions, often linked to parasite infections (Bernabucci et al., 2005; Lykkesfeldt & Svendsen, 2007).

Endoparasites may have the capacity to induce chronic stress and pain (Goddard et al., 2010). Evaluating chronic stress necessitates a multidisciplinary approach, considering parameters such as physiological, immunological, and productive ones (Dwyer & Bornett, 2004).

Hair analysis proved to be a valid indicator of sheep welfare through the retrospective assessment of long-term HPA activity (Fürtbauer et al., 2019); in addition, being a non-invasive technique, it can avoid biases in results due to blood collection (Weavera et al., 2021).

The aim of this study was to investigate the relationship between gastrointestinal nematode (GIN) burden, blood parameters and hair cortisol as indicators of health status in a farm of Zerasca sheep.

Materials and Methods

The study was conducted in compliance with European Commission regulations and received favourable opinion from the Ethics Committee of the University of Pisa (Italy) with decision N.31/2022.

The survey was carried out from August 2022 to March 2023 on a farm housing Zerasca sheep, a breed listed as endangered (FAO, 2019). The farm is located in the Zeri district (Massa Carrara, Italy) at an altitude of 900 meters above sea level (44°19' N, 9°47' E).

The examined flock comprised around 60 heads. In the farm, animals were raised following an extensive management system, allowing the animals to open pastures and grasslands for almost the entire year. The animals had access to the pasture during the good weather and came back to the shelter for the night. The primary source of nourishment for the sheep was the pasture, consisting of grass, shrubs, and bushes. Additionally, daily supplementation with hay and corn was provided.

The farm refrained from administering treatments against gastrointestinal parasites to the flock for the last ten years. Instead, the farm implemented ongoing monitoring, supervised by a veterinarian, and adopted sustainable strategies such as rotational grazing and hygiene of shelter. In instances of alterations in egg per gram (EPG) levels, the veterinarian determined whether individual treatment was necessary or if further analysis should be conducted.

Twelve multiparous ewes were chosen through a random selection for the study involving the collection of faeces, blood, and wool. Three sampling were performed in the morning in August 2022, November 2022, and March 2023. The veterinarian assessed the overall health status of the ewes during the sampling process.

Faecal samples were collected directly from the rectal ampulla of each selected ewe and examined using the concentration McMaster technique (Permin, and Hansen, 1988) to estimate the Faecal Egg Count (FEC) of gastrointestinal strongyles corresponding to Eggs per Gram (EPG). EPG were divided in three groups following the scheme of Ambrosi (1995).

Blood samples were taken from the jugular vein by a veterinarian using Vacutainer® test tubes and kept in a bag with dry ice (+5 °C) until the arrival at the laboratory.

The complete blood count was measured by the Veterinary Clinical Pathology at the Department of Veterinary Sciences (University of Pisa, Italy) using a CELL-DYN 3500® automated haematology analyser (Abbott, Minneapolis, USA). The following parameters were analysed: red blood cells (RBC), hematocrit (HCT), haemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), reticulocytes (RETIC), white blood cell count (WBC), neutrophils (NEU), lymphocytes (LYM), monocytes (MON), eosinophils (EOS), basophils (BAS), blood platelets (PLT).

A portion of serum was frozen and sent to the "Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana (IZSLT)" for the analysis of the following metabolic and immunological parameters: alanina-aminotransferase (ALT), aspartate-aminotransferase (AST), nitrogen ureic (BUN), beta-hydroxybutyric acid (BHBA), non-esterified fatty acids (NEFA), total proteins (TP), creatinine (Creat), calcium (Ca), chloride (Cl), and potassium (K) were measured by an automatic biochemical analyser (Olympus AU 400, San Diego, USA). Oxygen free radicals (OFR) were determined by a commercial colorimetric method (Diacron International S.r.l.).

Wool samples were collected at the top of the shoulders between the scapular bones, as close as possible to the skin using scissors. The wool samples were stored in aluminium foil and labelled envelopes at room temperature until the analysis was performed by the "Etovet" laboratory at the Department of Veterinary Sciences (University of Pisa, Italy). Wool samples were processed following the procedure reported by Mariti et al. (2020) for the evaluation of hair cortisol utilizing an Elisa method.

Moreover, BCS was recorded using the scale of Russel et al. (1969) which goes from one (emaciated) to five (obese).

ANOVA test was analysed to check the variability of blood parameters and hair cortisol in relation to EPG levels (level

1 = 0-300; level 2 = 301-600; level 3 = >600 EPG) (Ambrosi, 1995).

The correlation between BCS and EPG level was performed by a non-parametric test (Spearman's Rho).

JMP statistical software was employed (SAS, 2002).

Results

The overall mean FEC value was 589 EPG, which closely aligns with findings from previous studies conducted on Zerasca sheep in the same farm (533 EPG) (Benvenuti et al., 2011). In more detail, only a fair percentage (22%) fell within the level 3 requiring health attention of animals, but the majority (53%) fell within the level 1 assuring the absence ok risks for the health and the productions (Ambrosi, 1998).

The RBC and HCT levels were significantly influenced by EPG level ($p<0.05$) and fell below normal ranges, particularly in correspondence with the highest EPG levels. A negative and significant correlation between EPG and BCS was recorded ($p=0.0051$, $r=-0.4551$). Several parameters, including RBC, HGB, PLT, Ca, and Creat consistently fell below the reference range in each EPG level, in contrast, ALT consistently exceeded the range, indicating problems unrelated to EPG levels (Tab. I).

The statistical model did not show hair cortisol dependence ($p=0.0839$) on the EPG level, suggesting a lack of chronic stress in the inspected sheep.

	EPG levels								
	1		2		3				
Parameter	Mean	SE	Mean	SE	Mean	SE	P	UM	Reference Range*
RBC	8.84↓	0.306	8.10↓	0.446	7.58↓	0.472	0.0492	M/µl	9.49-15.12
HCT	27.35	1.010	24.99↓	1.467	24.49↓	1.556	0.0494	%	27.0-42.0
HGB	9.33↓	0.315	8.74↓	0.457	8.24↓	0.485	0.1634	g/dl	10.0-14.9
MCV	31.04	0.783	30.64	0.952	32.37	0.963	0.3237	Fl	24.4-32.5
MCH	10.57	0.195	10.87	0.237	10.91	0.240	0.4021	Pg	8.5-11.8
MCHC	34.20	0.516	35.36	0.627	33.62	0.635	0.0152	g/dl	32.3-42.0
RETIC	4.03	1.935	8.37	2.812	5.25	2.982	0.1651	K/µl	0-15.0
WBC	8.94	0.502	7.71	0.729	8.15	0.773	0.6391	K/µl	5.06-14.12
NEU	2.26	0.254	2.09	0.369	2.82	0.392	0.3953	K/µl	1.17-6.11
LYM	4.24	0.256	3.82	0.372	3.88	0.395	0.8104	K/µl	2.54-9.60
MON	1.17↑	0.097	0.99	0.141	0.85	0.150	0.6103	K/µl	0.10-1.01
EOS	1.19↑	0.130	0.70	0.189	0.48	0.201	0.1128	K/µl	0.05-0.95
BAS	0.09	0.020	0.10	0.029	0.12	0.030	0.8770	K/µl	0-0.12
PLT	192.31↓	29.300	222.67↓	43.589	260.12↓	46.233	0.6111	K/µl	301-922
ALT	21.17↑	1.422	21.11↑	2.011	23.12↑	2.133	0.6947	(U/l)	<18
AST	85.50	3.360	88.78	4.752	84.87	5.040	0.8811	(U/l)	40-128
BUN	14.33	1.369	18.00	1.936	14.25	2.053	0.3537	mg/dl	8.0-20.0
BHBA	5.26↓	0.675	5.05↓	0.954	7.32	1.012	0.2661	mg/dl	5.7-20
NEFA	275.83	66.151	132.40	93.552	158.87	99.227	0.5469	µmol/l	102-450
TP	7.29	0.158	7.02	0.223	6.87	0.237	0.2667	g/dl	6-7.90
Creat	0.82↓	0.023	0.77↓	0.032	0.80↓	0.034	0.5384	mg/dl	1.2-1.9
Ca	9.37↓	0.172	9.30↓	0.244	9.60↓	0.259	0.0041	mg/dl	11.5-13
Cl	104.50	0.819	108.44	1.158	108.37	1.229	0.1579	mmol/l	90-110
K	5.08	0.162	4.80	0.229	5.07	0.244	0.6976	mmol/l	4.8-7
OFR	47.67	4.192	50.33	5.929	53.00	6.289	0.9016	**U.CARR	44-88
Hair cortisol	21.9	1.850	19.09	2.616	25.72	2.775	0.0839	pg/mg	

Table I. Metabolic, immunological and hair cortisol related to EPG levels. * Reference ranges were provided by the laboratories that conducted the analyses.

Discussion

The EPG level distribution, as well as mean EPG value, were aligned with a previous study carried out in the same area (Benvenuti et al., 2006; Benvenuti et al., 2011).

The negative correlation between EPG and BCS revealed that BCS can be considered as an indirect indicator of the level of infection. Utilizing targeted selective treatments, i.e. treating only those animals exhibiting signs of high infection, such as low BCS and high FaMaCha (Kenyon & Jackson, 2012), is a possible approach to reduce anthelmintic treatment where not necessary. Specifically, a BCS≤2 is considered the optimal criterion for identifying ewes with FEC≥750 EPG (Soto-Barrientos, 2018).

In the present study, HCT emerged as an indicator influenced by EPG levels and remained below the threshold in animals with FEC exceeding 300 EPG, highlighting a negative effect of high parasite levels, in accordance with the study by Theodoropoulos et al. (2000). Other authors observed a decrease in RBC, HCT, and TP in animals with high gastrointestinal parasite infections (>600) (Baihaqi et al., 2020). The same authors reported similar data compared to our results for HCT values (26.8 and 24.6% for low and highly infested animals, respectively). However, Baihaqi et al., (2020) did not align with our results for TP, which remained within the normal range in the three groups, as well as HGB, which was below normal in the three groups.

Some authors (Bello and Tsado) suggested that dietary proteins of not high quality lead to haemoglobin values below the normal range. This can happen in extensive farming systems where pasture composition can be inconsistent, a common situation in the area of the present study. Furthermore, Oramari et al. (2014) reported that breed has a significant effect on HGB and HCT. They noted that Karadi sheep had higher values of HGB and HCT than Awassi and Naimy sheep. This condition has never been described for Zerasca sheep.

The RBC, a parameter known to be affected by parasite infections (Ngetich et al., 2019), exhibited lower values with high infection levels, supporting the results of Kozat et al. (2006) and Ebrahim (2018). A similar trend, though not significant, was observed for HGB, in line with Ngetich et al. (2019). Both parameters consistently remained below the normal ranges, suggesting factors beyond parasite burden occurred (Theodoropoulos, 2000).

Contrary to expectations, EOS were above the threshold in the group characterized by low infection level. This condition did not confirm the literature that indicates an increase of EOS (Theodoropoulos, 2000) in case of high infection; however, the recorded value is only slightly over the reference range. Eosinophilia is mentioned as consequence of *H. contortus* infection together with anaemia and hypoproteinaemia (Paddock, 2011). The present study showed the presence of a slight anaemia but not hypoproteinaemia.

The level of Ca was significantly affected by the EPG level, and remained below the reference range. Hypocalcaemia is most seen in the late stage of the gestation period and in early lactation (Calin et al., 2020), but the sheep included in the trial were not in that physiological condition, consequently, we can hypothesize that hypocalcemia could be explained by nutrient deficiency (Vijay et al., 2010; Ebrahim, 2018).

Stressful conditions can lead to excessive production of OFR. In the present study, OFR remained within the reference range, avoiding harmful effects on the cellular activity (Nazifi et al., 2010) pointing out that the presence of parasites did not represent a stressor for the animals or adaptive mechanisms can have limited excessive OFR in case of stress. Anyway, the good management practices could neutralize eventual stressors. Effective protective factors or antioxidant mechanisms may have neutralized excessive OFR production.

Interesting appeared the results about hair cortisol that, as indicator of sheep welfare through the retrospective assessment of long-term HPA activity, did not significantly varied in any group.

Unfortunately, the level of hair cortisol in sheep is not unanimously quantified, probably due to the many variables associated with the values: body location, sex, age, adiposity, fiber length, and color (Burnard et al., 2016). The same Author stated that hair cortisol in livestock was comparable to that derived from dogs and cats (2.1-206 pg/mg) and wildlife such as bears and wolves (0.6-53.3 pg/mg). Our data fall within this range, varying from 19.09 to 25.72 pg/mg.

The lack of statistical differences of wool cortisol between the three groups could suggest the ability of Zerasca sheep to tolerate the consequences of high GIN burden.

Conclusions

The study revealed only a few significant changes, particularly in RBC, HCT, and Ca levels associated with the EPG level. These minimal and marginal alterations, coupled with the lack of variations in immunological parameters and hair cortisol levels, suggest the inherent phenotypic resistance of Zerasca sheep to the investigated gastrointestinal nematodes (GIN).

This characteristic profile highlights a balance between host and parasite, as evidenced by the slight fluctuations in blood parameters in relation to the parasite burden. The absence of noticeable effects on health indicators suggests that this breed shows traits of resilience and overall good welfare within this specific context.

Further investigations on hair cortisol levels should be carried out in sheep to enable comparisons within this species.

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Institutional Review Board Statement

The study was conducted in compliance with European Commission regulations and received favourable opinion from the Ethics Committee of the University of Pisa (Italy) with decision N.31/2022.

Informed Consent Statement

Written informed consent has been obtained from the owner of the animals.

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Conflicts of Interest

The authors declare no conflicts of interest.

Author contribution

The authors contributed equally

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