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Contact: tel.: +421 915 984 669
e-mail: folia.veterinaria@uvlf.sk

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HORMONAL LEVELS AND FOLLICULAR DYNAMICS IN RELATION TO THE OESTROUS CYCLE IN BARB AND ARABIAN MARES, ALGERIA

Houssou, H.[1], Bouzebda-Afri, F.[1], Bouzebda, Z.[1], Haddouche, Z.[2]

[1]Laboratory of Animal Productions, Biotechnologies and Health
Institute Agronomic and Veterinary Sciences, Souk-Ahras University
[2]Reproductive manager at National Haras of Chaouchoua Tiaret
Algeria

houssouhind@yahoo.fr

ABSTRACT

This current study is an effort to understand the hormonal and follicular growth in the Barb and Arabian mares during the oestrous cycle; as mares are unique creatures. A total of 53 mares with 97 oestrous cycles were studied. The mares with a mean age of 10.38 ± 4.55 were examined by ultrasonography every day during their breeding season (2017). Two blood samples from each mare (n = 24) were obtained for progesterone (P4), oestrogen (oestradiol-17 beta) and follicle-stimulating hormone (FSH) determinations. The data revealed that the duration of the oestrous cycle was between 19 to 22 days. The pre-ovulatory follicle grew (3.02 ± 1.04) millimetre per day. The rate of cycles exploited in the mare (Arabian versus Barb) for conception was significantly different (P < 0.001). The maximal diameter of the follicle was 50.00 millimetre. The serum progesterone levels (P < 0.01) in mares were significantly higher in the luteal phase than those recorded during the time of oestrous. However, the levels of oestradiol and for FSH did not significantly change during the oestrous cycle in the mares. Determining the association between the size of the follicle and the hormone profiles were the most reliable criterion in the prediction of ovulation.

Key words: Algeria; hormone; mare; oestrous cycle; ultrasonography

INTRODUCTION

For the successful breeding of horses, an understanding of the physiological changes in the reproductive status of mares is critical. The most important factors influencing the breeding performance of horses are generally: the stallion, the mare, the genetic variability and its relation to the semen characteristics, and the management practices of the animal husbandry [3, 19].

Breeding records exist in many forms (foaling rates, pregnancy rates per cycle, or per season rates), but there is still a need to develop tests that can predict fertility with a reasonable degree of certainty [18]. With such knowledge of the physiological status of mares, horse breeders can

better manage the breeding and husbandry of the animals [39].

Follicular dynamics is defined as the process of continual growth and regression of antral follicles [6, 33]. It is very important to understand the reproductive cycles of a mare. Mares are exceptional due to their capacity to have a considerable follicular growth during the oestrous and can even arrive during the luteal phase. Occasionally, an antral follicle reaches the pre-ovulatory size and ovulates during the dioestrus without any sign of heat, so the determination of the presence of a large follicle on an ovary is a poor predictor of oestrous in the mare [7].

Since the initial report of Palmer and Driancourt [27], ultrasonography has proven to be a very useful method for visualizing the reproductive tract of the mare [20], enabling the early diagnosis of pregnancy and the timing of ovulation and the study of follicular dynamics and embryo characteristics [37, 38]. Ultrasonography is also complementary to traditional methods used for the diagnosis of the female reproductive status, such as hormonal assays [1, 11, 30, 36]. The mare has been considered a relevant comparative research model for follicle studies because of a striking similarity with human females concerning their follicle dynamics and hormonal changes during the inter-ovulatory interval [14].

Several hormones have been demonstrated as potential factors that control the follicle growth and subordination. During the breeding season, the non-pregnant mare will have recurring oestrous cycles. The oestrous cycle is defined as the period from one ovulation to a subsequent ovulation, with each ovulation being accompanied by signs of oestrous and plasma progesterone concentrations below 1 $ng.ml^{-1}$ [12, 25]. The determination of the moment of the oestrous cycle during which the ovulatory follicle was recruited became an object of a debate. Follicles tend to grow by waves, at the rate of one or two waves by cycle [10, 15].

Mares are unique creatures. Mares show a different pattern of oestrous cycle and ovulation events when compared to other species. Therefore, understanding the physiological conditions and reproductive events of mares are necessary to improve their pregnancy rates [39]. The horse conceptus is unique in that it does not make stable contact with the uterine epithelium until 40—42 days after ovulation [4, 21].

The aim of this study was to monitor the follicular changes and predict the ovulation and determine the degree of correlation between follicular diameters measured before ovulation by ultrasonography and the serum concentrations of the three main hormones implicated in the oestrous cycle of mares. We describe then the strategies to estimate the moment of the ovulation during the oestrous cycle.

MATERIALS AND METHODS

The study area

This study took place at the National Haras of Tiaret which was created in 1877 on a surface of 800 ha. The province of Tiaret is characterized as a continental climate with harsh winters, and hot and dry summers. Currently, the National stud farm of Tiaret serves as the main supplier of horses while maintaining the model and the original type.

Data collection

Animals studied

This study was conducted during the breeding season between February and April 2017. Fifty-three mares with 97 oestrous cycles, were studied from Barb mares (n = 17) with 32 oestrous cycles and Arabian mares (n = 36), with 65 oestrous cycles. The mares were all of known fertility with no uterine pathology. The age of these females varied between 4 and 20 years, and their body condition ranged from 4 to 6 on a scale of 9 according to Henneke et al. [17].

Ultrasonography

The same operator performed the trans-rectal palpation and ultrasound examinations of the reproductive system, always, with portable ultrasound equipment using a 5 to 7 MHz multi-frequency linear probe (DRAMINSKI). The ultrasound examinations were performed daily or twice-daily on follicles ≥ 15 mm, described with details by Lemma et al. [23]. The mares were judged in oestrous if they presented: a doughy uterus, uterine folds, a dominant follicle ≥ 30 mm in diameter, and a soft cervix [28].

Hormonal analysis

Blood samples were obtained by jugular venepunctures using dry vacutainer tubes to collect two samples (n = 24) (over eight-day intervals during April 2017) with each ul-

trasound examination. Thimonier [35] reported that an interval (7 and 11 days) between the samples allowed the characterizing of the physiological state of all the females in the domestic species. The dosage of oestrogens (oestradiol-17 beta), of follicle-stimulating hormone (FSH) and the progesterone (P4) were determined through use of a solid-phase radioimmunoassay (RIA) using a commercial diagnostic kit from Immunotech®, in the laboratory of doctor Bellil (El-Khroub, Constantine).

Statistical analysis

The data collected were subjected to various statistical methods using SPSS 20 and expressed as the mean ± standard error (SD) min and max. Oestrous and luteal data were analysed for period effects using repeated measure analysis of variance (ANOVA), followed by the Student Newman–Keuls multiple and significance were set at $P < 0.05$ and $P < 0.01$. The Student Newman–Keuls multiple comparison test was also used to study the effect of age on follicular growth and hormone profiles. The Pearson's correlation coefficients were performed to assess the association between the parameters studied.

Table 1. Descriptive statistics of the reproductive cycle of mares during oestrous

	Mean	SD	Min	Max
Age	10.38	4.55	4.00	20.00
Oestrous cycle	20.43	0.85	19	22
Follicular growth	3.02	1.04	2	4
Number of cycles	1.84	0.93	1	5

Ethical statement

All the experiments conducted for this study were done only after obtaining the ethical approval from of National Haras of Chaouchoua Tiaret.

The authors declare that there is no conflict of interest.

RESULTS

The mean value and the standard deviation for the diameters of the follicles during the oestrous cycle, the follicular growth as well as the number of cycles exploited for conception are presented in Table 1 for all mares, both Arabian and Barb. The ultrasound examinations revealed that the duration of the oestrous cycle in Arabian mares were from 19 to 22 days. On the other hand, the mean diameters of the follicles during the oestrous cycle was 20.43 ± 0.85 millimetre with a daily growth of 2 to 4 millimetres (Fig. 1.).

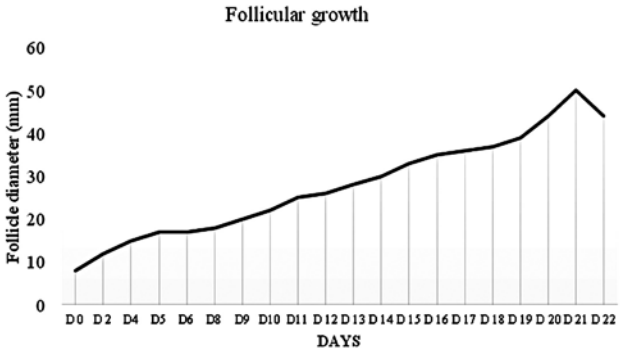

Fig. 1. Follicular dynamics of mare (aged 09 years).

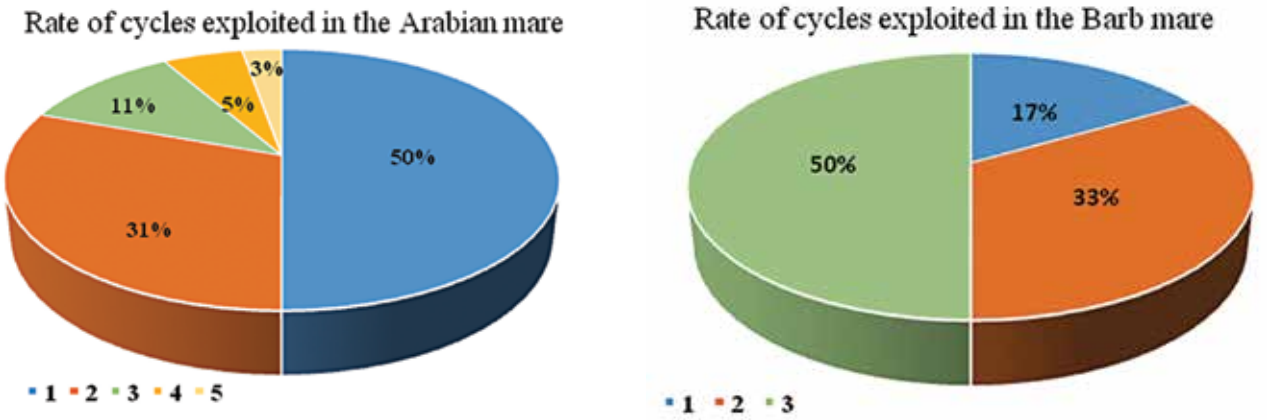

Fig. 2. Rate of cycles exploited in the mare (Arabian versus Barb) for conception ($P < 0.001$)

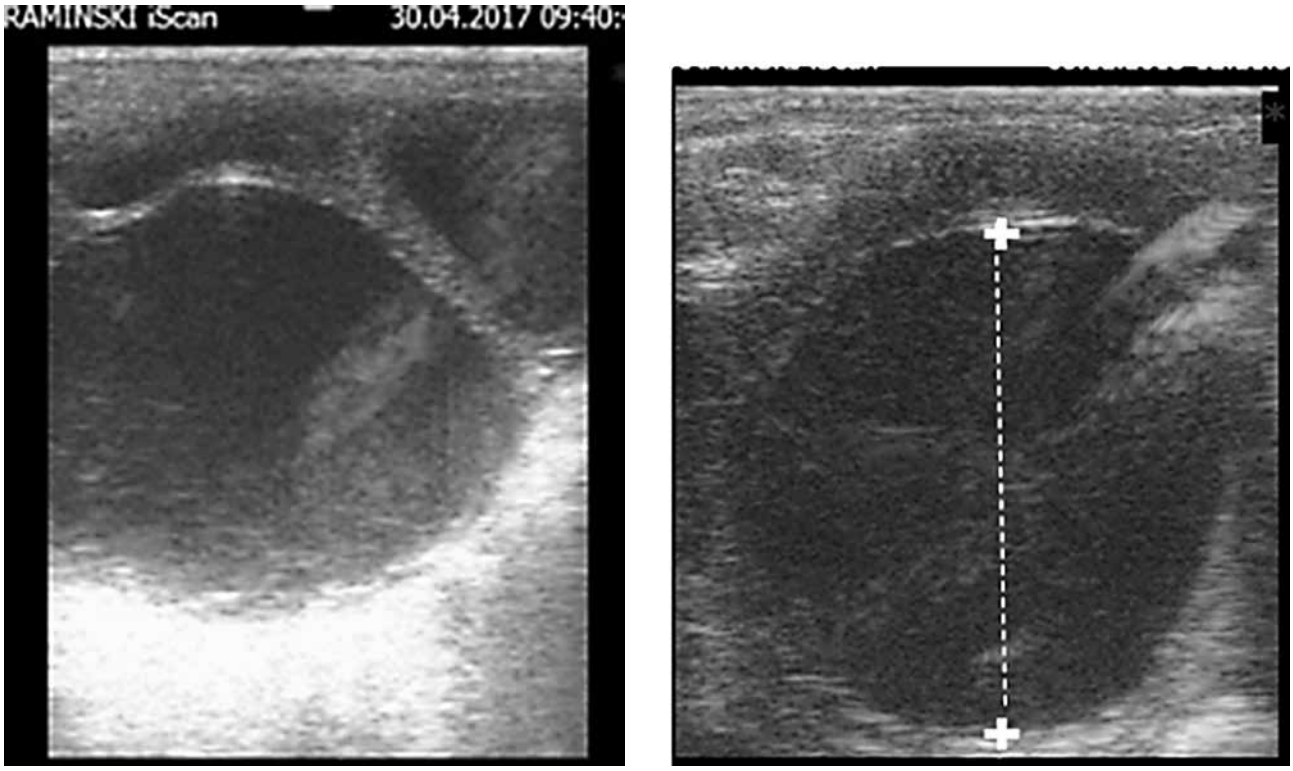

Fig. 3. The ultrasonographic echo of the dominant follicle

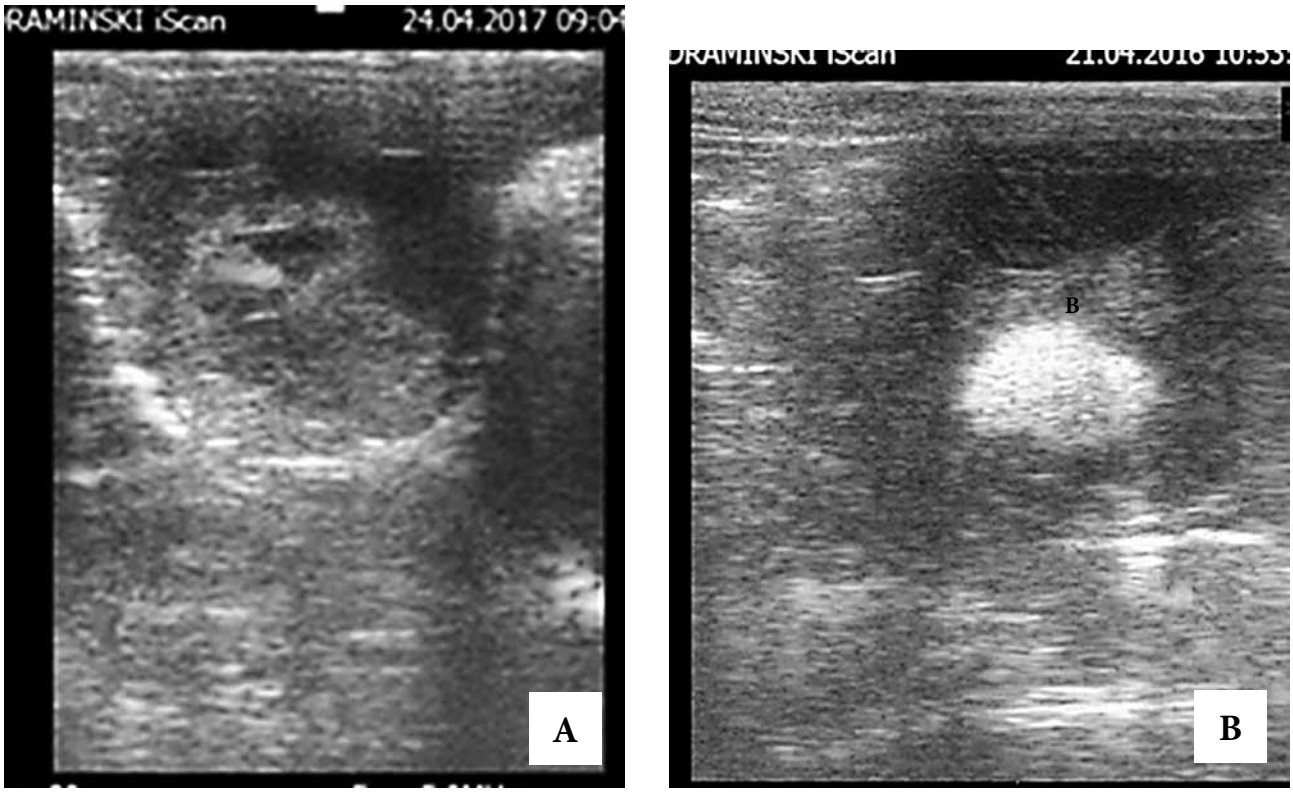

Fig. 4. Ultrasonographic images of corpus haemorrhage: recent ovulation (A); *corpus luteum* (B)

Table 2. Descriptive statistics of mares according the physiological stage

Variable	Physiological stage	Mean	SD	Min	Max	P value
Follicular diameter [mm]	E	31.47	14.54	15.00	50.00	0.036*
	D	27.00	14.94	15.00	47.00	
P4 [ng.ml^{-1}]	E	0.34	0.22	0.24	0.77	0.002**
	D	10.52	7.05	4.92	28.44	
Oestradiol [pmol.l^{-1}]	E	5.49	4.26	4.00	16.01	0.186
	D	7.36	2.45	4.00	9.00	
FSH [UI.L]	E	0.31	0.12	0.23	0.41	0.248
	D	0.29	0.21	0.21	0.37	

*—Correlation significant at P < 0.05; **—significant at P < 0.01; E—oestrous; D—dioestrus

Table 3. Coefficient of correlation among different parameter of mares during the oestrous

r	Age	Follicular diameter	P4 [ng.ml^{-1}]	Oestradiol [pmol.l^{-1}]	FSH [UI.l^{-1}]
Age	1				
Follicular diameter [mm]	–0.202	1			
P4 [ng.ml^{-1}]	0.396	0.49	1		
Oestradiol [pmol.l^{-1}]	0.139	0.06	–0.45	1	
FSH [UI.l^{-1}]	–0.230	–0.93**	–0.64	0.16	1

**—Correlation significant at P < 0.01

The number of oestrous cycles exploited for conception in the Arabian mares were five (50 % in the first cycle) versus three cycles exploited by the Barb mare (17 % in the first cycle), with a significant difference (P < 0.001) (Fig. 2.).

The descriptive statistics of the reproductive cycle of mares during oestrous is presented in Table 1.

The Figures 3 and 4 show respectively the ultrasonography of the dominant follicles and the *corpus luteum*.

The ultrasound examination demonstrated a significant difference in the growth of follicles between the mare in oestrous (15 to 50 mm) and dioestrus (15 to 47 mm) (P < 0.05) (Table 2, Fig. 3).

The serum progesterone levels (P4) in the mare, and follicular growth were significantly higher respectively (P < 0.01; P < 0.05) in the luteal phase of the oestrous cycle, then those recorded at the time of oestrous. However, the serum concentration of estradiol-17 beta and the FSH level did not significantly differ between oestrous and luteal phases of the mare oestrous cycle (P > 0.05) (Table 2).

The table 2 reveals the descriptive study of the hormonal and follicular parameters.

Table 3 shows a significant correlation between the level of FSH and the diameter of the follicular growth ($r = -0.93$; $P < 0.01$) during the oestrous cycle of mares. No difference was observed on the correlation among these parameters: follicular diameter, oestradiol-17 beta and P4.

DISCUSSION

This study demonstrated that the duration of the oestrous cycle of the mares were (19 to 22 days), with a follicular size between 15 and 50 mm. That was similar to the results reported by several other investigators [16, 24, 37, 39].

Warriach et al. [38] reported that the conception rate of the Arabian was 62 % in the first cycle mated in Pakistan; our results of a conception rate are different in the Arabian of 50 % and 17 % in the Barb. The higher conception rate in the Arabian mares may be due to a genetic trait of adaptability to warmer climates [38].

The ovulatory follicle grew at the rate of 2 to 4 mm per day, to arrive finally at the ovulatory stage; this is in agreement with the results found by others [5, 29, 30, 36]. Additionally, Blanchard et al. [7] reported that large follicles can be present during any stage of the oestrous cycle, so follicular size alone is not a reliable indicator of oestrous or dioestrus.

Mares in oestrous have an average level of the progesterone P4 0.34 ± 0.22 ng.ml^{-1}, during the oestrous, and (10.52 ± 7.05) during the dioestrus. The values of progesterone in this study are comparable to those reported by earlier authors [1, 2, 12, 22, 25, 26, 30].

The significant correlation of mares in oestrous were expressed between the rate of the FSH and the growth of the follicular size ($r = -0.93$; $P < 0.01$). The secretion of FSH was lower during the beginning of the oestrous because of the secretion of proteins inhibin-like products by the pre-ovulatory follicle, when the growing follicle reached the pre-ovulatory stage; it produced hormones, which inhibited the pituitary secretion of FSH [8, 9, 14, 24, 32, 34]. Furthermore, Tharasanit [34] reported that FSH declined when the size of the largest follicle reached approximately 13 mm.

The positive correlation between the follicular diameter and the progesterone P4 ($r = 0.49$; $P > 0.05$) was similar to the values reported by Tharasanit [34] when the follicle development remained during the elevated progesterone levels P4 [34]. A positive correlation between the FSH and the oestradiol level ($r = 0.16$; $P > 0.05$) was because of a synergic activity between the oestrogens and FSH by stimulation of the follicular growth [10]. A positive correlation between the growth of follicles and the rate of oestradiol was found ($r = 0.42$; $P > 0.05$). Numerous medium follicles can contribute to the increase of the concentration of oestrogens during the dioestrus; the *corpus luteum* can have a minor role in the increase of oestrogens during the dioestrus [10].

One study showed that no effect of mare age on oestradiol-17-beta serum, Ginther et al. [13]. Moreover, Rocha et al. [31] reported that, there was a lower oestradiol-17-beta serum concentration than the younger mares accompanied by smaller follicles at ovulation and a longer period from maximum follicle diameter to ovulation.

CONCLUSIONS

It was concluded that mares show a different pattern of oestrous cycle and ovulation, the presence of a large follicle by ultrasound on an ovary is a poor predictor of oestrous in the mare and large follicles can be present during any stage of the oestrous cycle, so follicular size alone is not a reliable indicator of oestrous or dioestrus. Consequently, the role of the hormone levels, especially the progesterone level, in the establishment of earlier pregnancy in the mare is very important. Finally, this study was taken to provide and to produce a guideline for veterinarians responsible for reproductive management of mares under Algerian conditions.

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LOCALISATION OF S100 PROTEIN AND ACETYLATED TUBULIN IN SHEEP PANCREAS

Marettová, E., Maretta, M.
Department of Morphological Disciplines,
University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice
Slovakia

elena.marettova@uvlf.sk

ABSTRACT

The pancreas plays a critical role in the control of nutritional homeostasis. It consists of two major parts, the exocrine pancreas, and the endocrine pancreas. In the present study S100 protein and acetylated α-tubulin were used to identify positive structures in both the exocrine and endocrine part of the ovine pancreas. In the exocrine part of the pancreas, a positive reaction to S100 protein was confined to centroacinar cells, intercalated, and intralobular ducts cells. In addition, the S100 protein was localized in the Schwann cells of nerve fibres. On the pancreatic islets, the S100 protein has been observed in Schwann cells of nerve axons, where they form a fine envelope that invests the islet surface. Inside the pancreatic islets, the Schwann cells positive for S100 protein envelope the endocrine cells of the islets. The difference in positivity of the S100 protein was found in relation to the endocrine cells. The relationship between endocrine cell positivity and positive exocrine duct cells was discussed. Acetylated α-tubulin (AT) was restricted to axons of the nerve fibres and was located within the connective tissue accompanying intralobular and interlobular ducts, and between secretory acini in close contact with secretory cells.

Key words: acetylated tubulin; immunohistochemistry; pancreas; S100 protein; sheep

INTRODUCTION

The pancreas consists of two main parts, the exocrine pancreas, which releases digestive enzymes; and the endocrine pancreas, which releases hormones such as insulin, glucagon, pancreatic polypeptide, and somatostatin, and maintains glucose homeostasis. Both parts of the pancreas are innervated by the sympathetic and parasympathetic nervous systems, and separate pathways regulate the exocrine and exocrine pancreas. Inside the pancreas, both types of autonomic fibres transmit nerve impulses to the acinar cells and to the pancreatic islets. The autonomic nervous system affects glucose metabolism in part through a connection to the pancreatic islet. Endocrine cells in the

Langerhans islets are well innervated by sympathetic, parasympathetic and sensory nerve fibres.

Adrenergic innervation in relation to the strong inhibitory effects of noradrenergic stimulation on insulin secretion has been studied by various authors [2, 16, 28]. It has been found that sympathetic islet innervation is increased in experimental diabetic mice [12] and that insulin secretion depends on the tone of the autonomic nervous system [28].

S100 proteins are involved in various intracellular and extracellular functions. They are involved: in the regulation of protein phosphorylation, transcription factors, Ca^{2+} homeostasis, dynamics of cytoskeleton components, enzyme activities, cell growth and differentiation, and the inflammatory response [14]. In addition, several authors have suggested the transduction of calcium signals by calcium-binding proteins, such as the S100 protein [3, 7]. Overexpression of the S100 protein has led to changes in the expression levels of several cytoskeletal proteins, including cytokeratins 8, 18, and 19 [33]. S100 proteins play an important role in tumour development and progression due to their multifunctional properties involved in various cellular and extracellular processes [11, 34].

S100 protein staining has been widely used in histological studies and diagnostic pathology to identify neuroectodermal derivatives, but has subsequently been shown to be present in normal non-nervous human tissues of various organs [17]. The S100 protein was localized in various exocrine and endocrine organs, where immunoreactivity to the S100 protein was limited to some secretory cells and to ductal cells. In human and animal pancreases, the S100 protein has been used to identify nerve structures and to identify cell types in the pancreatic islets [1, 25, 32]. S u n a m i et al. [30] demonstrated the three-dimensional architecture of peri-island nerve plexuses in the mouse pancreas by staining for the S100 protein.

Acetylated α-tubulin as a modified form of tubulin has been found to be present in a variety of cellular structures, but has also been found to be preferentially expressed in nerve fibres, both *in vivo* and *in vitro* [8]. It is known that microtubules in axons are generally richer in acetylated tubulin than dendritic microtubules; that acetylated tubulin is unevenly distributed throughout the length of the microtubules, and that microtubules differ in acetylated tubulin levels in different regions of the axons [6]. Currently, much attention is paid to pancreatic innervation during development in connection with beta-cell regeneration and neuronal competition [9, 13, 18] and the remodelling of pancreatic islet innervation in connection with diabetes.

The aim of this work was to study the protein S100 and acetylated α-tubulin related to exocrine and endocrine glandular tissues and nerve structures in the sheep pancreas.

MATERIALS AND METHODS

Our investigations were carried out on six adult clinically healthy sheep of both sexes weighting between 35 and 43 kg, age 2—4 years. The sheep were rendered insensible (anaesthetised) prior to slaughter at the University of Veterinary Medicine and Pharmacy in Košice, where they were used for education and scientific purposes. The samples of pancreas were dissected out of slaughtered sheep and the tissue samples were fixed in 10 % buffered formalin and embedded in paraffin. Then 5 μm thick paraffin sections were deparaffinised with xylene and dehydrated in a decreasing ethanol gradient. The sections were pre-treated with 3 % H_2O_2 in methanol to block endogenous peroxidase activity and pre-incubated with 2 % goat serum to mask the unspecific binding sites. The sections were incubated with the primary antibodies S100 protein (Sigma) and acetylated α-tubulin (Sigma), and washed in phosphate-balanced salt solution (PBS). Afterwards, the sections were incubated with biotinylated secondary antibody for 45 min, washed in PBS, and finely incubated with avidin-biotin-peroxidase complex (ABC kits, Vector Laboratories, USA). After washing in PBS, formation of the reaction product was achieved by incubation for 10 minutes at room temperature using a mixture of an equal volume of 0.02 % hydrogen peroxide and 0.1 % 3,3´-diaminobenzidine tetrahydrochloride, made in Tris buffer. For negative controls, the first antibody was substituted by PBS or by normal rabbit serum. Sections were counterstained with Mayer's hematoxylin and methylene blue.

Ethical considerations

The research was approved by the UVLF Ethics Committee in accordance with applicable national and international animal welfare legislation.

The authors declare that there was no conflict of interest.

RESULTS

The pancreas is innervated by sympathetic and parasympathetic nerve fibres. Sympathetic fibres cause vasoconstriction and inhibition of exocrine secretion and stimulate glucagon release but inhibit insulin release. Parasympathetic fibres induce secretion from acinar cells, which ultimately leads to the release of pancreatic juice, insulin and glucagon.

The pancreas is an encapsulated, tubuloacinary gland containing both exocrine and endocrine gland tissue. The exocrine pancreas forms lobules and consists of only two main cell types, namely acinar cells, which synthesize, store and secrete digestive enzymes, and ductal cells, which secrete chloride and bicarbonate. Cells in the endocrine pancreas are organized in pancreatic cell clusters, the islets of Langerhans. In the islets, insulin-secreting beta-cells are the predominant cell type. The remaining cells consist of alpha-cells that secrete glucagon, delta-cells that secrete somatostatin, and cells that secrete pancreatic polypeptide. The pancreatic stroma consists of a thin capsule that results in the formation of fine septa of connective tissue that separate the parenchyma into distinct lobules. Larger blood vessels, nerve fibres and excretory ducts take place in the septum of the connective tissue. Nerve protrusions enter the lobules and enter both exocrine and endocrine secretory cells.

In the exocrine part of the pancreas, a positive reaction to acetylated α-tubulin has been associated with nerve fibres found mainly in the septum of the connective tissue near the blood vessels and in the larger glandular ducts. Several nerve fibres run inside the glandular tissue along with the accompanying capillaries and fine cells and connective tissue fibres (Fig. 1). The unmyelinated nerve fibres of the pancreas gave fine branches that formed plexuses around the arteries and arterioles (Fig. 1). Nerve endings positive for acetylated tubulin and S100 protein were also observed between secretory acini near the basilar part of secretory cells (Fig. 1). A positive reaction to S100 protein was observed in centroacinar cells, intercalation (Fig. 2), and intralobular ducts while interlobular ducts showed weak immunoreactions. In relation to intralobular and interlobular ducts, some S100 protein-positive nerve fibres ended in close contact with the basement membrane of the duct cells.

In addition to the above exocrine gland cells, a positive response to S100 protein was associated with the surface of pancreatic islet endocrine cells. At the edge of the

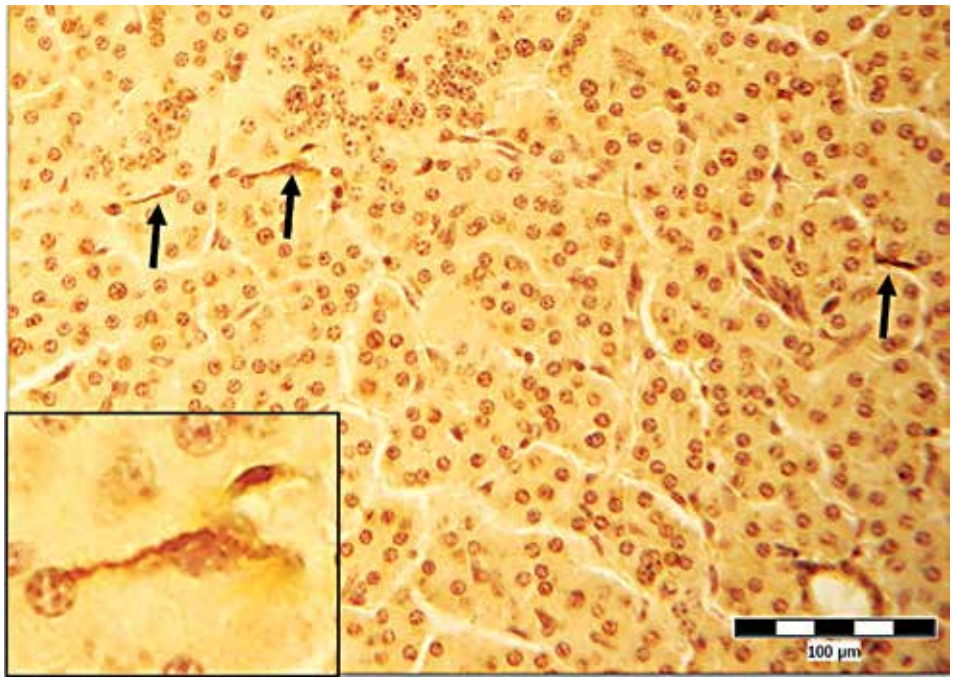

Fig. 1. Section of the pancreas stained with acetylated tubulin. Positive nerve fibres are located between the acini (arrow). Inside: Some nerve fibres get into the secretory cells

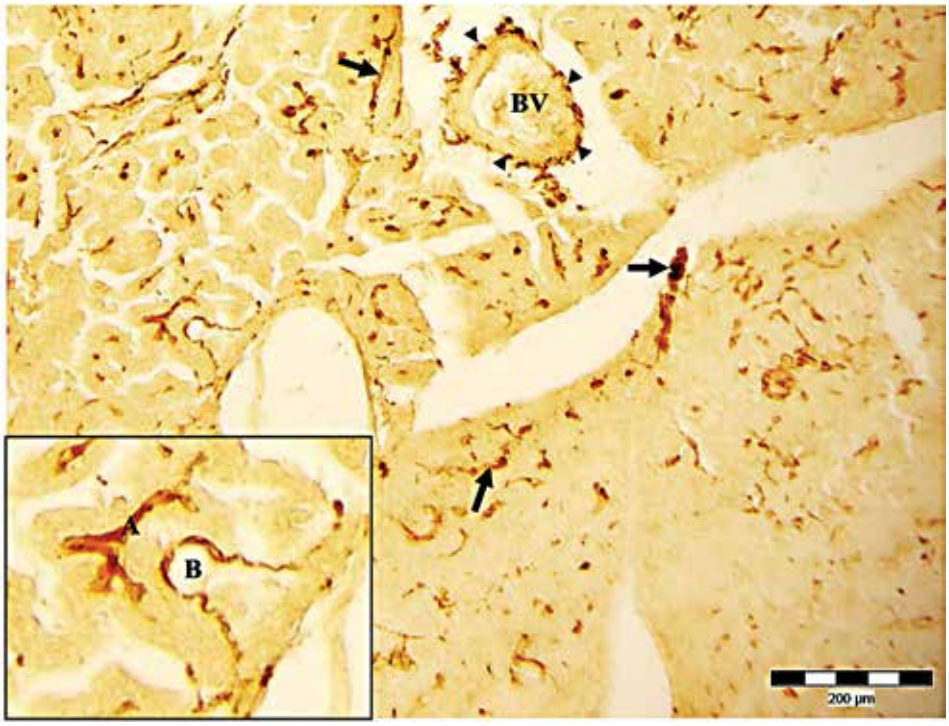

Fig. 2. Section of the pancreas stained for S 100 protein. Positive nerve fibres (arrowhead) located at the periphery of blood vessels (BV). Centroacinar cells (A), intercalated (B) duct give a positive reaction. The coarser nerve fibres run from the interlobular space. Thinner nerve fibres pass through the lobe (arrow)

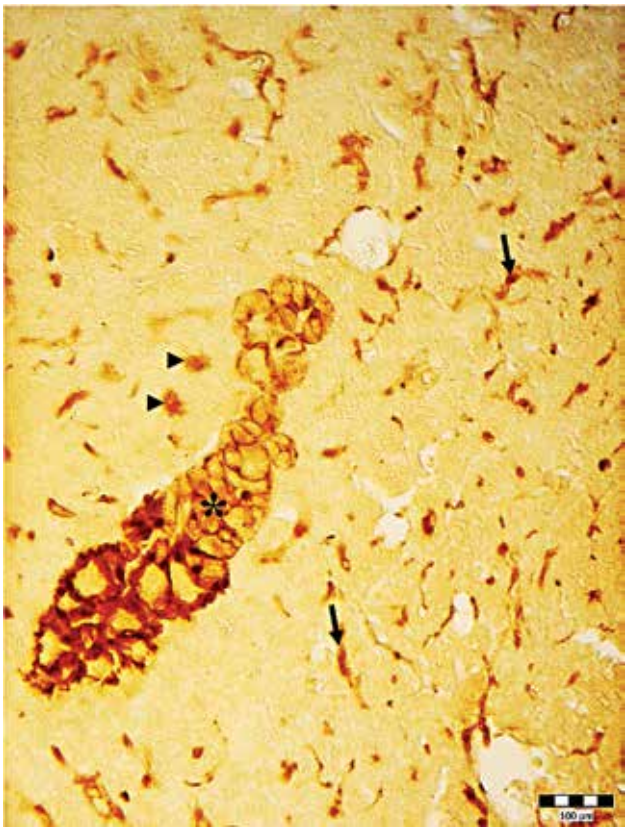

Fig. 3. Pancreas section stained for S100 protein. Numerous positive nerve fibres (arrow) and centroacinar cells (arrowhead) are visible. A positive reaction to S100 protein was also observed in Schwann cells of the surrounding secretory cells of pancreatic islet (asterisk). The difference in the intensity of the colouration of the pancreatic islets is present

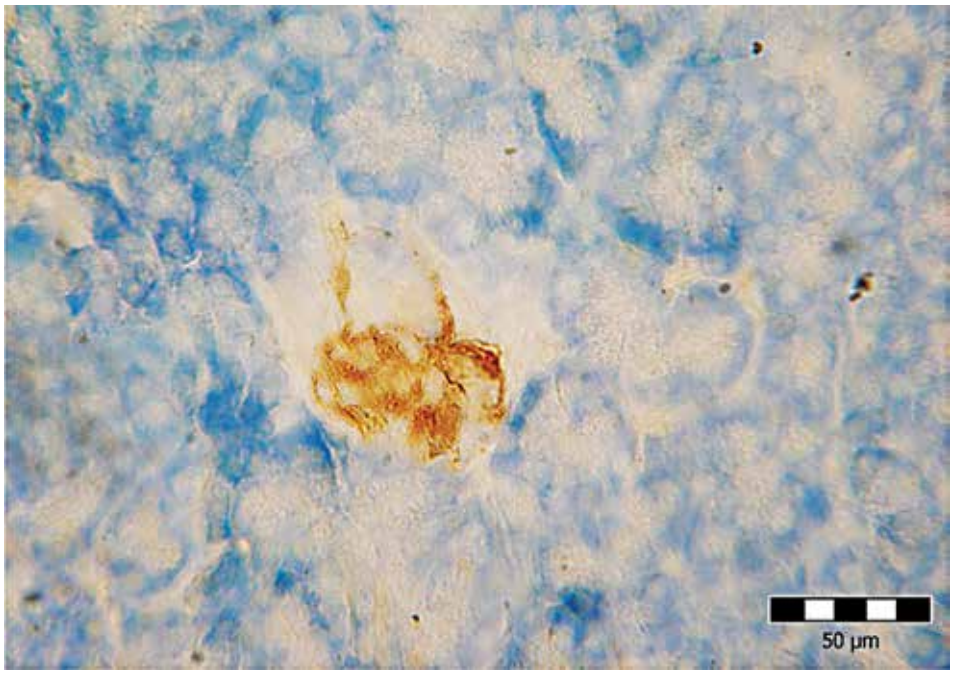

Fig. 4. Pancreas section stained for S100 protein. Some scattered islet secretory cells show a mild positive reaction

pancreatic islets and around the secretory cells, Schwann cells positive for the S100 protein formed a fine envelope that invested the surface of the islet. The pancreatic islet endocrine cells were coated by cytoplasmic processes of Schwann cells positive for S100 protein (Fig. 3 and 4). Differences in the intensity of the response were observed in some parts of the islets. Pancreatic islet endocrine cells were generally negative for S100 protein, although a slight staining reaction was observed in some endocrine cells.

DISCUSSION

The intrapancreatic nerves in sheep formed four plexuses: perivascular, periductal, periacinar, and peri-islets. In sheep pancreas, a positive reaction to protein S100 was found with different intensity in the exocrine and endocrine part of the pancreas. A positive response to S100 protein and acetylated tubulin was observed in nerve fibres, which usually ran with arteries in the interlobular septa of connective tissue. Similar nerve localization has been described in humans and some animal species [1, 10]. As in the mouse pancreas [33], the unmyelinated nerve fibres of the pancreas gave fine branches that formed plexuses around the arteries and arterioles. Although the nerve fibres of the networks were locally in close connection with the walls of blood vessels or the bases of secretory cells, no specialized axonal contacts were found with these tissues. In the pancreas of chinchilla [26], these structures were located in the parenchyma in close proximity to blood vessels and were also located in the walls of the pancreatic ducts and interstitial connective tissue. Adrenergic nerve fibres form a network in the adventitia of blood vessels, and individual fibres passed through the entire pancreatic parenchyma. Different extent in nerve endings has been associated with acini, islets, ducts, blood vessels, interlobular connective tissue, as well as intrapancreatic ganglion neurons in the ovine pancreas [4]. As we found in sheep, S100 protein and AT-positive nerve fibres were also observed between secretory acini in close contact with secretory cells.

The immunoreactive nerve endings of the vesicular acetylcholine transporter were distributed among the acini, while only individual cholinergic nerve fibres innervated the interlobular connective tissue. Colocalisation of vesicular acetylcholine transporter (VACh)T, tyrosine hydroxylase (TH), substance P (SP), and neuropeptide Y (NPY) has been found in different populations of nerve fibres located between acini [4]. The co-localization of vasoactive intestinal peptide (VIP) and NPY has been found

in nerve endings located around blood vessels and acini, and in connective tissue septa. Immunoreactivities against NPY, VIP, and SP have been observed to varying degrees in nerve endings associated with acini, islets, canals, blood vessels, interlobular connective tissue, as well as in intrapancreatic ganglion neurons [5].

It has been seen that the rich nerve fibres coated with Schwann cells positive for the S100 protein accompany the arteries towards the islets of Langerhans. A fine network of fibres has been observed around the islets of Langerhans [26]. Both myelinated and unmyelinated fibres are observed around the perimeter of the islets. In the sheep pancreas, S100 protein-positive Schwann cells were often found at the edge of the islets, defining the islet and exocrine parenchyma [23]. Networks of unmyelinated nerve fibres consist of axons with varicose veins and Schwann cells. Schwann's islet cells combined their thin and slender processes to form a fine network or dense plexus on the islet surface in the pancreas of mice [30]. The rich innervation of islets suggests that neural regulation of secretory function is mediated by the control of blood flow in the pancreas [33]. In adult mice, it has been seen that more sympathetic fibres located on the periphery of their islets enter the islets and reach the nucleus, where mainly beta cells are located [9].

When they reached the capillaries, the nerve fibres left the arterioles and formed very loose networks in the intercellular spaces [29]. Inside the islets, the endocrine cells were enveloped by Schwann cell cytoplasmic processes. The presence of nerve fibres with a synaptic structure has occasionally been noted on the surface of the endocrine cell. In this part, Schwann cells expanded their thin membrane processes, which directly covered the basal part of several endocrine cells as a whole. Numerous axons with varicose veins were usually found on the surface of this Schwann cell membrane, but they sometimes crawled under them [30]. Some of these nerves directly synchronized with endocrine cells and affect their activity [31]. Nerve fibres entering the pancreas form a network around the perimeter of the islets referred to as peri-island plexuses [22]. Some fibres were observed upon entering the islets and ending on the surface of islet cells and innervated smooth muscle cells of blood vessels located within the islet [27]. R o d r i g u e z - D i a z, C a i c e d o [29] classified these nerve fibres as sympathetic as invasive sympathetic axons, which preferentially innervated the blood vessel smooth muscle cells. This innervation pattern suggests that sympathetic nerves may direct hormone secretion by modulating blood flow in the islets instead of acting directly on endocrine cells.

Two types of S100 positive cells can be found in the human islets of Langerhans cells located on the periphery of the islets and cells located inside the islet. Cells located at the periphery have long processes running mainly along the periphery, while cells located inside the islets generally have no processes [25]. Interactions between thin nerve fibres and endocrine cells located separately or within islets predominated [25].

In exocrine glandular tissue, a S100 protein positive response was observed in centroacinar cells and in all secretory ducts. The strongest response was found in centroacinar cells and in small intercalated ducts, moderate in intralobular ducts and weakest in interlobular ducts. N a g a s a o et al. [24] described the relationship between intercalated ducts and islets in mammals. Their results revealed intercalated ducts that responded positively to S100 protein in and near the islets, with approximately 12 % of the islets having intercalated channels in the vicinity and approximately 1.5 % containing intercalated ducts [24]. This relationship was not found in the sheep pancreas.

It is now well known that the pancreatic endocrine cells differ from epithelial progenitors. In the human foetal pancreas, epithelial ductal cells express numerous transcription factors that regulate endocrine cell differentiation [19]. Differentiation endocrine cells temporarily retain epithelial markers and are often associated with the ductal epithelium. Common staining properties probably lie in their embryonic origin. It is well known that the structures of the nervous system originate from the neuroectoderm. Like other neuroendocrine cells, the islet cells might have originated from the neural crest. In the foetal life, their stem cells are located in the epithelium of the pancreatic ductuli [15]. K i r o v o v a et al. [21] demonstrated close integration between nervous system structures and endocrine cells in the human pancreas. As previously emphasized during the prenatal development of the pancreas, the nervous system regulates the proliferation and maturation of endocrine cells and contributes to the formation of islet architecture. It seems that contacts with the structures of the nervous system may be for the migration of epithelial progenitors into creative islets [21] and that the nervous system directly regulates the weight of endocrine cells and their maturation.

It can be assumed that the two types of S100 positive cells are important both in the morphology of the islets and in the regulation of hormones expressed by endocrine cells. The two cell types in the islets of Langerhans are distinguished in the early foetal period of the human pancreas, with two types of S100 positive cells appearing that differ in structure. Peripheral cells are morphologically similar to glial cells [25]. The cells inside the islets do not differ in structure from other endocrine cells. There were no such differences in sheep pancreas, but different intensity of antibody staining was observed between positive cells. P r o s h c h i n a et al. [25] found in humans small S100-reactive cells with thin processes on the periphery of some islets. The processes of these cells often cover or surround the nerve fibres passing to the islets [20, 25]. S100-positive cells with thin processes detected by the authors in human pancreas correspond to glial (Schwann) cells observed on the periphery of islets in other mammals [30, 33].

CONCLUSIONS

A strong response to acetylated tubulin and S100 protein has been observed in sheep pancreas in cells and nerve fibres in both exocrine and endocrine tissues of the glands. Positive nerves for both groups were complicated and were located in the connective tissue near the blood vessels accompanying the intercalate, intralobular and interlobular ducts. The nerve fibre branches enter the secretory lobes and are in close contact with secretory acini and cells. On the periphery of Langerhans islets 100-positive Schwann cells form a fine coat. The individual nerve fibres, along the blood capillaries, enter the islets and touch the endocrine secretory cells.

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PREVALENCE, ANTIBIOGRAM AND BIOFILM PRODUCTION OF *LISTERIA MONOCYTOGENES* FROM FAECES AND FOETUSES OF SLAUGHTERED PREGNANT COWS: ENVIRONMENTAL AND PUBLIC HEALTH IMPLICATIONS

Adesokan, H. K., Kehinde, E. G.
Department of Veterinary Public Health and Preventive Medicine
University of Ibadan, Ibadan
Nigeria

greaterglory2008@gmail.com

ABSTRACT

The indiscriminate slaughter of pregnant animals which characterizes most developing countries poses increasing environmental and public health risks from *Listeria monocytogenes* infections which are endemic in such settings. The available reports show increasing trends of *Listeria monocytogenes* infections in both humans and animals in Nigeria. This study examined the prevalence, antibiogram and biofilm production of *L. monocytogenes* from faeces and foetuses of slaughtered pregnant cows in Ibadan Central Abattoir, Nigeria. Faecal (n = 118) and foetal (n = 118) swabs were cultured and isolates tested for antibiotic susceptibility by Kirby-Bauer assay, while biofilm production was quantified following the standard procedures. The data were analysed using the Chi Square and Student's t-test at P < 0.05. *Listeria monocytogenes* were isolated from five (4.2 %) and three (2.5 %) faeces and foetus swabs, respectively, without significant association with sample type (P = 0.50). The isolates were resistant to all the antibiotics tested except gentamicin; with significantly higher production of biofilm by those from foetal samples (P = 0.012). The detection of widespread antibiotic-resistant *L. monocytogenes* from faeces and foetuses has important environmental and public health implications, given the risk of contamination through faecal shedding and foetal handling. The biofilm production by the pathogen connotes its ability to persist in the environment, suggestive of the challenging effects to its control. Campaigns against indiscriminate slaughter of pregnant animals, and proper hygiene are advocated to ultimately safeguard human and animal health.

Key words: abattoir; antibiotic resistance; *Listeria monocytogenes*; prevalence; public health

INTRODUCTION

Listeria monocytogenes is a facultative intracellular pathogen which causes infectious disease in many different animal species, especially in farm ruminants [37] as well as humans. Foetuses from pregnant infected animals are

very rich in *L. monocytogenes* [18]. Similarly, faeces play a significant role in the spread of listeriosis to humans and animals [35]. It is associated with severe foodborne infections in humans; thus, this organism is considered one of the most important pathogens responsible for foodborne infections. Listeriosis, caused by *L. monocytogenes* is a zoonotic disease which poses health risk to the immunocompromised and occupationally exposed individuals including abattoir workers and surrounding environments. In the present decennium, numerous serious outbreaks of foodborne listeriosis have been recorded in different countries and continents [23, 31, 51, 52], causing serious manifestations in the form of septicaemia and meningitis, principally in the immunocompromised and old populaces in addition to pregnant women, who may bring forth stillborn babies or seriously contaminated infants [29, 55].

These associated health problems could be more challenging particularly in most developing countries including Nigeria characterized with indiscriminate use of antibiotics in animals and consequent widespread cases of antibiotic resistance [3, 14]. Considering the delayed health care-seeking behaviour of abattoir workers and most rural dwellers in the surrounding environments, coupled with other associated immunocompromising practices, these occupationally and environmentally exposed individuals may be the worst hit. More so, the risk of zoonotic transmission of *L. monocytogenes* in the abattoir and surrounding environments could be potentiated since the organism could colonize surfaces, forming biofilms that remain attached to equipment used in food production [46]. Unfortunately, most abattoir workers in the country generally do not observe proper hygiene when processing slaughtered animals and are thus exposed, while serving as a source infection to others in their communities. Also, poor disposal of animal faeces which are often washed off into the surrounding environments threatens environmental health. As reported, cattle and many other mammals including humans, can be asymptomatic shedders of the pathogen [54].

In order to assess the magnitude of this risk, we sought to determine the prevalence, antibiotic susceptibility profiles and biofilm production of *Listeria monocytogenes* in slaughtered pregnant cows in Ibadan Central Abattoir, southwestern Nigeria.

MATERIALS AND METHODS

Study setting

This cross-sectional study was carried out at the Ibadan Central Abattoir, Ibadan, southwestern Nigeria between May and December, 2018. Ibadan is the third most populous city in the country and the largest by area. It has a population of over 3 million people. The city is projected to increase to about 5.03 million inhabitants by 2025, considering an average annual growth rate of 4.6 % during the period 2010—2020 [50]. Ibadan Central Abattoir was chosen as it is the only abattoir in the city recognized by the government of the state where food animals are slaughtered. It slaughters an average of 450 heads of cattle per day aside from goats, sheep and pigs and has the capacity of holding a human population of about 800 people. The abattoir supplies meat to the entire citizenry of the city and neighbouring environments. It thus provides a milieu for zoonotic transmission of diseases given the human-animal interface in the setting, coupled with unguarded contacts between humans and animals as well as poor hygienic practices of the abattoir workers.

Study population and sample size

The study population included all slaughtered pregnant cows at the abattoir during the period of study. Pregnancy state in the slaughter animal population was determined following evisceration after slaughter, since routine ante-mortem examination was not enabled in the setting. Following the 6.9 % prevalence of *L. monocytogenes* in livestock animals [26], sample size was calculated using the formula by Thrusfield [49]. This gave a minimum sample size of 110 animals including the addition of 10 % level of attrition.

Animal sampling

The purposive sampling technique was used in the collection of samples by sampling every identified pregnant cow slaughtered on each day of visit to the abattoir. Swabs of foetuses and faeces of every slaughtered pregnant cow were aseptically collected using sterile swabs sticks. These samples, upon collection were immediately placed in swab stick casings containing listeria transport medium (Oxoid Limited, United Kingdom). All the samples were collected following this procedure each day of the visit to the abattoir until the sample size was reached. The abattoir was

visited twice a week throughout the period of the study. Upon collection each day, the swab samples were transported in a transporting flask containing icepack to the Bacterial Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan for processing. Samples were processed within three hours post-collection.

Sample processing

A 10^5-fold serial dilution of the samples was carried out. Thereafter, the samples were incubated at 37 °C for 18 to 24 hours and then plated on *Listeria* selective agar (Oxoid Limited, United Kingdom). After inoculation onto the agar, the Petri dishes were turned upside down and incubated at 37 °C for 24 to 48 hours. After incubation, the growth on the medium were observed. On the primary medium used, the most distinct colonies showing perfect characteristics of *Listeria* were picked and aseptically sub-cultured in freshly prepared *Listeria* selective medium and incubated to obtain pure colonies. This was done as soon as possible to ensure optimum viability of the organisms. After the second round of culture on the selective medium, the bacterial colonies on the media were then aseptically stored on a nutrient agar slant (Oxoid Limited, United Kingdom) and refrigerated. The *L. monocytogenes* were identified following the earlier described protocols [15] using conventional biochemical methods which included Gram staining, catalase, aesculin, triple sugar iron (TSI) reaction, motility, urease, sugar fermentation tests (lactose, sucrose, mannitol and xylose), motility and CAMP tests.

Antibiotic susceptibility testing

All of the *L. monocytogenes* isolates obtained were subjected to antibiotic susceptibility testing by the disk diffusion method as described by Clinical and Laboratory Standards Institute [20]. Briefly, 3 ml of sterile normal saline was used to emulsify an inoculum of each pure bacterial isolate and the density was then adjusted to the 0.5 McFarland standard. Using a sterile cotton swab dipped into the standardized suspension of bacterial cultures, inoculations were made into the Mueller-Hilton Agar (MHA) plates (Oxoid, England). The plates were then allowed to dry. Antibiotic disk containing amikacin (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefuroxime (30 µg), gentamicin (10 µg), vancomycin (10 µg), tetracycline (30 µg), co-trimoxazole (25 µg), ciprofloxacin (5 µg), meropenem (10 µg), chloramphenicol (30 µg) (Antibiotic Becton Dickinson and Company, Sparks, USA) were placed onto MHA plates.

These antimicrobial agents were chosen on the basis of their importance in treating human or animal *Listeria* infections; their use in animals and on the basis of their ability to provide diversity for representation of different antimicrobial agent classes. The plates were thereafter incubated at 37 °C for 24 hours. The zone of inhibition was measured in millimetres and zone diameters were interpreted as susceptible, intermediate and resistant with respect to standards on the basis of the critical points recommended [20]. *L. monocytogenes* ATCC7644 was used as a reference strain.

Biofilm production and quantification

Biofilm production and quantification by the identified *L. monocytogenes* were done according to an earlier described procedure [42]. One colony of each overnight grown *L. monocytogenes* culture were transferred into 9 ml tryptose soy broth (Oxoid Limited, United Kingdom) and incubated at 37 °C for 24 h was used for the biofilm formation on the glass vials [42]. Non-inoculated tryptose soy broth served as the negative control. The glass vials (4.5 ×191.4 cm; Fisher Scientific) which were used as glass surface were washed with alkaline detergent (Alconox®) and rinsed thoroughly using deionized water. They were thereafter air-dried and then autoclaved at 121°C for 30 min. Then, 5 ml of the pure cultures were placed into the glass vials and incubated at 37 °C for 24 h in order to allow the cells in the broth to attach to the glass vials. The glass vials were covered with lids in order to prevent evaporation of the broth. Following the incubation period, quantification of *L. monocytogenes* attached to glass surface was done using a crystal violet binding assay [42] with some slight modifications. The broth cultures were withdrawn and the glass vials were rinsed three times with sterile distilled water to remove loosely attached cells.

Using a Bunsen burner, the glass vials were air-dried and fixed by passing them over the flame three times. Thereafter, the fixed cells on the surfaces were stained with 2 ml of 1 % crystal violet (Fisher Scientific, United States) for 1 min. The glass vials were placed under running tap water in order to rinse off excess stain until there was no visible stain in the washed water. Further, the glass vials were air-dried for 5 min at 60 °C. The dye bound to the

adherent cells was re-solubilized using 200 µl of 33 % (v/v) acetic acid per vial. Each of the isolates were run in triplicate. The optical density (OD) of each of the glass vials and the controls were measured at 450 nm using a spectrophotometer (Novaspec II). To determine the actual value, the absorbance of the negative control was subtracted from the absorbance values [42] and the means of the respective values were obtained for each isolate.

Data analysis

The data obtained were coded in Microsoft excel and subjected to descriptive statistics. Frequencies and percentages were calculated as appropriate for the prevalence of *L. monocytogenes* per sample type. Chi-square was used to test for association between outcome variable (*L. monocytogenes* positivity) and sample type. The Student's t-test was used to compare the means of biofilm produced by *L. monocytogenes* between faecal and foetal samples using STATA 12.0 software. The level of significance was put at $P \leq 0.05$.

RESULTS

Isolation and identification of *Listeria monocytogenes*

A total of 236 samples were collected, 118 each from faeces and foetuses. Of these, *Listeria* spp. were present in 95 (80.5 %) and 79 (66.9 %) of the cow faecal and foetal swabs, respectively. The mean count for the faecal swabs was 0.532 ± 0.24 log CFU.ml^{-1}, with the highest and lowest counts being 1.839 log and 0.30 log CFU.ml^{-1}, respectively. The mean count for the foetal swabs was 1.136 ± 0.32 log CFU.ml^{-1}, with the highest and lowest counts being 1.477 log CFU.ml^{-1} and 0.301 log CFU.ml^{-1}, respectively. Further, 5 (4.2 %) and 3 (2.5 %) of the total samples obtained from faecal and foetal swabs, respectively, were confirmed as *L. monocytogenes*, with one isolate per sample (Table 1). Overall, a total of eight *L. monocytogenes* isolates were obtained.

Antibiotics susceptibility

The mean disk diffusion zones obtained indicated that the *L. monocytogenes* isolates from the faecal samples showed reduced susceptibility (as measured by disk diffusion zone sizes) compared to foetal sample isolates for amikacin, gentamicin and chloramphenicol (Table 2).

All the isolates from both faecal and foetal swabs exhibited 100 % resistance to all the antibiotics tested except to gentamicin where 60.0 % and 40.0 % intermediate resistance and susceptibility were recorded for isolates from faecal samples as well as 100 % susceptibility for foetal isolates. Similarly, 80.0 % and 20.0 % of the faecal isolates as well as 66.7 % and 33.3 % of the foetal isolates demonstrated resistance and intermediate resistance, respectively to chloramphenicol (Table 3).

Biofilm production

The means and standard error of the means of the biofilm production by the *L. monocytogenes* from faecal and foetal swabs are presented in Table 4. It revealed that the strains from the foetal swabs produced significantly higher quantity of biofilm than those from the faecal samples ($P = 0.012$).

DISCUSSION AND CONCLUSIONS

Listeria monocytogenes constitutes a serious global public health threat, with food animals and their products serving as the most important vehicles for the majority of cases of infections [34, 36]. The present study was performed at the Ibadan Central Abattoir, southwestern Ni-

Table 1. Prevalence of *Listeria* spp. and *Listeria monocytogenes* from faeces and foetuses of slaughtered pregnant cows at Ibadan Central Abattoir, Ibadan, southwestern Nigeria

Type of samples	No. of samples collected	*Listeria* spp. n [%]	Mean counts Log CFU.ml^{-1} [Mean ± SEM]	*Listeria monocytogenes* n [%]
Faecal swabs	118	95 (81)	0.532 ± 0.24	5 (4.2)
Foetal swabs	118	79 (67)	1.136 ± 0.32	3 (2.5)

Table 2. Mean disk diffusion zones and resistance breakpoints for *Listeria monocytogenes* from faecal and foetal swabs of slaughtered pregnant cows at Ibadan Central Abattoir, southwestern Nigeria

Antibiotics	Breakpoint [mm]	Mean disk diffusion zone of inhibition [mm]	
		*Faeces [n = 5]	Foetus [n = 3]
AMK (amikacin)	≤ 14	11	13
CTX (cefotaxime)	≤ 14	0.0	0.0
CTR (ceftriaxone)	≤ 19	0.0	0.0
CRX (cefuroxime)	≤ 14	0.0	0.0
GEN (gentamicin)	≤ 12	14.2	20
VAN (vancomycin)	–	0.0	0.0
TET (tetracycline)	≤ 14	5	0.0
COT (co-trimoxazole)	≤ 10	0.0	0.0
CIP (ciprofloxacin)	≤ 15	0.0	0.0
MEM (meropenem)	≤ 13	0.0	0.0
CHL (chloramphenicol)	≤ 12	8	10

*n—Number of *Listeria monocytogenes* isolates

Table 3. Resistance/susceptibility of *L. monocytogenes* isolated from faecal and foetal swabs of slaughtered pregnant cows in Ibadan, Nigeria

Antibiotics	*Breakpoints [mm]			Isolate [%]					
				**Faeces [n = 5]			Foetus [n = 3]		
	R	I	S	R	I	S	R	I	S
Amikacin	≤ 14	15—16	≥ 17	80	20	0.0	66.7	33.3	0.0
Cefotaxime	≤ 14	15—22	≥ 23	100	0.0	0.0	100	0.0	0.0
Ceftriaxone	≤ 13	14—20	≥ 21	100	0.0	0.0	100	0.0	0.0
Cefuroxime	≤ 14	15—22	≥ 23	100	0.0	0.0	100	0.0	0.0
Gentamicin	≤ 12	13—14	≥ 15	0.0	60	40	0.0	0.0	100
Vancomycin	–	–	–	100	0.0	0.0	100	0.0	0.0
Tetracycline	≤ 14	15—18	≥ 19	100	0.0	0.0	100	0.0	0.0
Co-trimoxazole	≤ 10	11—15	≥ 16	100	0.0	0.0	100	0.0	0.0
Ciprofloxacin	≤ 15	16—20	≥ 21	100	0.0	0.0	100	0.0	0.0
Meropenem	≤ 13	14—15	≥ 16	100	0.0	0.0	100	0.0	0.0
Chloramphenicol	≤ 12	13—17	≥ 18	80	20	0.0	66.7	33.3	0.0

* R—Resistant; I—Intermediate; S—Susceptible; **n—Number of *Listeria monocytogenes* isolates

Table 4. Biofilm quantification of *Listeria monocytogenes* from faecal and foetal samples (450 nm)

Isolates from faecal swab samples	Mean ± SD	P value
101A	0.046 ± 0.01	
57A	0.046 ± 0.01	
99A	0.044 ± 0.01	
39A	0.045 ± 0.01	
63A	0.047 ± 0.01	0.012
Isolates from foetal swab samples		
54B	0.047 ± 0.01	
36B	0.049 ± 0.01	
65B	0.048 ± 0.01	

geria, an animal processing facility which is increasingly recognized as a melting pot for food pathogens. The cattle being slaughtered at the abattoir are mostly trade animals drawn from different farms across the country and are often transhumant in nature, moving from one point to the other in search of water and pasture. Importantly, this study observed a higher prevalence of *L. monocytogenes* from faecal than foetal swab samples, with the strains isolated being resistant to the majority of antibiotics studied, as well as, all being biofilm formers. Hence, strong social and commercial links between such mobile cattle herds and the local communities, their transverse can contribute to efficient distributions of the zoonotic agent from animals to humans [53]. Besides, the strong exposure of the abattoir workers and the surrounding abattoir environment to this pathogen is a matter of serious public health concern.

Our findings reiterate cattle as an important carrier of *L. monocytogenes* and agree with previous reports that this foodborne pathogen is quite prevalent in animals and their products [9, 30, 38]. More specifically, this study demonstrated that cow faeces and foetuses remained a potential source of *L. monocytogenes* infection to meat processors and handlers. This is in line with the study [25] that reported cattle as a potentially important reservoir for *L. monocytogenes*. The present finding is of serious public health importance given the poor hygienic practices of most abattoir workers [27]. Cross contamination of carcasses with faeces and unhygienic handling of foetuses from slaughtered pregnant animals during animal processing is a common occurrence particularly in developing countries. Thus, both the meat processors, and consumers through contaminated meat are potentially exposed to the infection. It is also plausible that the cattle might have been shedding the organisms while in the respective herds from which they were sourced, thus exposing both livestock handlers and other animals to the infection and also causing environmental contamination.

In addition, the bacterial isolation of *L. monocytogenes* from foetuses of slaughtered pregnant cows in this study portends public health challenges considering the reported practice of eating and selling gravid uterus and foetuses as meat in Nigeria [4]. This concern is potentiated by the practice of eating raw or improperly cooked meat which is common amongst meat handlers in the country [5, 28] and amongst Africans in general [32, 48]. As such, there is a high risk of human infection with *L. monocytogenes* among these meat handlers and other potential consumers. In addition, poor meat/food handling practices characteristic of meat handlers and most households in developing countries, including Nigeria [7, 17], could as well, enhance the transmission of *L. monocytogenes*. A previous report has shown that food contamination from raw meat remains an important cause of foodborne disease outbreaks or food poisoning [44]. Such contaminations do occur when food that does not require cooking such as salad is prepared on the same chopping board already used to prepare raw meat without adequate washing [1]. Cross-contamination can also occur when raw meat is stored above ready-to-eat meals. Thus, the practice of selling foetuses to and poor hygienic handling by unsuspecting buyers connotes considerable public health issues.

Besides, the fate of cow faeces from slaughtered cattle in the abattoir is a matter of concern. Most abattoirs in Nigeria lack adequate facilities for proper waste handling and disposal. As a result, the faeces and other wastes generated from slaughter processes are often disposed of around the abattoir premises, thereby constituting social, environmental and public health hazards to the surrounding environments. More importantly, during the rainy season, these waste materials are washed off, traversing kilometres away from the abattoir and contaminating water wells, thus exposing such neighbouring environments to bacterial contaminations. A d e s o k a n, S u l a i m o n [8] revealed in their study that almost 75 % of slaughterhouse workers in a major meat processing facility in Ibadan, Nigeria discharged slaughterhouse wastewater into surrounding streams. Such a practice as previously reported, could contaminate surface and underground waters [2] with resultant pathogenic organisms, including *L. monocytogenes* in surrounding well water [11, 24]. This practice inadvertently impacts with associated severe health risk to such poor communities located near the river which use it for domestic activities, such as cooking, washing, and bathing [8]. Further, the practice of using cow faeces as manure in farming in Nigeria portends a matter of public health importance as the pathogens could be transmitted from one place to the other with potential effects on both crops and humans. Vegetables, for instance have been identified as a major vehicle for listeriosis due to their direct contamination with decaying vegetation, soil surfaces, rivers, animal faeces used as manure, and effluents from sewage treatments, improper harvesting and handling procedures,

improper sanitary conditions of equipment, and transportation practices [16, 19]. Previous studies have reported isolation of *L. monocytogenes* from vegetables [10, 45].

Furthermore, the levels of resistance exhibited to the antibiotics used in this study calls for public health action. Since the majority of these antibiotics are commonly shared with humans, the current levels of resistance do imply that such antibiotics might prove ineffective against infections in humans when the need arises. This finding is similar to the reports of other studies [6, 13, 47] which indicated that *L. monocytogenes* were highly resistant to important antibiotics. Our observation might be buttressing a previous report of widespread abuse of antibiotics by farmers and animal health workers [12]. In this study, the isolated *L. monocytogenes* were resistant to amikacin, cefotaxime, cefuroxime, ceftriaxone, tetracycline, vancomycin, co-trimoxazole, ciprofloxacin and meropenem indicating resistance to nine commonly used antibiotics. This is similar to the report of S a r a n g i, P a n d a [47] whereby resistance towards oxytetracycline, penicillin G, tobramycin, cefotaxime, cephalexin and ceftriaxone was observed. P e t e r et al. [43] similarly indicated that 16 isolates of *L. monocytogenes* from pork, beef, and chicken were resistant to amoxicillin, augmentin, cloxacillin, and tetracycline, although susceptible to gentamicin, co-trimoxazole, erythromycin, and chloramphenicol. Further, L e e et al. [33] reported that all strains of *L. monocytogenes* which were isolated from ready-to-eat seafood and food processing environments were resistant to benzyl penicillin, clindamycin, and oxacillin.

In this study, all the isolated *L. monocytogenes* from foetal and faecal swab samples produced biofilms. This is in line with the report [41] that *L. monocytogenes* can evolve a biofilm and are able to persist in the food processing environment. As previously reported by O l a i m a t et al. [39] in their review of the emergence of antibiotics resistance in *L. monocytogenes* isolated from food products, most *L. monocytogenes* strains possess a strong biofilm forming capability. Generally, biofilm forming bacteria pose a great challenge to the food industry because of its inherent resistance to the action of disinfectants; thus, contributing to *L. monocytogenes* protection against cleaning and sanitation in food processing environment. Biofilms continue to pose concerns to food manufacturers as they are one of the major reasons for limiting the shelf life and favouring pathogen contamination of food products [22]. The growth of biofilms in food processing environment is one of the main sources of repeated bacterial food contaminations [21], making it challenging to control bacterial contaminations in such an environment. Though significantly higher biofilms were produced by the *L. monocytogenes* from foetal samples, all the isolates obtained in this study produced biofilm. In general, *L. monocytogenes* in the biofilm state shows a reduced susceptibility to antimicrobial agents [22]. This might explain the observed high level of resistance of *L. monocytogenes* to the majority of antibiotics evaluated in our present study.

Our findings notwithstanding, this study had some limitations. One, the serotypes of the *L. monocytogenes* isolated were not determined, as this would have provided more insights into the findings of the study. Two, samples from the abattoir environmental surfaces were not obtained as analysis of such would have enabled comparison of the strains between those isolated from the faecal/foetal samples of the slaughtered animals and those from the environment.

Despite these limitations, the present findings have established a higher prevalence of *L. monocytogenes* in faeces than foetal swabs, indicating important environmental and public health implications since faecal shedding poses a risk of contamination of the abattoir environment and exposed humans. In addition, the level of resistance to major antibiotics by the isolates is worrisome considering the increasing health challenges the world is facing today with resulting resistance in humans when the need to use such antibiotics arises. This observation of widespread resistance to major antibiotics by *L. monocytogenes* therefore, requires all hands to be on deck towards mitigating the resulting rise in resistance profile of the pathogen in humans. As reported, it is anticipated that global deaths from infections caused by antibiotic resistant pathogens will increase from 700,000 to 10 million annually, and costs are predicted to reach US $100 trillion by 2050 [40]. Formation of biofilm by the *L. monocytogenes* strains indicates their ability to persist in the abattoir environment, thus resisting sanitizers with attendant challenging effects to its control. The need for extensive educational programme for abattoir workers on proper hygiene and against slaughter of pregnant animals is hereby advocated.

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EXTERIOR EVALUATION OF SELECTED BREEDS OF PIGEONS: OWLS AND FRILLS

Adamčík, M.[1], Zigo, F.[1], Kolenič, P.[2], Ondrašovičová, S.[3]

[1]Department of Nutrition and Animal Husbandry
University of Veterinary Medicine and Pharmacy, Komenského 73, 041 81 Košice
[2]Slovak Breeders Association, Krížna 44, 824 76 Bratislava
[3]Department of Biology and Physiology, University of Veterinary Medicine and Pharmacy
Komenského 73, 041 81 Košice
Slovakia

frantisek.zigo@uvlf.sk

ABSTRACT

Owl and frill pigeons are considered one of the oldest breeds of domestic pigeons and for breeders they mean the embodiment of beauty, pride, elegance and temperament. The common feature of the whole group of owl and frill pigeons is the presence of vertically growing feather adornment—frill and a significant refinement of exterior features on the head. The main goal of this study was to record the current situation in the exhibition sector and to compare the exterior of selected breeds of owl and frill pigeons with the relevant European standard at top breeder's exhibitions. Altogether 722 short-beaked owl and frill pigeons (Oriental frill, African owl and Turbit) raised by breeders from seventeen European countries were evaluated at five important exhibitions in Europe. The examination of the exterior showed that the most common exterior faults on the body of oriental frills were defects in colour pattern or lacing, poorly developed frill, faults in figure as well as defects in positioning and body posture. Exterior faults observed on the heads of oriental frill included: short top or forehead, defects in shape or length of the beak, as well as defects in its line. In addition to the faults in the oriental frills, there were observed imperfections in the rounding of the head and in the length of the wings and tail in the African owls. Turbits frequently exhibited deficiencies with respect to the length of the top or forehead. The exterior evaluation of owl and frill pigeons for breeders in the future shows the need for systematic elimination of deviations from physiological and physical development with culling of individuals transmitting morphological defects.

Key words: African owls; assessment; oriental frill; pigeon breeds; standard; Turbits

INTRODUCTION

The aim of modern pigeon farming is to preserve individual breeds of pigeons, which are without a doubt part of our cultural and social heritage. The task of enthusiastic breeders is to refine the characteristic breeding traits in

Fig. 1. Feathered ornaments of owl pigeons—frill and crest
Photo: Adamčík (2018)

Fig. 2. Oriental frill
Photo: Adamčík (2019)

accordance with the relevant breed standards and also enhance and extend the wide range of breeds. Pigeon breeders are currently divided into two groups [4, 7].

The first group consists of breeders of carrier pigeons. The most important selection and breeding criterion in the breeding of carrier pigeons is their performance, i.e. the speed at which the pigeon can return from the race to the loft [16]. The second, much more diverse group consists of breeders of fancy breeds of pigeons. The most important selection and breeding criterion in this type of breeding is the need to comply with the prescribed standard of the respective breed in terms of exterior and, in the case of high-flyer and roller pigeons, also in terms of their performance [9, 11].

According to the current division of pigeon breeds based on the differences in exterior features and uses, experts have divided breeds of pigeons into ten groups which currently include 576 breeds in Europe [15].

The above division is binding for all member states of the European Breeders' Association (Entente Européenne d'Aviculture et de Cuniculture—EE) and the European Pigeon Standards Book complies with this division. According to this division, pigeons are displayed at all exhibitions organized under the auspices of EE [5].

The main groups of pigeons include utility pigeons (e.g. Texan Pioneer, Carneau, French Mondain, Runt, Piestanau giant pigeon and other) with homer pigeons (e. g. Giant Homer, Show Racer, German Beauty homer and other), wattle pigeons (e. g. Scandaroon, Dragoon, Barb, carrier and other), utility pigeons—Huhntauben (e. g. King, Modena, German Modena, Maltese and other), pouters and croppers (e. g. Slovakian Cropper, Pommeranian

Fig. 3. African owl
Photo: Adamčík (2019)

Fig. 4. Turbit
Photo: Adamčík (2019)

Cropper, Bohemian Steller cropper, Hana Pouter and other), colour pigeons (e. g. Gimpel, Bohemian Wingpigeon, Czech spot whitetail, Franconian field pigeon and other), trumpeters (e. g. Arabian trumpeter, German double crested trumpeter, Bokhara trumpeter, Bohemian trumpeter and other), structure pigeons (e. g. Chinese owl, Fantail, Frillback, old Dutch Capuchine, Jacobin), owls and frills (e. g. Oriental frill, African owl, Italian owl, French owl, English owl), tumblers, highflyers and rollers (e. g. Slovak highflyer, Wiener highflyer, Kosice highflyer, Kosice roller, East Slovakian roller and other) [1].

Due to the large number of breeds, this study focuses on the description and evaluation of the standards of three breeds included in the eighth group of pigeons, which are owl and frill pigeons. Owl and frill pigeons are considered one of the oldest breeds of domestic pigeons (*Columbia livia domestica*). The common feature of all owl pigeons is the presence of a vertically growing feather adornment—frill, and a significant refinement of the exterior features on the head (Fig. 1). Owl and frill pigeons are an object of interest of top specialists who have brought their appearance to its current form. They are popular all over the world but they are becoming increasingly rare [13].

Owls and frills breeds are divided according to the length of the beak to short-beaked breeds and breeds with middle-size beaks. Short-beaked breeds include: Oriental frill (Fig. 2), African owl (Fig. 3), German shield owl, German colourtail owl, Turbit (Fig. 4), Turbiteen, Polish owl, Russian owl, Bulgarian shield owl, Anatolian owl, English owl and Domino frill. Middle-size beak breeds include: Antverp Smerle, Flanders Smerle, Old Dutch Turbit, Old German owl, Aachen luster shield, Italian owl, Hamburger

Sticken, French owl, Ghent owl, Luttich owl, Swedish owl, Tunesian owl, Figurita frill, Barbarisi owl, and Barbet of Liège [8, 10, 13].

Of these breeds, the best-known and most popular breed is undoubtedly the oriental frill pigeon without which no major exhibition on all continents of the world can do [12]. Exhibitions are one of the most effective measures aimed at improvement of the level of pigeon breeding. The determination of the breeding value by assessing pigeons has always been a major issue. The breeding efforts involving any species of animals require certain limits within which the given field must be oriented. The relevant indicators are the standards of breeds that serve as the tools which help the breeders to achieve a more optimal exterior as similar as possible to the ideal of the breed [2].

The main goal of this study was to point out the most common exterior deficiencies of owl pigeons and summarize the faults of selected short-beaked owl breeds observed at top breeding events.

MATERIALS AND METHODS

Selection of owl pigeon breeds

Due to the high number of owl and frill pigeon breeds and the extensive definitions of their standards, three breeds of short-beaked owl and frill pigeons were selected for this study. They are those most often presented at exhibitions and represent the most valuable breeding core of the whole group. Our study included short-beaked breeds: Oriental frill pigeon (Satinette and Blondinette), African owl pigeon and Turbit. The exterior of 722 owl pigeons of selected breeds was judged at 5 European exhibitions during the years 2018 and 2019. Their breeders came from seventeen European countries. Of the 722 owl pigeons on display, 591 were Oriental frills, 113 were African owls and 18 were Turbits (Table 1).

Table 1. Representation of exposed pigeons from individual countries

Country/pcs	Oriental frill	African owl	Turbit
Slovakia	114	36	7
Czechia	9	10	4
Hungary	109	5	0
Poland	48	24	0
Austria	23	0	0
Germany	61	6	1
Denmark	27	8	6
Romania	69	8	0
Bulgaria	63	12	0
Belgium	21	0	0
Italy	10	0	0
Netherlands	19	0	0
Norway	0	4	0
Croatia	6	0	0
Bosnia and Herzegovina	4	0	0
Serbia	2	0	0
Macedonia	6	0	0
Total	**591**	**113**	**18**

Assessment of owl pigeons

The assessment of pigeons at these shows takes place before the start of the show without public access. Unlike in exhibitions of other animal species, in small-animals (e. g. poultry, rabbits) exhibitions the judge does not know the name of the owner of the animal, which in some cases contributes to the objectivity of the report [17].

Pigeons are evaluated by trained assessors who have successfully passed theoretical and practical assessment exams, participate in trainings organized by the breeders' association and are active pigeon breeders. The assessment of a pigeon is a detailed comparison of a specific exposed individual with the relevant European standard. For a correct assessment of individual details and body proportions, many years of experience, a refined eye and knowledge of the specifics of individual breeds, plumage pigmentation and colour pattern, as well as the current trend or direction in breeding, are required. All exterior errors and advantages are processed in the judges' evaluation sheets and recorded on a pigeons' assessment card which is placed on each cage [13].

The European system for the assessment of the pigeon's exterior at shows emphasizes the main advantages and does not only point out the faults of the exhibited individuals. Each individual at the show is assigned a pigeons'

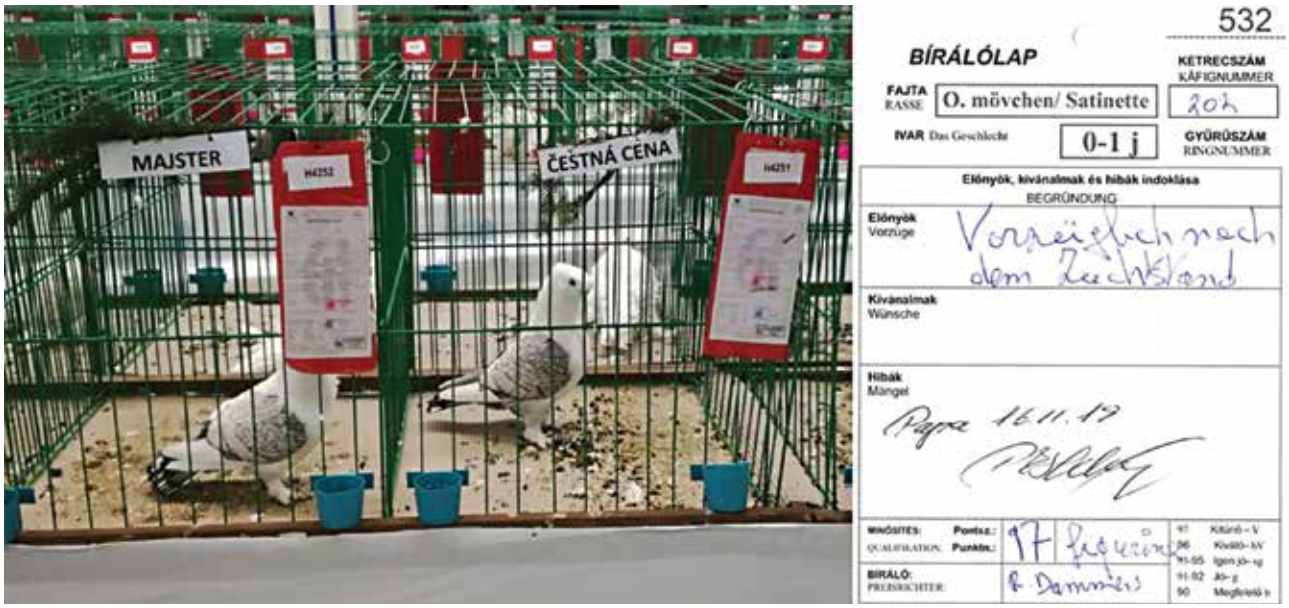

Fig. 5. Assessment of the pigeon's exterior with description of advantages and the final score
Photo: Adamčík (2019)

Table 2. Method of filling the assessment card—European approach

Points	Symbol	Verbal expression	Positive points	Wishes	Faults	Disqualifying faults in condition
97	E	excellent	4 and more	–	–	–
96	F	fine	3	1	–	–
95	VG	very good	3	2	–	–
94	VG	very good	3	3	–	–
93	VG	very good	2	4	–	–
92	G	good	2	2	1	–
91	G	good	1	3	2	–
90	S	satisfactory	1	3	3	–
0	US	unsatisfactory	–	–	4	1
0	NR	not rated	–	–	–	1

Source: B o c k o [12]

assessment card on which the following is listed: cage number, ring number (it also shows the year of hatching), sex (given by the breeder), breed, colour character, advantages, recommendations (small faults), deficiencies (major faults), exclusion deficiencies or deficiencies in breeder's care, evaluation (verbal), points and any award obtained, stamp of the show, date and stamp of the judge (Fig. 5).

Table 2. shows the method of filling the assessment card—European approach. The evaluation system is set so that the highest possible award that a pigeon can get without even one recommendation is 97 points with a verbal expression on the assessment card—a typical representative of the contemporary breeding [2].

Statistical analysis

Exterior faults and positive points in judged breeds were summarized and statistically compared from the judges' evaluation sheets. Statistical analysis was performed using software Microsoft Excel 2007. Chi square test ($\chi 2$ test) was used to compare the individual position between the selected breeds of pigeon. The dependence of the individual signs was tested at a significance level of $P < 0.05$.

The authors declare that there is no conflict of interest.

RESULTS

Tables 3 and 4 summarize the exterior deficiencies and advantages on the body and the head of the evaluated owl and frill pigeon breeds from the judges' evaluation sheets. The most common exterior faults on the body of oriental frill pigeons included defects in colour pattern or lacing, a poorly developed frill, deficiencies in positioning and body posture, and long wings and tail. Exterior faults observed on the head of oriental frill pigeons included a short

Table 3. Summary of the most common exterior faults and advantages on the body of selected breeds

Exterior faults	Oriental frill		African owls		Turbits		P
	pcs (591)	%	pcs (113)	%	pcs (18)	%	
Poorly developed frill	275	46.5	57	50.4	9	50.0	P < 0.05
Faults of other feathered ornaments	154	26.1	–	–	1	5.6	NS
Defects in colour pattern or lacing	309	52.3	11	9.7	4	22.2	P < 0.05
Faults in figure	179	30.2	52	46.0	8	44.4	P < 0.05
Defects in positioning and body posture	248	42.0	37	32.7	9	50.0	P < 0.05
Long wings and tail	130	22.0	12	10.6	4	22.2	P < 0.05
Drooping wings	7	1.2	2	1.8	–	–	NS
Exterior ideals and advantages							
Correct frill shape	316	53.5	56	49.6	9	50.0	P < 0.05
Correct breast width	412	69.8	61	54.0	10	55.6	P < 0.05

NS—non-significant

Table 4. Summary of the most common exterior faults and advantages on the head of selected breeds

Exterior faults	Oriental frill		African owls		Turbits		P
	pcs (591)	%	pcs (113)	%	pcs (18)	%	
Short top or forehead	238	40.2	31	27.4	11	61.1	P < 0.05
Long top	12	2.0	–	–	–	–	NS
Low forehead	159	26.9	–	–	–	–	NS
Defects in shape or length of the beak	139	23.5	34	30.0	8	44.4	P < 0.05
Defects in the beak line	156	26.4	30	26.5	3	16.7	P < 0.05
Defects in eye colour	55	9.3	3	2.7	–	–	NS
Exterior ideals and advantages							
Correct length of the top and forehead	341	57.8	78	69.0	7	38.9	P < 0.05
Correct height of the forehead	432	73.1	79	70.0	10	55.6	P < 0.05

Percentage and statistical significance ($P < 0.05$) are calculated from selected breeds of owls: 591 oriental frills, 113 African owls and 18 Turbits. NS—non-significant

top or forehead, defects in shape or length of the beak as well as defects in its line. It should be noted that more than 50 % of frills had correct frill shape with optimal length of the top and forehead.

The ratio of head to torso length must correspond to certain limits. The top and forehead of an Oriental frill pigeon are not as short as those in the African owl pigeon but not as long as in the Turbit. In correlation with the total length of the torso it represents a certain "middle path" in the typology of these breeds. In many cases, the course of the beak line (the dividing gap between the upper and lower beak jaws) which passes through the lower edge of the eyebrow and ends at the tip of the crest is some indicator of the correct head length.

DISCUSSION

Our study is based on 722 short-beaked owl and frill pigeons raised by breeders from seventeen European countries, evaluated at five important exhibitions in Europe. From this group, Oriental frills are currently the most popular, which was confirmed by the number of individuals (591) displayed at all exhibitions. It was evident that neither breeders nor judges prefer owl pigeons with extremely and unnaturally large heads, with faulty formation of the frill or stance or posture deficiencies, as had been the case in the past.

In some Oriental frill pigeons too long forehead was observed and cross-breeding of Turbits in past generations. It was evident in these individuals and it is a very correct action by the judge to point to this fault despite the fact that excessive elongation of the head of Oriental frill pigeons has become a trend for many leading breeders. In order to preserve the typology of the head of short-beaked owl pigeons it is essential that these deficiencies are addressed to also at smaller exhibitions. According to the experience of H e l w e g [6], the Danish expert on owl and frill pigeons, if these birds have excessively long head they also have a long torso of body. To compensate for such fault, the breeders try to obtain offspring with correct body length by cross-breeding with shorter individuals. In such cases, cross-breeding with width breast individuals may appear helpful as the visual perception of the figure (long body) changes.

The judges of Oriental owl and frill pigeons D a m e r s [3], V e s e l ý [13], emphasized that the correct proportionality of individual body parts and strong vitality and elegance of the exhibited individuals should be the essential criteria. In addition to the incorrect ratio of the individual parts, a frequent exterior deficiency was a poorly shaped frill or figure as well as defects in colour pattern or lacing which indicates that some breeders pay less attention to frill formation while refining the colour pattern and lacing or vice versa.

A number of exhibited oriental frills (Blondinettes) had figure deficiencies shaping and moderate faults in the purity of colour and pointing, particularly with respect to the arrow pointing on tips of their wings that must be pronounced in laced of frill pigeons. Another recommendation for breeders is to achieve the best possible improvement in the formation of the correct colour pattern or lacing and in the correct formation of feathered ornaments, body posture or height of the forehead.

Similar to oriental frill pigeons, defects in figure, shape, length and in the line of the beak, proportionality of individual body parts, formation of frill and deviation from proper posture were also observed in African owls. Our observations showed that many African owls raised by Danish, Bulgarian and German breeders had considerably longer body, long neck and some of them also longer posterior portion (back). Deficiencies in the formation of the frill and a more horizontal beak line were frequent particularly in German lines.

One can assume that these faults indicate cross-breeding with oriental frill pigeons with the aim to enlarge the head. On the contrary, the exhibited African owls raised by Slovak, Czech and Romanian breeders had short and wide bodies, full and wide forehead, full cheeks and the heads of these birds were properly rounded with a wide set beak. Their deficiencies included faults in formation of the frill and posture. Results of our evaluation allowed us to state that the Slovak and Czech lines of African owl pigeons were advantageous with respect to physique formation compared to other countries. Although the pigeons raised there had bigger heads but also had too long body and the posterior portion.

Unfortunately, the Turbit breed is currently the least bred breed of the selected three breeds of short-beaked owl and frill pigeons. Achieving the required head length in Turbit pigeons while maintaining a harmonic ratio between the head and body length seems to be a breeder's "tough nut", but it was achieved in some individuals

as demonstrated in exposed individuals. There are very few high quality Turbit lines in the world, but they can be found mainly among Slovak and Czech breeders. Some Turbit breeders resort to interbreeding to refresh the blood of their pigeons. Some lines of oriental frills with a long crest and forehead and a more horizontal beak line and the show racer breed seem to be suitable for this purpose [14].

According to W h i t e [14], the method of measuring the length of the head and the length of the sternum could be very helpful in future refining of Turbits. Almost 61 % of the representatives of this breed had too short crest and forehead and 50 % of individuals had fault in figure (long narrow body) and formation of frill. In addition, many individuals had defects in length and line of beak.

CONCLUSIONS

Owl and frill pigeon breeders should keep in mind that the breeding should focus primarily on the health of their birds and their natural resistance and vitality. Related to this is the need for the culling of individuals with morphological defects, i. e. the need for systematic eliminations of deviations from physiological development of pigeon figures. The European standards of the investigated breeds are set in a way that allows the breeders to use purposeful targeted refining activities in order to achieve animal lines ensuring success at exhibitions. This involves breeding activities ensuring harmonic body shape and conformity of proportions without the need to achieve unnatural dimensions of the head as was the trend in the past.

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OCCURRENCE AND PREVALENCE OF MACROPARASITES OF AFRICAN GIANT RATS (*CRICETOMYS GAMBIANUS*) IN A SAVANNA REGION OF NIGERIA

Amaechi, E. C.[1], Ade-Akanbi, P. A.[1], Olagunju, I. T.[1]
Okorie, C. A.[2], Ejike, B. U.[3]

[1]Department of Zoology, Faculty of Life Sciences, University of Ilorin
[2]Department of Animal and Environmental Biology, University of Port Harcourt
[3]Department of Biology/Microbiology, Polytechnic Aba, Abia State
Nigeria

ebubeamechi@yahoo.com

ABSTRACT

Rodents play an important role in the transmission of zoonotic diseases since they serve as reservoirs of these infections. A survey of ecto and intestinal parasites of the African giant rat (*Cricetomys gambianus*) was carried out between January and May, 2018 in Ilorin to access their potential as reservoirs of zoonoses. A total of 60 African giant rats were caught live using single catch rat traps which were set around bushes, near human habitation. The giant rats were euthanized with chloroform and thoroughly examined for ectoparasites. The giant rats were dissected and the alimentary canal removed. Direct smear floatation and sedimentation methods were used to detect endoparasites in the animals. The prevalence of different types of endoparasites were: *Hymenolepis nana* (33.3%), *Hymenolepis diminuta* (25.0 %), *Aspicularis tetraptera* (29.2%), and *Acanthocephala* spp. (12.5%), while the ectoparasites were: *Ixodes rasus* (37.9%), *Xenopsylla cheopis* (44.8%) and *Ornithonyssus bacoti* (17.2%). African giant rats harbour quite a number of infections that can be spread to humans especially in developing countries where most communities are economically disadvantaged, thus the need to properly enlighten the populace.

Key words: macroparasites; occurrence; rodents; savanna; zoonoses

INTRODUCTION

The African giant rat (*Cricetomys gambianus*) is a known reservoir of zoonotic diseases [2, 4, 6]. Rat-borne diseases are responsible for more deaths of humans than all of the wars in history [10, 15]. Rats destroy much of the food crops stored by the farmers. Zoonotic diseases are transmitted directly by rats through bites, urine and faeces [2, 5, 6, 12]. Indirect transmission of diseases can also take place through rat-borne ectoparasites such as the flea, *Xenopsylla cheopis*. Rodents are the most abundant order of mammals and are one of the few groups of ani-

mals that flourish in close associations with humans [7]. It is well known that members of the order Rodentia globally harbour different ecto and intestinal arthropods, of which many are vectors for diseases of medical and veterinary importance [10, 14]. Rats have successfully exploited a wide range of habitats and environments throughout the world [8]. The African giant rats (*Cricetomys gambianus*) are known to be a successful group of mammals basically as a result of its adaptability, high reproduction frequency and its ability to survive in a variety of ecological niches while exploiting a variety of food materials [4, 9, 14]. Rodents are known to pose great devastating risk to man acting as reservoirs of diseases thus increasing the possibility of acquiring rodent-borne zoonosis which is of major health concern to man [1, 3, 8]. Human parasitic infections acquired through transmission from wild rats such as African giant rats present a huge problem in tropical countries like Nigeria [8, 13]. The giant rat has been found to mainly live in the savanna region and also in the edges of the forest and in mountain areas 3,500 m above the sea level [4].

In Nigeria, only a few studies have been carried out on the ecto and intestinal parasitic fauna of the African giant rat, *Cricetomys gambianus* [1, 2, 3, 6]. However, there are no documented information on the ecto and intestinal parasitic fauna in *Cricetomys gambianus* in Ilorin, a savannah region of Nigeria. Data gathered from studies on the dynamics of parasitic lifecycle and the role of wild rats as vectors is essential for epidemiologist to carry out proper control efforts in managing rat-borne diseases.

This paper reports on the ecto and endo parasitic fauna of *Cricetomys gambianus* and their possible public health potentials in Ilorin, north central Nigeria.

MATERIALS AND METHODS

Ilorin, the capital of Kwara State, north central Nigeria is located within Longitudes 4°30′ and 4°5′E and Latitudes 8°25′ and 8°40′N. The soil of the area is typically coarse and sandy. The climate is tropical, with a mean annual temperature of 27 °C, relative humidity of 76 % and rainfall of 1,800 mm. The presence of tall trees and grasslands characterize the area. The general environmental conditions are suitable for the growth and survival of *C. gambianus*, which results in a high number in the study area.

Ethical statement

Ethical approval was sought and granted by the University of Ilorin Ethical committee.

The authors declare that there is no conflict of interest.

Animals

Altogether sixty (60) African giant rats were included in the study. The animals caught were kept in cages where they were properly fed with animal feed and water and monitored. Of the sixty rats examined, 48 (80 %) were males while 12 (20 %) were females..

Ectoparasite collection

The African giant rats captured were subjected to morphometric examinations. The rats were identified based on the descriptions from T a y l o r et al. [17]. Morphometric measurements of head, tail, ear and hind leg were recorded. The fur of each specimen was combed with a fine-tooth comb to dislodge any ectoparasite onto a white paper. Fine forceps were used to remove ticks and mites from the skin when it was difficult to dislodge them by combing. The contents on the white paper were examined carefully with a hand lens and later placed into specimen bottles containing 70 % alcohol, except for ticks which were mounted for identification. Mites and lice were cleared in lactophenol and mounted for identification. Morphometric characteristics such as weight and length of the animals were used to ascertain the parasitic abundance.

Endoparasites collection

The entire gut was removed and dissected into sections and placed in separate petri dishes that had saline solutions. The stomach and caecum contents were filtered and all endoparasites recovered were collected, counted and preserved in 70 % alcohol before further identification. Helminths were processed according to the different types. Endo and ectoparasites were examined, identified and determined using direct microscopic examination. Ectoparasites species was determined based on morphological characteristics while helminths were identified using keys of T a y l o r et al. [17].

Statistical analysis

The data were entered into Microsoft excel spreadsheet 2007 and a descriptive analysis was used to determine the prevalence, while the Chi-square test was employed to de-

termine the prevalence amongst the animals. All statistical analysis was performed using a statistical package for social sciences (SPSS) software package version 20.

RESULTS

Figure 1 shows the overall prevalence in the intestinal and ectoparasites in the sixty African giant rats. Of the rats examined, 24 (45.3 %) were infected with intestinal parasites, while 29 (54.7 %) had ectoparasites.

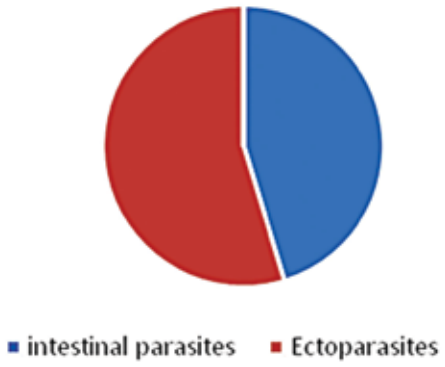

Fig. 1. Overall prevalence of intestinal and ectoparasites isolated from the giant rats in Ilorin

Prevalence of intestinal parasites in relation to sex

Of the 48 males studied, *Hymenolepis diminuta* had the highest prevalence of 20.8 %. Of the 12 female rats studied, *Hymeolepis nana* showed the greatest prevalence (16.7 %). *Acanthocephalan* was the least prevalent in males with 4.2 % while *Hymenolepis diminuta* was least (4.2 %) in females. The prevalence of ectoparasites in relation to sex, revealed three species identified infesting African giant rats, *Xenopsylla cheopis* had the highest prevalence of 44.8 %, while *Ornithonyssus bacoti* had the least prevalence of 17.2 % (Table 1).

The prevalence of ectoparasites in relation to sex, revealed three species identified infesting African giant rats, *Xenopsylla cheopis* had the highest prevalence of 44.8 %, while *Ornithonyssus bacoti* had the least prevalence of 17.2 % (Table 2).

Prevalence of intestinal and ectoparasites in relation to the length of the animal

The measurements of the body condition have long been used to infer the impacts of parasitism on an animal's nutritional state or overall wellness. If these conditions reflect the host health, then examining relationship between animal weight, length and infection status is a convenient way to measure parasite impacts on their hosts.

Table 1. Prevalence of intestinal parasites stratified by sex

Parasite isolated	No. examined	Total No. infected [%]	Sex	
			Male [%]	Female [%]
Hymenolepsis nana	60	8 (33.3)	4 (16.7)	4 (16.7)
Hymenolepsis diminuta	60	6 (25.0)	5 (20.8)	1 (4.2)
Aspicularis teraptera	60	7 (29.2)	4 (16.7)	3 (12.5)
Acanthocephala spp.	60	3 (12.5)	1 (4.2)	2 (8.3)

Table 2. Prevalence of ectoparasites stratified by sex

Parasite isolated	No. examined	Total No. infected [%]	Sex	
			Male [%]	Female [%]
Ixodes rasus	60	11 (37.9)	7 (63.6)	4 (36.4)
Xenopsylla cheopis	60	13 (44.8)	10 (34.5)	3 (10.3)
Ornithonyssus bacoti	60	5 (17.2)	2 (6.9)	3 10.3)

Table 3 shows the prevalence level in relation to the length of the rats. The number of intestinal and ectoparasites were found to increase slightly with an increase in the body size. Parasites with a body length of 62—64 cm length showed the greatest prevalence with 25.0 % in the intestinal parasites and 27.6 % in the ectoparasites.

Prevalence of intestinal and ectoparasites in relation to the weight of the animal

Table 4 shows the prevalence level in relation to the weight of the rats. There was a marked increase in the prevalence of the intestinal parasite in relation to the increase in the body weight. Rats weighing between 1.58—1.63 kg had the highest prevalence of 29.2 %, while rats with 1.38—1.42 kg had the lowest prevalence with 12.5 %.

DISCUSSION

This study is the first detailed account of intestinal and ectoparasites of public health importance in the Giant rats captured in Ilorin, north central Nigeria. African giant rats are known to harbour various types of parasites of which some are zoonotic and can cause death to humans [16]. These rats have adapted to living in close association with humans where they utilize human agricultural products

Table 3. Prevalence of parasitic fauna in relation to length of the animal

Length [cm]	**No. examined**	**Intestinal parasites** [%]	**Ectoparasites** [%]
47—49	9	2 (8.3)	2 (6.9)
50—52	12	3 12.5)	3 (10.3)
53—55	12	4 (16.7)	3 (10.3)
56—58	10	4 (16.7)	6 (20.7)
59—61	9	5 (20.8)	7 (24.1)
62—64	8	6 (25.0)	8 (27.6)
Total	**60**	**24 (47.2)**	**29 (54.7)**

Table 4. Prevalence of parasitic fauna in relation to the weight of the animal

Weight [kg]	**No. examined**	**Intestinal parasites** [%]	**Endoparasites** [%]
1.38—1.42	10	3 12.5)	3 (10.3)
1.43—1.47	14	4 (16.7)	5 (17.2)
1.48—1.52	14	4 (16.7)	6 (20.7)
1.53—1.57	12	6 (25.0)	7 (24.1)
1.58—1.63	10	7 (29.2)	8 (27.6)
Total	**60**	**24 (47.2)**	**29 (54.7)**

and waste as their food resources and buildings as their homes [11]. The need to understand the implications of the parasites harboured by the giant rats to humans and their environment is needed, thus the need for the present study. In this study, a total prevalence of 88.3 % of macro parasites (endo and ectoparasites) were recorded. This is higher than a prevalence of 55.3 % in Enugu south eastern Nigeria [4]. The high parasites prevalence recorded in this study is indicative of the possible transmission of zoonotic helminths from rodents to humans. This could occur as a result of consumption of uncooked or improperly cooked food contaminated with the infective larvae, eggs or metacercariae. Ectoparasites recorded in the study were 54.7 %, with flea (*Xenopslla cheopis*) having the highest occurrence. Other researches in other parts of this country reported similar findings [3, 9]. *Xenopslla cheopis* is known to be an important vector of plague, endemic typhus and parasitic cestodes such as *H. diminuta* and *Dipylidium caninum* [4]. *X. cheopis* is a potential intermediate hosts for *H. diminuta* usually found in grain storage facilities or in farms where grains are being stored. Ectoparasites were unevenly distributed on the host body and were found to be more predominant on the anterior trunk of the body than any other region of the body. Ectoparasites were not found in the tail region probably due to little amount of blood flow and/or reduced fur in this region.

In the present study, ectoparasites were more observed than intestinal parasites. This finding disagrees with the observation of M b a y a [9] in Maiduguri, north eastern Nigeria. The relatively high abundance of ectoparasites could be linked to the relative conducive environmental condition that allows the parasites to thrive. These high burdens indicate the importance of this rodents in the transmission of arthropod-borne diseases in the study area [7]. In the gut of the rats, *H. nana* had the highest occurrence which disagrees with the work of O k o y e, O b i e z u e [14] in Enugu, south east Nigeria. Both *H. nana* and *H. diminuta* are zoonotic cestode helminths, although *H. diminuta* is not common in humans. *H. diminuta* is transmitted to humans by the ingestion of *Tribolium confusum* (flour beetle, an intermediate host), with infested cereals, or by the faecal oral route. Faecal contents of rats infected with *H. diminuta* are attractive in some manner to beetles, and this is an evidence that a tapeworm is able to enhance its transmission chances by influencing the foraging of its intermediate host. *H. nana* is transmitted through faecal-oral contact (eggs) or by accidental ingestion of intermediate hosts harbouring cysticercoids [6]. In relation to sex, the male rats harboured more endoparasites than the female counterparts, a result similar to M b a y a [9] in Maiduguri. The higher intensity of infection seen in males could be suggestive of high activeness in search of food thereby making them more predisposed to eggs, cysts and larvae of parasites. Also, in terms of ectoparasites, more male rats harboured more parasites than their female counterparts. This finding is in agreement with the work of E k e h, E k e c h u k w u [4]. African giant rats with longer lengths tend to harbour more macro parasites (endo and ectoparasites). This agrees with the findings of E k e h, E k e c h u k w u [4]. This suggests that the longer the rats the higher their surface exposure to parasites. In relation to weight, rats with higher weights harboured more macro parasites. Larger species ingest more endoparasites and have more surface area for ectoparasites. Animals that eat larger volume of food have more exposure to parasites. This explains why larger and heavier giant rats harboured more parasites. Rodent species are ubiquitous and may serves as bridges between many different environments and parasite populations. As a consequence, a good number of rodent species have higher parasite loads. Hosts with larger size occupy larger ecological niches in which several parasites are present.

CONCLUSIONS

The information presented here updates our understanding of the major parasitic infections that African giant rats harbour and can be transmitted to humans and other animal populations in Ilorin, north central Nigeria. The possibility of these rats contaminating the environment, food and water sources with these parasites poses a public health threat since these rats live in close association with humans. Rat control measures should be applied to control giant rats as it has a public health risk.

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EFFECTS OF ORGANIC ACID BLEND ON GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY AND CONCENTRATION OF VOLATILE FATTY ACIDS IN THE FAECES OF YOUNG PIGS

Bujňák, L., Naď, P., Mihok, T.
Department of Animal Nutrition and Husbandry
University of Veterinary Medicine and Pharmacy in Košice
Komenského 73, 041 81 Košice
Slovakia

lukas.bujnak@uvlf.sk

ABSTRACT

The objective of this experiment was to evaluate the effects of a feeding diet containing a dry organic acid blend (lactic acid—ammonium formate—ammonium propionate—citrate—sorbate) in young pigs on their: performance, the apparent total tract digestibility of nutrients and the concentration of volatile fatty acids in their faeces. A total of 12 crossbred pigs (Slovakian White × Landrace) with an initial live weight of 12.78 ± 1.86 kg were divided into two dietary treatments. The experimental period lasted 28 days. Pigs were fed a control diet (control group) or a diet supplemented with a dry organic acid (OA) blend, 0.6 g per 100 g feed (experimental group). Compared with the control group, the average daily gain (ADG) was improved (P < 0.05) by OA blend over the period of the investigation (0—28 days). For the apparent total tract digestibility determination, ash which is insoluble in hydrochloric acid was used as a marker. The apparent total tract digestibility of crude protein and total ash was improved (P < 0.05) by the OA blend in the experiment. Compared with the control group, the concentration of the total volatile fatty acid in the faeces increased (P < 0.05) in pigs supplemented with the OA blend. The concentration of butyric acid in the faeces tended to be higher (P < 0.01) in pigs supplemented with the OA blend compared with the control group. In conclusion, the addition of 0.6 g per 100 g feed had a positive effect on: growth performance, total tract digestibility of crude protein and the concentration of volatile fatty acids in the faeces of young pigs.

Key words: digestibility; growth performance; organic acids; pigs; volatile fatty acids

INTRODUCTION

Young pigs, especially after weaning are exposed to various multiple stressors, including changes in diet composition, surrounding environment, and microbial challenges [6]. In-feed antibiotic growth promoters have been widely used for reducing harmful microbial activity and gastrointestinal disease in pigs after weaning [8, 27]. In

January 2006, antibiotic growth promoters were prohibited for use in animal feeds in the European Union. Due to the increasing safety concerns about the risk of spreading antibiotic resistance in the environment, and the presence of chemical residues in animal products, using organic acids to replace antibiotic in the diet of farm animals has increased considerably in recent years [19]. The inclusion of dietary organic acids as an alternative to antibiotic addition was evaluated in several studies using weaned pigs and in growing and finishing swine [1, 5, 24]. A number of studies have highlighted the potential effects of organic acids in improving digestion, nutrient digestibility and the promotion of growth performance in pigs [13, 14, 17].

The most commonly used organic acids include formic acid, fumaric acid, lactic acid, propionic and citric acids. Lactic acid has been reported to reduce gastric pH and to be more effective than other organic acid in improving the growth performance of pigs [25].

The results of organic acids blends are not always consistent, and the response to dietary organic acid blend could be affected by: the type of organic acids, dosage, feed formula, and the age of the animals [19]. The inconsistent responses to dietary acidifiers could be explained by: feed palatability, sources and composition of the diet, supplementation level of acidifier and the age of the animals [11].

This fact motivated us to determine the effects of feeding a diet containing dry organic acid blend in young pigs on: performance, total tract digestibility of nutrients and concentration of volatile fatty acids in the faeces.

MATERIALS AND METHODS

Animals, diets and experimental design

A total of 12 crossbred pigs (Slovakian White × Landrace) with an average body weight (BW) of 12.78 ± 1.86 kg were used for a 4-week trial (28 days). Pigs were divided into two groups; the control group (CG) and the experimental group (EG) with 6 pigs in each group. Both groups contained equal numbers of females (2) and castrated males (4).

The same feed components for the control and the experimental groups were used in the experiment: corn, wheat, barley, soybean meal, whey derivative, rapeseed oil and premix of minerals and vitamins. The diets used in this experiment were formulated to meet the requirements of the NRC [18]. The experimental group diet was supplemented with dry organic acid (OA) blended in an amount of 0.6 g per 100 g diet. The characteristics of the supplemented OA blend were as follows: lactic acid, ammonium formate, ammonium propionate, citrate, and sorbate (ash 39.0 %).

Diet analysis

The diets were analysed for their dry mater (DM), crude protein (CP), crude fibre (CF), ether extract (EE) ash as well as ash which is insoluble in hydrochloric acid according to the EC Commission Regulation [4]. The nitrogen free extract (NFE) was mathematically calculated according to the formula NFE = DM – CP – EE – CF – Ash. The concentrations of dietary metabolizable energy were calculated according to Šimeček et al. [23]. The results of the diet analyses are shown in Table 1.

Table 1. Chemical composition (g.kg^{-1}, as fed basis) of diets

	Control diet	Experimental diet
	Analysed content [g.kg^{-1}]	
DM	894.8	892.5
CP	185.5	188.5
EE	34.7	34.6
CF	32.7	33.0
Ash	49.5	56.9
NFE	592.4	579.5
ME [MJ.kg^{-1}]	13.65	13.60

DM—dry matter; CP—crude protein; EE—etheric extract; CF— crude fibre; NFE— nitrogen free extract; ME—metabolizable energy

Both groups (CG and EG) were fed twice per day. The pigs had free access to water *ad libitum*. Water was also provided directly in the trough during meals. Feed consumption and pigs' weight were recorded weekly to determine the average daily gain (ADG) and the feed conversion ratio (FCR) which were calculated at the end of the experiment. The investigation was carried out in the animal quarters of the Department of Animal Nutrition and Husbandry at the University of Veterinary Medicine in Košice.

Nutrient digestibility

For the apparent total tract digestibility determination, acid-insoluble ash was used as a marker. The faeces were taken directly from the rectum at the end of the investigation individually from each animal. The samples were immediately frozen at –20 °C until analysis. Each sample of faeces was pre-dried at 60 °C. All samples were ground to pass through a 1-mm sieve before analysis. Faecal samples were analysed in the same way as for diets.

Volatile fatty acid concentration

Fresh faecal samples collected on day 28 were used for the analysis of volatile fatty acids (VFA). The quantitative determination of the main volatile fatty acids (VFA): acetate, propionate, and butyrate, were done by the method of isotachophoresis employing a two-capillary analyser EA100 (VILLA LABECO, Slovakia). The total volatile fatty acid (TVFA) concentration in the faeces was calculated as a sum of acetate, propionate and butyrate concentrations.

Statistical methods

All data were reported as the mean ± SD (standard deviation). The differences between means were determined according to the unpaired t-test using Graph-Pad Prism software, USA). By conventional criteria, the differences $P < 0.05$ were considered to be of statistically significance.

Ethical statement

The research was approved by the UVLF Ethics Committee in accordance with applicable national and international animal welfare legislation.

The authors declare that there was no conflict of interest.

RESULTS

Growth performance

The means and standard deviations for growth performance, initial live weight of animals (day 0) and live weight at the end of the experiment (day 28) are given in Table 2. Compared with the control group, the pigs fed the experimental diet supplemented with a dry organic acid blend had greater ($P < 0.05$) average daily gains (ADG) (+45 g.day^{-1}). The feed efficiency in pigs was measured via feed consumed per unit of gain. The feed conversion ratio (FCR) which was measured as the feed intake over a period divided by the average daily gain (ADG). The FCR was a little higher in pigs fed the control diet (+0.02 kg feed.kg^{-1} gain) compared to the experimental group.

Table 2. Effects of organic acid blend supplementation on the performance of the pigs (means ± SD)

Diet	Control (CG) n = 6	Experimental (EG) n = 6
	Live weight [kg]	
Initial (day 0)	12.83 ± 1.82	12.72 ± 1.89
Final (day 28)	30.20 ± 2.87	31.35 ± 2.90
ADG [g]	620.4^{A} ± 35.2	665.3^{B} ± 34.3
FCR gain [kg feed.kg^{-1}]	1.79	1.77

ADG—Average daily gain; FCR—Feed conversion ratio; SD—Standard deviation; A, B—significant at $P < 0.05$

Apparent nutrient digestibility

Our findings showed a tendency for an improvement of the apparent total tract digestibility of nutrients after supplementation of the diet with the mixed organic acids in young pigs (12 to 30 kg of live weight). Compared with the control group, pigs fed with the dry organic acid blend increased ($P < 0.05$) the apparent total tract digestibility of crude protein and ash, by approximately 2.9 % and 4.6 % respectively (Table 3). It is generally considered that dietary organic acids lower the gastric pH, resulting in increased activity of proteolytic enzymes and thus improved the protein digestion. As regard to ash, it may be also due to the higher content of ash in the experimental diet after the dry organic acid blend administration.

The apparent total tract digestibility of fat, crude fibre

Table 3. Coefficients of digestibility (CD) in pigs (Mean ± SD)

Parameter [%]	Control group	Experimental group
CP	78.50^{A} ± 2.02	81.40^{B} ± 1.77
Fat	65.70 ± 2.83	67.20 ± 2.92
CF [%]	33.50 ± 2.88	34.50 ± 2.62
Ash [%]	39.30^{A} ± 2.98	43.90^{B} ± 2.77
NFE [%]	85.20 ± 2.06	87.50 ± 1.80

CP—crude protein; CF—crude fibre; NFE—nitrogen free extract
A, B—significant at $P < 0.05$

and nitrogen free extract were also improved (fat +1.5 %, crude fibre +1 % and nitrogen-free extract +2.3 %) in pigs supplemented with a diet containing a mixed of organic acids compared to the control group; but these differences were not statistically significant. The NFE represents soluble carbohydrates and other digestibles and easily utilizable non-nitrogenous substances in the feed.

Volatile fatty acids in the faeces

Compared with the control group, the concentration of the total volatile fatty acids (TVFA) in the faeces was improved ($P < 0.05$) in pigs supplemented with the organic acid blend (Table 4). The content of acetic and propionic in the faeces were also increased by the supplement of the organic acids blend compared with the control group, but these differences were not statistically significant. We observed higher ($P < 0.01$) concentration of butyric acid in the faeces of pigs from the experimental group compared with the control group.

Table 4. Effects of organic acid blend on the volatile fatty acids concentration in the faeces (g.kg^{-1}) (means ± SD)

	Control group	Experimental group
Acetic acid	3.73 ± 0.38	4.15 ± 0.40
Propionic acid	2.46 ± 0.21	2.76 ± 0.30
Butyric acid	1.22^{A} ± 0.10	1.46^{C} ± 0.09
TVFA	7.41^{A} ± 0.69	8.37^{B} ± 0.79

TVFA—Total volatile fatty acids;
A, B—significant at $P < 0.05$; A, C—significant at $P < 0.01$

DISCUSSION

Acidifiers are often used as alternatives to antibiotic growth promoters because of their ability to create a favourable intestinal environment for the beneficial microbes which may result in increased nutrient digestibility and increased growth performance [12]. Dietary acidifiers may be organic or inorganic acids or salts of acids [21, 22]. In our study, we reported the effects of feeding a diet containing a dry organic acid blend on the: performance, total tract digestibility of nutrients and concentration of volatile fatty acids in the faeces of young Slovakian White x Landrace pigs (12 to 30 kg of live weight). The applied organic acid (OA) blend consisted of a mix of: lactic acid, ammonium formate, ammonium propionate, citrate, and sorbate.

In the results of the current study, ADG was positively significantly affected by dietary inclusion of dry organic acid blend in the experimental diet (0.6 g OA blend in 100 g diet). Also the FCR parameter was improved in pigs fed the experimental diet.

This is in agreement with G r i l i et al. [7], who reported that the addition of a mixture of citric acids and sorbic acids resulted in improved growth performance of pigs. Likewise, the addition of a blend of organic acids (fumaric, lactate, citric, propionic, and benzoic acids) followed by a blend of phosphoric, fumaric, lactic, and citric acid improved the growth performance of newly weaned pigs in the study of W a l s h et al. [26].

Similarly, Ø v e r l a n d et al. [20] also reported that weaned pigs fed with a diet containing formic acid had a significantly improved ADG and feed efficiency compared with pigs fed a diet without OA supplementation. The study of the L o n g et al. [13] demonstrated that organic acids supplementation, which contained butyrate, sorbic acid combined with other short and medium chain fatty acids significantly improved ADG and feed efficiency in weaned pigs compared with a control group during all periods of the experiment.

The improved performance in pigs fed diets containing OA blend in the present study could be associated with greater digestibility of the organic nutrients and total ash. The improvement in apparent total tract digestibility (ATTD) of nutrients for pigs fed with OA blend in this study compared with the control group is in agreement with the results of M r o z et al. [16], who reported that the ATTD of crude protein was increased by OA (especially formic acid) supplementation, likely because OA can improve the activity of pepsin and trypsin.

It is generally known that dietary acidifiers lower gastric pH, resulting in an increased activity of proteolytic enzymes and improved protein digestibility [11]. Although the organic acid supplementation was initially targeted for weaned piglets, there is growing evidence that dietary acidification may also be beneficial for the performance of fattening pigs [22]. Some previous results have indicated that in fattening pigs, organic acids improved the apparent ileal digestibility of protein and amino acids [10, 15, 16] and absorption of minerals [9].

The volatile fatty acids, produced by microbial fermentation of carbohydrates in the gastrointestinal tract, are beneficial to the animal [2]. In the current study, OA blend as a feed additive resulted in a significantly higher TVFA content in the faeces. Although the differences among the coefficients of digestibility of CF and NFE were not statistically significant, the higher apparent total tract digestibility of CF and NFE (+1 % and 2.3 % respectively) in pigs from the experimental group indicated that the OA blend promoted the microbes to utilize carbohydrates to produce volatile fatty acids.

The results of the L o n g et al. [13] study indicated that the concentration of TVFA in faeces was significantly improved in pigs supplemented with mixed OA and the content of acetic, propionic and butyric acid in faeces were also increased by the supplement of organic acids compared with the control group.

The volatile fatty acids, especially butyric acid, produced by fermentation of carbohydrates in the large intestine, had a positive effect on the epithelial cell growth and absorptive functions in pigs [2, 3], which partly agrees with our results.

CONCLUSIONS

The present study demonstrated that the overall growth performance was improved in pigs fed diets supplemented with mixed organic acids when compared with pigs fed with the control diet without organic acid blend. The results could be due to the improvement in apparent total tract digestibility of total proteins and ash by mixed organic acids supplementation. The results suggested that an organic acid blend can be used with positive effects on: the performance, protein digestibility and total volatile fatty acids content in the faeces in the category of young pigs (12 to 30 kg of live weight).

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CONTAMINATION OF SLOVAK BILBERRY (*Vaccinium myrtillus* L.) WITH RADIOCAESIUM ^{137}Cs IN SELECTED SLOVAK LOCATIONS

Beňová, K.[1], Gašpareková, I.[2], Dvořák, P.[2], Havelková, A.[3]

[1]University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice Slovakia

[2]Faculty of Veterinary Hygiene and Ecology, University of Veterinary Sciences Brno Palackého 1946/1, 612 42 Brno

[3]Faculty of Medicine, Masaryk University, Kamenice 753/5, 625 00 Brno Czechia

katarina.benova@uvlf.sk

ABSTRACT

The aim of this study was to determine the activity of post-Chernobyl ^{137}Cs in Slovak bilberry (*Vaccinium myrtillus* L.) from selected locations of Slovakia depending upon: the season, location and the soil-plant transfer factor (TF). The ^{137}Cs activity was determined in samples of forest soil and bilberry bushes (stems, leaves and fruits) growing on the soil in three locations: Arboretum Mlyňany (1), Hodruša-Hámre (2) and Javorníky (3). Altogether we collected 35 samples; namely 15 samples from Arboretum Mlyňany, 10 samples from Hodruša-Hámre and 10 from Javorníky. The samples of soil were examined also for the activity of ^{40}K. The samples were collected in the spring, summer and autumn in the years 2019 and 2020 and were analysed by a gamma-ray spectrometry method. The highest levels of ^{137}Cs activity concentrations were determined in Hodruša-Hámre in September where they reached 161 ± 13 Bq.kg^{-1} in the soil, 3.95 ± 1.07 Bq.kg^{-1} in the stems and 14.7 ± 4.26 Bq.kg^{-1} in the leaves. The lowest levels were determined in the Arboretum Mlyňany in October where the ^{137}Cs activity in the soil reached 13.1 ± 1 15 Bq.kg^{-1}, while in the stems, leaves and fruits it was lower than the minimum detectable level. In the latter location, we also determined the highest activity of ^{40}K, the radioactive isotope physiologically and metabolically similar to ^{137}Cs. The mass activity of ^{137}Cs in all samples of bilberries were lower than the minimum detectable activity (MDA). The comparison of the results obtained in this study with the limit for total mass activity of radiocaesium (^{134}Cs + ^{137}Cs) in the food (600 Bq.kg^{-1}) allowed us to conclude that the fruits, stems and leaves of Slovak bilberries present neither health nor environmental risk.

Key words: gamma-ray spectrometry; radioactivity; radiocaesium; *Vaccinium myrtillus* L.; transfer factor

INTRODUCTION

Slovak bilberry (*Vaccinium myrtillus* L., Ericaceae) belongs among the boreal bushes with densely entwined

roots and individual plants occupying sometimes an area of several square metres.

Bilberry fruits have been an important part of local diets in many countries, including Slovakia. They are valued for their pleasant taste and aroma and are often processed into jams, preserves, juices, and alcoholic beverages. They are rich in anthocyanins which make for the intense dark purple coloration of the fruit, as well as all processed foods made from the berries [26]. Their high market value is caused by their relatively difficult availability—bilberry bushes only grow in the wild, montane areas. It is not possible to cultivate them due to very specific soil demands and the fruit harvesting is a tedious, tiring work, as it is done using either by hands or small harvesting rakes [18, 28].

European Medicines Agency considers bilberry fruits a traditional herbal medicinal product that is used either for treatment of mild diarrhoea and oral mucosa inflammation, or for the treatment of capillary fragility and heavy legs caused by venous circulatory disturbances [8]. Fruits are used in teas, juices, wine, tinctures, and capsules also against fever, cold and night blindness and stomach diseases. Tea from bilberry stems and leaves have been used as a supportive therapy in mild forms of *diabetes mellitus* [14].

Even decades after the Chernobyl nuclear accident, radiocaesium ^{137}Cs can under some conditions pass to plants and bushes growing on ^{137}Cs contaminated soil. Radiocaesium (mainly ^{134}Cs and ^{137}Cs) are the predominant contributors to radiation exposure after nuclear accidents because they have a relatively long half-life—^{134}Cs has a half-life of 2.06 years and ^{137}Cs 30.2 years—highly transferable, and wide distribution in the environment [25]. Higher levels of ^{40}K in the growth media are known to reduce translocation of radiocaesium to plants [19].

The collection and consumption of bilberry plants and fruits are very popular in Slovakia which stimulated our interest in their health safety [2]. Bilberry bushes are capable of accumulating radiocaesium [5] and due to their spreading over large areas and easy identification and sampling they are considered as a bioindicator plant [24].

The uptake of radionuclides from soil by plants is expressed by means of a soil-plant transfer factor (TF), a tool in the form of a mathematical equation. TF is essential for environmental transfer models, which are useful for the prediction of radionuclide concentration in agricultural crop [7].

The aim of our study was to determine by means of gamma-ray spectroscopy the mass activity concentration of radiocaesium ^{137}Cs in samples of bilberry plans and soil collected from selected locations in Slovakia with dependence on: the date of collection, character of collection sites and soil-plant transfer factor.

MATERIALS AND METHODS

Our investigations focused on the determination of mass activity level of ^{137}Cs (Bq.kg^{-1}) by gamma-ray spectrometry in samples collected in selected mountain and sub-mountain locations in different seasons of the year—spring, summer, autumn. No samples were collected in winter due to the absence of plant parts and limited access. The selected locations differed in composition of soil and other factors that could affect the soil-plant transfer factor. The samples of soil collected in our study were analysed also for the activity of ^{40}K due to the similarity of this isotope with ^{137}Cs and its influence on plant uptake of Cs.

The samples of Slovak bilberry bushes and soil were collected from 3 locations in the Slovak territory: 1—Arboretum Mlyňany (land register Vieska nad Žitavou, close to the village of Zlaté Moravce); 2 —Hodruša-Hámre (Protected landscape area Štiavnické vrchy); and 3—Javorníky (Fig. 1). These locations were selected on the basis of the presence of forest bilberries. Bilberry prefer soils with acidic pH [4]. Geophysical maps of soils in Slovakia was provided by the State Geological Institute of Dionýz Štúr which allowed us to identify the suitable areas. Within them, we subsequently identified areas with strong areal activity concentration of ^{137}Cs, i. e. the area of Štiavnické vrchy, the vicinity of the village of Zlaté Moravce and Javorníky.

Because one of the selected areas was situated in the Protected landscape area (site 2), according to the Act No. 543/2002 Coll. on Nature and Landscape Protection we had to obtain a permit to collect fruits and plant parts from this area. A similar situation applied to location 1 where sampling was conducted after obtaining permission from the Arboretum owner.

In the year 2019 we collected samples from the area in autumn (October, November) and in 2020 in the spring and summer (May, June, September) using the standard procedure – manual collection to glass or plastic sampling bottles (fruit and soil) or to cloth bags (shoots) in order to ensure the ventilation and breathing of the plants.

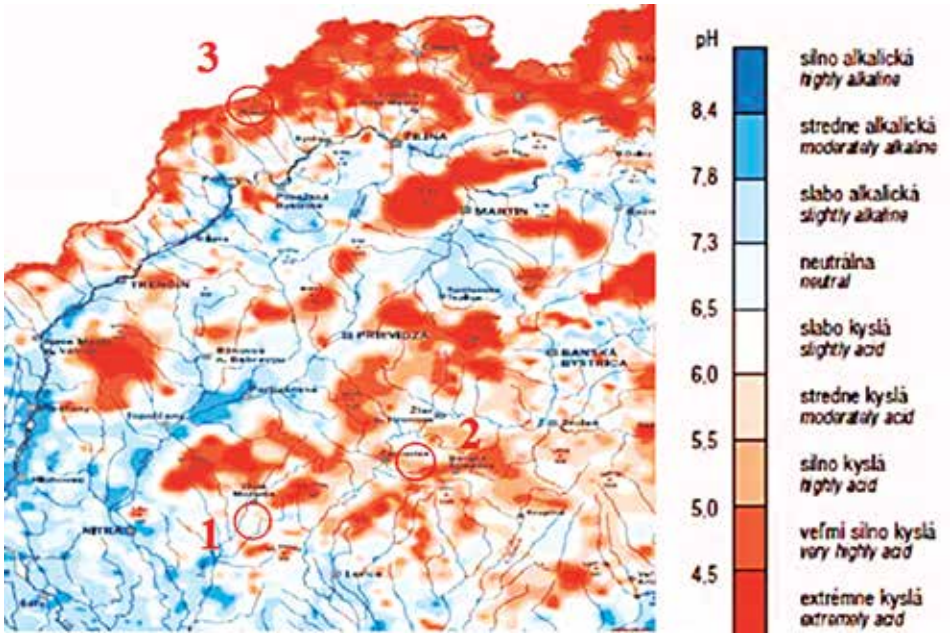

Fig. 1. Sites of collection of samples of soil and bilberry bushes: 1—Arboretum Mlyňany; 2—Hodruša-Hámre); 3—Javorníky. Elevation in metres above sea level (MASL): 1 = 165; 2 = 375—800; 3 = 905—1070

First we collected soil samples close to the growing bushes to the depth of 5—10 cm from the area of about 100 cm^2 (10 × 10 cm) by means of a garden spatula (total volume approx. 500 ml). Then we collected shoots of individual bushes to cloth bags by garden scissors or knife. We collected material from several places in order to sample the biggest possible area. Berries were collected in autumn in glass bottles. The volume of the samples were sufficient to fill up the measurement vessels. We collected all together 35 samples; i. e. 15 from Arboretum Mlyňany, 10 from Hodruša-Hámre and 10 from the sampling site Javorníky.

Dirt, stones, vegetation residues and other impurities were removed from the soil samples. Shoot samples were divided to stems and leaves and stem samples were subsequently cut to about 2 cm long pieces. Plant samples were frozen and transported together with soil samples to the Veterinary and Pharmaceutical University in Brno, the Czech Republic to the Central workplace for utilization of ionizing radiation where the relevant gamma-ray spectrometric determinations were carried out.

For examination, the samples were placed in Marinelli vessels of volume 450 ml or 200 ml vessels (samples from site 1). Two path gamma-ray spectrometry was used to determine the activity of gamma radiation emitting radionuclides ^{137}Cs and ^{40}K. The measurements were based on the use of a germanium detector (HPGe) with relative effectiveness—HPGe GC2020 20 % and HPGE GC 4018 40 %, with resolution of both detectors equal to 1.8 keV. Software Genie (Canberra) and Gamwin (Nuwia Třebíč) were employed. Individual paths were checked by the Czech Metrological Institute.

In addition to mass activity concentration, also total combined standard uncertainty (u_a) was calculated according to the equation:

$$u_a = (u_E^2 + u_p^2 + u_y^2 + u_s^2 + u_{ss}^2 + u_r^2 + u_t^2 + u_A^2 + u_M^2)^{½}$$

where

- u_E relative uncertainty of effectiveness,
- u_p relative uncertainty of peak area,
- u_y relative uncertainty of yield,
- u_s relative uncertainty of sum of temporal coincidences,
- u_{ss} relative uncertainty of electronic stability,
- u_r relative uncertainty of decay,
- u_T relative uncertainty relative uncertainty of time,
- u_A relative uncertainty relative uncertainty of self-absorption,
- u_M relative uncertainty of reproducibility.

The transfer factor (TF) as a value determining the availability of ^{137}Cs from the soil for the root system of plants was determined. It was calculated as a proportion of the activity of the ^{137}Cs in the plant tissue and the activity of ^{137}Cs in the soil (expressed Bq.kg^{-1}) according to B u r g e r and L i c h t s c h e i d l [4].

$$\text{Transfer factor} = \frac{\text{Cs activity in plant (Bq.kg}^{-1})}{\text{Cs activity in soil (Bq.kg}^{-1})}$$

The statistical evaluation was carried out by means of MS Excel software comparing individual results that reached values exceeding MDA. The ^{137}Cs activity in samples collected in individual locations in different seasons was compared using ANOVA Single factor test. Differences in results of soil, stems and leaves from the sites Hodruša-Hámre and Javorníky were evaluated by the paired t-test. The level of $P < 0.05$ was considered significant.

The authors declare that there is no conflict of interest.

RESULTS

The results of the mass activity concentration of ^{137}Cs in individual samples collected from soil and Slovak bilberry bushes grown in the soil are presented in Tables 1—3. In addition to ^{137}Cs, the tables contain also results of the mass activity concentration of ^{40}K detected in the soil samples.

The comparison of the activity concentration of ^{137}Cs in bilberry plant parts and fruits detected in our study showed that this radionuclide level was much lower than the current Slovak limit for the total mass activity of radiocaesium (^{134}Cs + ^{137}Cs) in food (600 Bq.kg^{-1}) [23].

The statistical evaluation of our results showed that there were no significant differences in the activity of ^{137}Cs between the locations of Hodruša-Hámre and Javorníky.

Although no significant differences in the activity of ^{137}Cs in soils were observed between the seasons, decreases in the mass activity of ^{137}Cs in the soil in October – November was observed in all locations.

Table 1. Summary of the mass activity concentration of ^{137}Cs and ^{40}K in samples obtained from collection site 1—Arboretum Mlyňany

Date of sampling	Sample type	^{137}Cs [Bq.kg^{-1}]	Standard uncertainty [Bq.kg^{-1}]	^{40}K [Bq.kg^{-1}]	Standard uncertainty [Bq.kg^{-1}]
4th Oct., 2019	Soil	19.4	1.87	79	6.29
	Soil	13.1	1.15	291	23
	Stems A	< 3.80	0.291	–	–
	Stems	< 21.0	1.16	–	–
	Leaves	< 7.90	0.614	–	–
	Leaves	< 9.10	0.714	–	–
	Leaves A	< 16.0	1.28	–	–
	Fruit A	< 15.0	1.14	–	–
7th June, 2020	Soil	13.4	0.732	293	13
	Leaves A	< 22.0	1.68	–	–
	Stems	< 8.70	0.674	–	-
7th Sept., 2020	Soil	40.1	3.71	625	49.5
	Stems	< 16.0	1.27	–	–
	Leaves A	< 8.40	0.653	–	–
	Fruit A	< 5.00	0.788	–	–

A—longer detection time due to small sample; <—indicates value below minimum detectable activity (MDA)

Table 2. Summary of the mass activity concentration of ^{137}Cs and ^{40}K in samples obtained from collection site 2—Hodruša-Hámre

Date of sampling	Sample type	^{137}Cs [Bq.kg^{-1}]	Standard uncertainty [Bq.kg^{-1}]	^{40}K [Bq.kg^{-1}]	Standard uncertainty [Bq.kg^{-1}]
23rd Nov., 2019	Soil	95.2	4.98	54.6	2.49
	Stems	5.35	1.99	–	–
	Leaves	< 150	11.5	–	–
15th June, 2020	Soil	48.2	3.96	< 89	6.9
	Stems	< 19.0	1.48	–	–
	Leaves	< 25.0	1.98	–	–
7th Sept., 2020	Soil	161	13.0	< 230	18.4
	Stems	3.95	1.07	–	–
	Leaves	14.7	4.26	–	–
	Fruit	< 9.80	0.767	–	–

<—indicates value below minimum detectable activity (MDA)

Table 3. Summary of the mass activity concentration of ^{137}Cs and ^{40}K in samples obtained from collection site 3—Javorníky

Date of sampling	Sample type	^{137}Cs [Bq.kg^{-1}]	Standard uncertainty [Bq.kg^{-1}]	^{40}K [Bq.kg^{-1}]	Standard uncertainty [Bq.kg^{-1}]
3rd Nov., 2019	Soil	54.1	2.54	89.2	4.25
	Stems	< 5.70	0.44	–	–
	Leaves	< 17.0	1.32	–	–
3rd May, 2020	Soil	112	5.18	184	8.71
	Stems	4.02	1.19	–	–
	Leaves	2.05	0.392	–	–
15th, Sept., 2020	Soil	80.5	3.73	234	11
	Stems	< 16.0	1.25	–	–
	Leaves	< 30.0	2.32	–	–
	Fruit	< 29.0	2.24	–	–

<—indicates value below minimum detectable activity (MDA)

The results of the calculation of the transfer factor (TF), the parameter expressing the uptake of radionuclides from soil by the plants, are presented in Table 4. This factor was calculated from the results above the minimum detectable activity.

The transfer factor was determined only for samples with the activities of radiocaesium above MDA, i. e. from the sampling site 2—Hodruša-Hámre collected in November 2019 and September, 2020, and 3—Javorníky, collected in May, 2020, when the activities of radiocaesium were above the MDA. Because the activities in the leaves collected in site 2 in November 2020 were below MDA, the TF was calculated only for soil-stems transfer. For the same reason, the transfer factor was not determined for samples collected in the sampling site 1—Arboretum Mlyňany.

Table 4. Transfer factor calculated for collection sites 2—Hodruša-Hámre and 3—Javorníky

Collection site and date	Transfer factor	
2—Hodruša-Hámre 23rd Nov., 2019	soil—stems soil—leaves	0.056 –
2—Hodruša-Hámre 7th Sept., 2020	soil—stems soil—leaves	0.024 0.091
3—Javorníky 3rd May, 2020	soil—stems soil—leaves	0.036 0.018

DISCUSSION

The Chernobyl nuclear accident resulted in a massive release of large amounts of radioactive material into the atmosphere and its spreading by wind. This radioactive contamination reached also the Slovak territory. The number of deposited particles and the intensity of the settling radionuclide aerosol were affected by many factors including the explosion itself (force, height) and meteorological situation at the affected territory (particularly precipitations) [6]. The long half-life (30.2 years) of ^{137}Cs resulted in long persistence of this radionuclide, its low degradability and long-term biological availability [22].

Despite the fact that the Chernobyl accident occurred 35 years ago, traces of radioactive contamination are still detected in Slovakia as indicated by our investigation of soil and bilberry plants and fruit. The aim of this study was to point to the factors, either natural or anthropogenic, that can affect the process of migration of ^{137}Cs in soil or transfer from soil to plants and can result in potential differences in mass activity of this radionuclide between individual locations or yearly seasons. Thus, we tried to confirm or reject the hypothesis that there may exist significant differences in the mass activity concentration of ^{137}Cs, determined by gamma-ray spectrometry, in relation to location and the time of sample collection.

When analysing environmental samples, the measurement of radioactivity of samples depends on several factors, namely: type and energy of radiation, activity, size and shape of samples, etc. The total effectiveness of methods is affected not only by the effectiveness of the respective detector but also the so-called geometry of measurement. Geometry of measurement is a spatial relationship and configuration of the measured sample or radioactive beam in relation to the detector [27].

The < MDA designation used for some samples in enclosed tables indicates small amount, weight or size of the sample and geometry of measurement which may have affected the radionuclide activity determination.

According to our results there were no significant differences in the activity of ^{137}Cs between locations Hodruša-Hámre and Javorníky. In the location Arboretum Mlyňany the values were below MDA, i. e. the ^{137}Cs activity was low. The samples originated from a botanical institution with collection of woody species and all-year round care in the form of manuring, supplementation of nutrients or other maintenance interventions. According to B u r g e r, L i c h t s c h e i d l [4], the key factors affecting the content of radiocaesium include not only soil composition but also its amendment and cultivation in the form of various anthropogenic activities such as turning, hoeing and others. Such activities are expected to support penetration of caesium to deeper layers of the soil and its migration. This may be important for shallow-rooted plants reaching to the depth of up to 10 cm, thus also bilberries [16], as the content of caesium in surface soil layers may be reduced by diffusion or mass flow. This could serve as an explanation why the activity of ^{137}Cs in soil in Arboretum Mlyňany was lower in comparison with locations 2 and 3. Another specific feature of this location was the highest level of ^{40}K. G u i l l é n et al. [10], A l m a h a y n i et al. [1], S a l t, M a y e s [20] reported that ^{137}Cs is physiologically and metabolically similar to potassium. Plants take up this nutrient by their root system. There exists a threshold for potassium concentration in soil above which the plants prefer potassium to caesium. Potassium fertilizers can thus be used as an effective mean for decreasing translocation of radiocaesium by saturation of soil with nutrient chemically similar to this radionuclide. Thus, it is possible that the above anthropogenic interventions and supplementation of soil with nutrients in the form of fertilizers could reduce the mass activity of ^{137}Cs and its soil-plant transfer. We observed variations in the activity of ^{40}K in location 1. The highest activity of this radionuclide in the soil was detected at the beginning of September and the lowest in October. According to S a l t, M a y e s [20], the seasonal changes in concentration of potassium in plants with their growth are related to atmospheric conditions, supply of nutrients and developmental stage of the plant.

The highest activity of ^{137}Cs, either in the soil or in plants was detected in location 2. This result corresponds

to the final transfer factor which was insignificantly higher compared to location 3. Results for soil-stems transfer were higher in November than in September. The nutrient requirements in individual stages of the development of plants and fruits vary. The transfer factor is high in the initial stages, gradually decreases as fruits began to develop and is the lowest during their ripening [27]. The transfer factor between locations 2 and 3 may differ owing to different soil properties or atmospheric conditions that affect the growth and development of plants and their fruit [10]. According to these authors, the very low level of ^{40}K should be reflected in increased activity of ^{137}Cs in plants. The sampling sites in locations 2 and 3 were sites exposed to minimal human intervention so persistence of ^{137}Cs in upper soil layers was expected. It should be mentioned that samples originating from locations 2 and 3 were collected in a coniferous forest. According to Daniel et al. [6], higher levels of ^{137}Cs are expected in forests with presence of fallen leaves and fir-needles that increase the contamination of upper soil layers. The activity of ^{137}Cs in the bilberry plant and fruit are affected by: soil properties, content of minerals and pH, as mentioned by Matsuoka et al. [17]. These authors reported that low pH of soil can increase radiocaesium levels in bilberry plants and fruit. In the investigated locations silt soil with varying amounts of sand and clay prevailed. According to Sanchez et al. [21], low pH causes mineralisation of organic matter in the soil and NH^{4+} ions are produced. They accelerate mobility of ^{137}Cs in soil solution making it more available to plant roots.

In comparison with Hodruša-Hámre and Javorníky, less acidic soil prevails in Arboretum Mlyňany and this is reflected also in the activity of ^{137}Cs which was very low. According to Daniel et al. [6], the mobility of ^{137}Cs is the lowest at soil pH ranging from 4 to 7. The transfer and migration of this radionuclide gradually slows down with time in relation to texture and type of soil or mobility of soil microorganisms. Guillén et al. [10] also mentioned that the accumulation of radiocaesium in stems, leaves and fruits of berries may differ depending upon the atmospheric conditions, e.g. temperature and precipitations. During dry and hot periods the activity of ^{137}Cs decreases due to more rapid maturation of plants and fruit in comparison with more humid and colder seasons. It should be mentioned that the activity of ^{137}Cs is also affected by the altitude—the higher the elevation, the higher the activity of ^{137}Cs [6]. The height above sea level affects indirectly radiocaesium transfer which is affected also by other direct factors such as soil properties. Forest soil at higher elevation generally contains more humus and its pH is lower. The mobility of radiocaesium and its availability to plants is thus higher in such soils [5]. The radiocaesium activity detected in our study was higher in locations 2 and 3 located at higher elevation than in location 1.

According to Daniel et al. [6], Guillén et al. [10], the content of clay in soil is another important factor with respect to radiocaesium activity in soil. This is in fact the key factor, as caesium can be irreversibly adsorbed to clay and its transfer to plants cannot occur. Clay serves as the main sorption site for radiocaesium.

The mass activity concentration in the investigated samples of stems, leaves and fruits was considerably lower than that determined in soil samples. Study by Grabovskyi et al. [9] showed a gradual decrease in contamination of vegetative and generative organs of plants with ^{137}Cs over time. A considerably higher reduction was recorded in plant parts and fruits of bilberries in comparison with soil samples. The authors explanation is that the availability of ^{137}Cs molecules present in soil for plant roots decreases. Should this tendency in contaminated areas persist in future, the activity of ^{137}Cs in free nature will be rapidly decreased. Reduced mobility and transfer of ^{137}Cs to plants is indicated by the study of Kruyts, Delvaux [15] due to the mixing of organic matter with minerals owing to intensive biological activity, for example by movement of soil fauna.

The analysis of samples collected in locations Hodruša-Hámre and Javorníky showed no significant differences. The insignificance of differences could be ascribed to different atmospheric conditions, soil moisture and type or pH in these locations [9, 10, 13, 21].

Although no significant differences in the activity of ^{137}Cs in soils were observed between the seasons, a decrease in mass activity of ^{137}Cs in soil in autumn was observed at all locations. According to Guillén et al. [10], de Boulois et al. [3], the transfer of radionuclides is affected by the presence of mycorrhisae fungi living in symbiosis with the roots of the host plants. Their function involves transfer of carbon from plant to fungi and of mineral nutrients (particularly phosphorus and nitrogen) from fungi to the plant. Thus the mycorrhisae fungi serve as a "filter" for the host plant as they accumulate non-essential elements, such as for example caesium, and thus

limit toxicity and biological availability of the contaminant and its transfer from soil to plant. It is possible that this was one of the reasons why in autumn, the season favourable for fungi, a decrease in the mass activity of ^{137}Cs in soil was detected. The highest levels of radiocaesium were recorded in September (location Hodruša-Hámre and Arboretum Mlyňany) and in May (Javorníky). According to Grabovskyi et al. [9], Kenzo et al. [11], seasonal deviations may be caused by the age of plants. The increase in the activity of ^{137}Cs immediately after the growth of shoots indicates strong accumulation of ^{137}Cs in young tissues. Young plants thus exhibit higher concentration of ^{137}Cs in leaves than the old ones collected from the same site. This could explain the lower concentrations of ^{137}Cs in the older parts of plants collected in autumn compared to younger plants sampled in spring and summer. According to Salt. Mayes [20], soil moisture is an important factor affecting the activity of ^{137}Cs. The high activity recorded in September may be attributed to the increasing soil moisture at the end of August which could stimulate the plant growth in comparison with summer and resulted in increased concentrations of ^{137}Cs in September. This could explain the sudden increase of radiocaesium in Hodruša-Hámre and Arboretum Mlyňany in September in comparison with the levels at the other sampling dates. According to Kenzo et al. [12], a decrease in the activity of ^{137}Cs from spring to summer may be attributed to several processes, e. g., to dilute this radionuclide as a result of biomass increase over the growing season, leaching due to precipitation or translocation to other organs together with potassium.

A comparison of results of mass activity concentration of radiocaesium in Slovak bilberry plants and soil obtained in this study with the limit for total mass activity of radiocaesium (^{134}Cs + ^{137}Cs) in food (600 Bq.kg^{-1}) [23] allowed us to conclude that the consumption of investigated fruits, or use of plant parts presents no health risk. Also the level of this radionuclide in soil raises no environmental concern.

CONCLUSIONS

Our research demonstrated that samples of Slovak bilberry and the relevant soil collected from 3 selected locations in Slovakia contained radionuclide ^{137}Cs even 35 years after the Chernobyl nuclear accident. According to expectations, the highest activity concentrations of ^{137}Cs were detected in location 2—Hodruša-Hámre (elevation 375—800 m), that was least affected by anthropogenic activities and movement of people. The lowest ^{137}Cs activity was determined in Arboretum Mlyňany, situated at lower elevation with higher content of ^{40}K in the soil. The temperatures in this location are higher and anthropogenic activities more intensive. No significant seasonal influence or dependence of mass activity concentration of ^{137}Cs on location was detected. The results obtained indicate that the mass activity concentrations of ^{137}Cs in the investigated fruits, stems and leaves of Slovak bilberries present neither health nor environmental risk.

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ZOONOTIC PARASITOLOGICAL FINDINGS IN A PUPPY: THE COURSE AND THERAPEUTICAL EFFICACY

Burcáková, Ľ.[1, 2], Štrkolcová, G.[2], Könige, A.[1], Várady, M.[1]

[1]Department of Experimental Pharmacology, Institute of Parasitology
Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice
[2]Department of Epizootiology, Parasitology and Protection of One's Health
University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice
Slovakia

gabriela.strkolcova@uvlf.sk

ABSTRACT

Considering the close contact of companion animals and humans, gastrointestinal parasitic zoonoses are very widespread and represent a high risk of transmission with the potential of severe consequences affecting the digestive tract of both humans and other animals. In this study we focused on enteric zoonoses caused by *Toxocara canis* nematode, *Dipylidium caninum* tapeworm and *Giardia duodenalis* protozoa. Our primary aim was to observe *Toxocara canis* egg excretion within the 27 consecutive days before and after orally treatment (2 Caniverm® tablets) on Day 13 in a naturally infected puppy. An average egg per gram (EPG) of *T. canis* detected by coprological quantitative McMaster method was 4558.33 and 666.66, before and after treatment, respectively. The percentage of faecal egg count reduction (%FECR) in *in vivo* Faecal Egg Count Reduction Test (FECRT) has confirmed an 85.37 % efficacy against *T. canis*. Secondly, the efficacy of Caniverm® against the tapeworm *Dipylidium caninum* was also determined. No *D. caninum* proglottides were detected on Day 14. The data showed 100 % effectiveness of the anthelmintic treatment. Metrobactin® 250 mg has been tested as experimental therapy against *Giardia duodenalis* on Day 3. On day 10, no cysts were observed in the faeces after *per os* ¼ tablet administration twice a day for 7 days.

Key words: *Dipylidium caninum*; efficacy; egg excretion; EPG; FERT; *Giardia intestinalis*; McMaster; *Toxocara canis* treatment; zoonoses

INTRODUCTION

The most prevalent zoonotic gastrointestinal parasites in dogs are the nematodes *Toxocara* spp. and the protozoans *Giardia duodenalis*, and *Cryptosporidium parvum* [5, 29, 40]. Clinical presentation of enteric parasitoses is closely associated with the age and immunological status of the affected animal depending on the intensity of the infection or due to the presence of coinfections with other pathogens. Barutzki, Schaper [4] reported *T. canis* and *G. duodenalis* coinfection in young dogs above 6 weeks

of age. Furthermore, M o s k v i n a, Z h e l e z n o v a [31] proved, that endoparasites affect the most often puppies and young dogs under 1 year of age. The diseases caused by the enteric endoparasites are mainly manifested by anorexia, anaemia, diarrhoea, emaciation, vomiting and even death. Asymptomatic infections can also develop [3, 13, 47]. The monoxenous protozoa *G. duodenalis* has high pathogenetic effects and orofaecal transmission can occur through contaminated food or water [32]. On the other hand, though one of the most prevalent canine nematode, *T. canis* undergo several infection routes in a host body, such as tracheal, somatic and transplacental migration, and transmammary transmission, its life cycle is direct. In addition, a lactating bitch can be infected as they ingest of immature fourth stage larvae from vomit and faeces of the puppies or transmission through paratenic host may be encountered [34, 36, 37, 52]. The most typical and important infection route for puppies up to 3 months of age is the tracheal migration. Based on this, toxocarosis is primarily an important problem for puppies, which emphasizes the need for control, monitoring and treatment [11, 24].

A rarely occuring zoonotic parasite that also inhabits the alimentary tract is the *D. caninum* tapeworm, known as the cucumber seed or double pore tapeworm. The detection and diagnosis of this parasite is rather challenging, as its life cycle requires an intermediate host (e. g. *Ctenocephalides canis* flea) and irregular excretion of proglottids in the faeces, which makes diagnosis difficult [22, 46, 55, 61]. Dipylidiasis is mainly asymptomatic [16, 56]. If clinical signs occur, they are mostly non-specific, similarly to giardiasis and toxocarosis, apart from the scooting behaviour, that is typical for dipylidiasis [19, 44, 46, 47]. Considering the occurrence, mode of infection and the life cycle of enteric parasites, it is necessary to establish an accurate diagnostics with aimed therapy. In general, the treatment is mainly prophylactic without previous coprological examination, which increases the risk of persistent patent period and resistance with reduced antiparasitic efficacy [39].

Our primary aim was to monitor the day-dependent *T. canis* eggs excretion before and after treatment in a puppy within and to determine the efficacy of the administered therapy using the FECR test according to C o l e s et al. [10]. Parasitological examination revealed the presence of other zoonotic endoparasites, namely *G. duodenalis* protozoa and *D. caninum* tapeworm, that we set ourselves to observe and medicate.

MATERIALS AND METHODS

The animal

The 8-week old mixed breed female puppy was selected due to the previous diagnosis of toxocarosis with a medical history of vaccination, deworming, flea treated and no previous severe diseases. The animal was born in a shelter and has been housed indoors with another 6 puppies at about the same age. They had daily access to a garden in a fenced circular enclosure with a diameter of 2 m. The physical examination revealed: the weight of 2.4 kg, anorexia and cough with dyspnoe mainly at night, during exercise or in stressful situations. Watery, mucous diarrhoea with a blood content and abdominal distension with mild pain on palpation in this area were observed. We measured a slightly increased body temperature (38.5 °C) and respiratory rate (34 breaths.min^{-1}) before treatment. The heart rate values were in the normal physiological range. The fleas (*Ctenocephalides canis*) were found scattered along the hair coat mainly around the neck. The inspection of the perianal region dry cucumber seed shaped proglottids attached to the hair coat were noticed. The animal showed scooting behaviour.

Parasitological diagnostics methods

Toxocara canis

Qualitative copromicroscopic flotation method [23] had been conducted to confirm *T. canis* infection. For a more detailed description of the level of infection we applied the coproscopical quantitative McMaster technique according to T a y l o r et al. [54] using the flotation solution with specific gravity 1.240, where values of eggs *per gram* (EPG) defined the intensity of the infection. Results were evaluated in McMaster chambers via coefficient ×50, which shows sensitivity > 50 EPG for individual faecal samples.

Dipylidium caninum

The canine tapeworm *D. caninum* was diagnosed on the basis of the finding of white or light pink coloured proglottids in a fresh faecal sample (Fig. 1). The latter were separated from the faeces and preserved in 70 % alcohol solution for further microscopic examinations.

D. caninum packs of cells we diagnosed microspopically by pressing proglottides through two microscopic slides in order to push the content out [45] (Fig. 2).

Fig. 1. ***Dipylidium caninum*** **proglottids found in fresh faeces**

Fig. 2. ***Dipylidium caninum*** **egg packets**

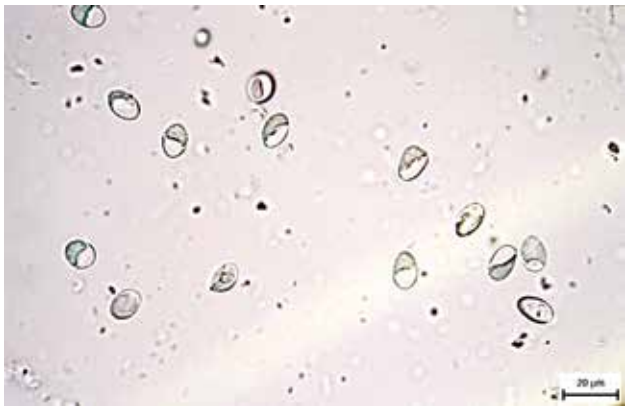

Fig. 3. ***Giardia duodenalis*** **cysts**

Giardia duodenalis

In order to identify *G. duodenalis* cysts we used the centrifugal-floatation technique with zinc sulphate solution (1.180 specific gravity) according to Faust (Fig. 3) [15].

FECRT (Faecal egg count reduction test) [10]

The FECRT was conducted according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) instructions. The animal was treated by the recommended dose of fenbendazole 37.5 mg-pyrantel embonate 36.0 mg-praziquantel 12.5 mg combination/1 tbl. (Caniverm® mite tbl 6 × 1.75 g, Bioveta, Czech Republic) following the instructions for use of the corresponding product depending on the weight and age. The percentage of faecal

Table 2. Drug schedule in chronological order within the experimental period

Parasite	Day	Drug/active substance	Drug administration
Giardia duodenalis	3	Metrobactin® 250 mg/ metronidazole	*per os*
Ctenocephalides canis	4	Frontline Combo Spot-on on dogs/ fipronil, s-methoprene	spot-on
Toxocara canis	13	Caniverm® mite tbl 6 × 1.75 g/ fenbendazole-pyrantel embonate-praziquantel	*per os*
	18	Caniverm® mite tbl 6 × 1.75 g/ fenbendazole-pyrantel embonate-praziquantel	
Dipylidium caninum	13	Caniverm® mite tbl 6 × 1.75 g/ fenbendazol-pyrantel embonate-praziquantel	*per os*

egg count reduction (%FECR) of *T. canis* eggs was counted using the method: %FECRT = 100 – [(post-treatment EPG count/pre-treatment EPG count) × 100], where a shortage of treatment efficacy was presumed if %FECRT > 90 %.

Drug schedule

The puppy carried three different zoonotic diseases caused by three cathegories of parasites (*G. duodenalis* protozoa, *T. canis* nematode, *D. caninum* tapeworm). *C. canis* flea infestation also occured. The administered drugs are summarized in Table 2.

Ethical considerations

The research was approved by the UVLF Ethics Committee in accordance with applicable national and international animal welfare legislation.

The authors declare that there is no conflict of interest.

RESULTS

Toxocara canis

Our study lasted 27 days and a total of 84 individual faecal samples had been examined as part of the daily monitoring of the dynamics of *T. canis* infection before and after *per os* administration of Caniverm® deworming therapy. In order to verify the efficacy of the antihelmintic treatment, egg shedding during the experimental trial, followed by the evaluation of the results using the *in vivo* FECR test [10] were used.

Monitoring of the intensity of infection before and after treatment using the modiffied McMaster method [54]

The experimental period was divided into two phases, before and after the treatment. The first phase took 12 days, where *T. canis* eggs were detected using the McMaster method and egg counts assesed. *T. canis* egg excretion before treatment is represented in Fig. 4. The irregular egg excretion is clearly seen. The first 7 days the chart shows a slightly fluctuating tendency, while Day 8 indicated a significant decrease in the production with the lowest count on the Day 9. On Day 10, egg shedding increased markedly with the peak of 6800 EPG on Day 11. The mean (arithmetic) value 4558.33 EPG ranging from 900 epg to 6800 EPG was determined in the first 12 days of the experimental period.

The animal was treated with a single dose (2 tablets) based on a fenbendazole 37.5 mg-pyrantel embonate 36.0 mg-praziquantel 12.5 mg combination (Caniverm® mite tbl 6 × 1.75 g, Bioveta, Czech Republic) on Day 13. The patent period persisted for another 2 days after antihelminthics administration (Fig. 5). Egg shedding dropped sharply from 2500 EPG to 700 EPG without egg production on the Day 15 directly after treatment. The following day, egg shedding reappeared and was on the uptrend over the next few days. Due to persistent patent period, we repeated the therapy on Day 18 and Day 13.

The EPG counts decreased gradually with no finding of *T. canis* eggs on Day 23. The assessment was extended by 4 days for post-therapeutic control. No eggs were found

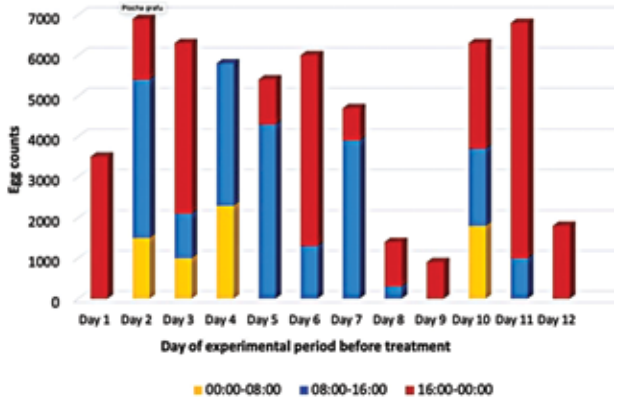

Fig. 4. *Toxocara canis* egg excretion before treatment in the course of time

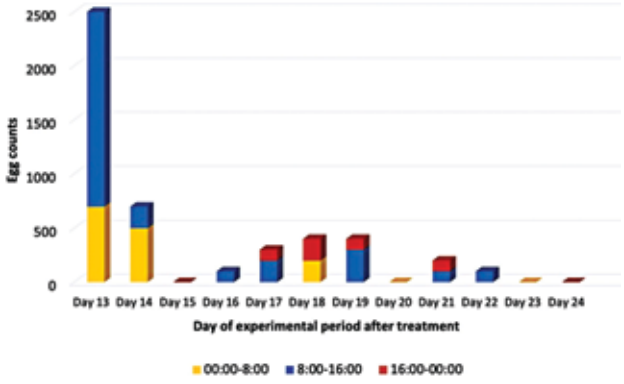

Fig. 5. *Toxocara canis* egg excretion after treatment in the course of time

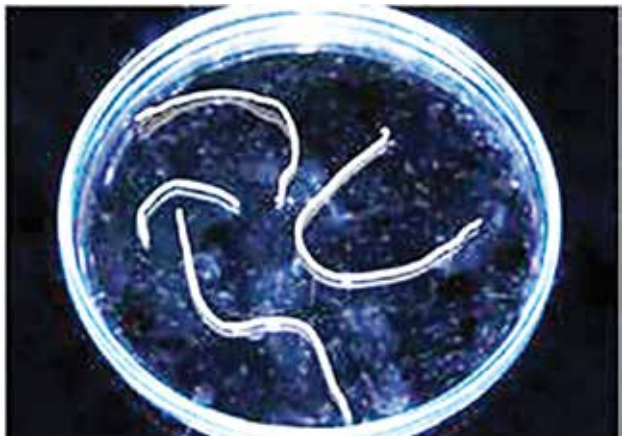

Fig. 6. *Toxocara canis* larvae found in the faeces samples after treatment

in the faeces samples during the control days. The mean (arithmetic) value 666.66 EPG ranging from 100 EPG to 2500 EPG was quantified in the post-treatment phase including Day 13 to Day 18.

Table 1. Egg counts within the trial and mean values before treatment *Toxocara canis* egg excretion. Mean (a) value is comprised of the egg excretion within the before treatment (Day 1—12) phase. Mean (b) includes range of Day 13—18 after treatment

Before treatment		After treatment	
Day	**n**	**Day**	**n**
1	3500	13	2500
2	5800	14	700
3	6300	15	0
4	5800	16	100
5	5400	17	300
6	6000	18	400
7	4700	19	400
8	1400	20	0
9	900	21	200
10	6300	22	100
11	6800	23	0
12	1800	24	0
Mean (a)	**4558.33**	**Mean (b)**	**666.66**

n—summary of egg counts per day; a—mean (arithmetic) egg counts during pre-treatment trial; b—mean (arithmetic) egg counts during post-treatment trial

Toxocara canis larvae finding after treatment

We found 14 *T. canis* larvae in the faeces sample directly after the first drug administration in ranged 1.9—11.7 cm in size (Fig. 6). Larvae with the size of 10.2 cm and 11.7 cm we diagnosed as adult females. After the second Caniverm® drug administration, we detected 4 larvae ranged from 2.4—5.8 cm.

Determination of antihelmintic efficacy using *in vivo* Faecal egg Count Reduction (FECR) analysis [10]

To evaluate the antihelmintic efficacy, we considered the mean (arithmetic) values (Table 1) during pre-treatment (from Day 1 to Day 12) and post-treatment (from Day 13 to Day 18) phase. The efficacy of Caniverm® against *T. canis* was 85.37 %, which means the shortage of drug efficacy. This finding is also substantiated by the fact of persistent patent period of *T. canis* eggs after the first drug administration. The irregular curve of egg shedding within the whole experimental period is showed in Fig. 7.

Dipilidium caninum

Faecal proglottids excretion and therapeutical approach

We have confirmed *D. caninum* infection by the positive finding of proglottides in a faeces (Fig. 1). Microscopic examination using the pressing method to extrude the proglottides content revealed typical egg packets (cocoons). Fleas *C. canis* (n = 15) were found and examined due to the presences of larvocyst cysticercoids, which showed a negative result. In total, 39 gravid tapeworm segments were found in the faecal samples before treatment. Fig. 8 shows the course of proglottides excretion within the pre-treatment phase. A sharp drop of excretion on the sixth day is rather significant with interruption from Day 7 to Day 12 included. Caniverm® was chosen as a treatment against *D. caninum* on Day 13. After drug administration we detected 3 proglottides in the faeces. Within the deworming against dipylidiasis it is also necessary to eliminate ectoparasites. Thus, the flea infestation was treated by the spot-on application pipette based on fipronil 67 mg, s-methoprene 60.30 mg (Frontline Combo Spot on on dogs 2—10 kg, 1 × 0.67 ml, MERIAL, France) on Day 4 of the experimental period.

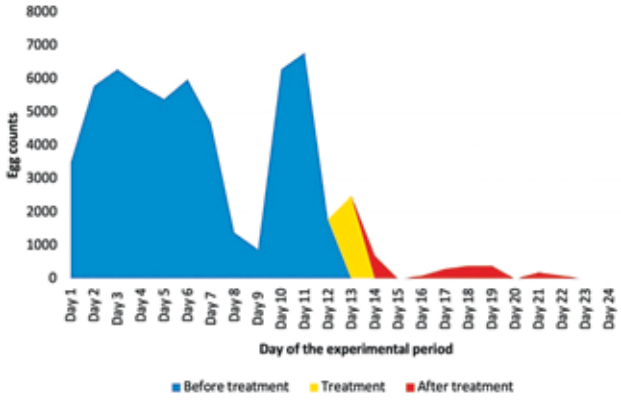

Fig. 7. *Toxocara canis* egg excretion during experimental period

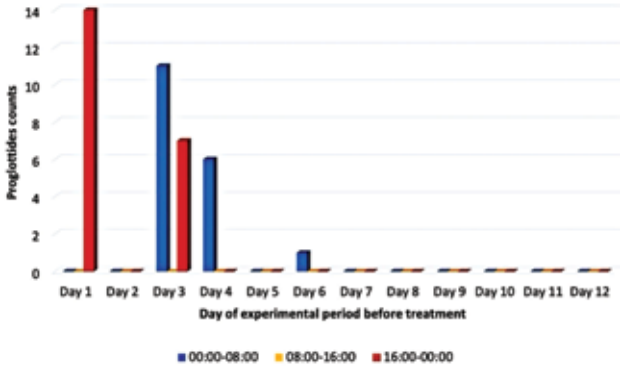

Fig. 8. *Dipylidium caninum* proglottides excretion before treatment

Giardia duodenalis

Therapeutical approach

The protozoan parasitic species *G. duodenalis* was detected using the concentration flotation method [15]. The puppy was orally given Metronidazole 250 mg (Metrobactin® 250 mg, Le Vet Beheer B. V., Holand) at the dose of ¼ of tablet twice a day for 7 days on the third day of the experimental period. During seven days of metronidazole administration, we performed one follow-up examination, where a significant decrease in a number of cysts was detected. The last control microscopic assesment we performed as a confirmation of the successful treatment. No evidence of *G. duodenalis* cysts on Day 10 was found.

DISCUSSION

The gastrointestinal tract of dogs may be affected by a variety of endoparasites with consequences encompassing no clinical signs up to loss of appetite, diarrhoea and even death. Zoonotic enteric diseases are particularly a serious threat due to their pathogenicity and possible transmission to humans [39]. Basic microscopic parasitological assessment of faecal samples using the flotation method remains still crucial in the identification of intestinal parasitic disorders [27, 30]. Due to the increased need for more accurate and precise diagnostics, assorted modifications of the McMaster method have been obtained [10, 18, 39, 41, 58, 60]. Based on this, quantification of egg counts per gram in the faeces by the McMaster method played a key role in determining the level or phase of parasitic infections. This knowledge enhanced the treatment of the patients and antihelmintic resistance were reliably determined [55] using FECRT to determine drug efficacy [10]. Pereckiene et al. [39] reported higher sensitivity of FECs (Faecal Egg Counts), assuming that the McMaster method procedure involved a centrifugation. He also confirmed that more accurate results are acquired using solutions with a higher specific gravity (range of 1.200-—1.270) and counting in a minimal of two grids of the McMaster chamber. Although the McMaster method is very accurate, it does not give a real picture of worm burden at a certain time, as eggs are excreted only by adult females, thus adult males and immature worms are not embraced. The other factors influencing the FECs are: female egg productivity and fecundity, nematode species and its biology, females and males ratio, the amount of faeces daily passed or egg concentration in a faeces sample. We must take into consideration age, immune and physiological state of the host or other ongoing diseases. As regards all these aspects, the results of McMaster method may differ [6, 7, 43, 57]. During an experimental period of our study, a circadian rhythm of *T. canis* eggs excretion using the McMaster method has been conducted. The technical procedure included centrifugation and flotation solution with 1.240 specific gravity and multiplication coefficient ×50 for more accurate EPG values, as mentioned above. It is well known that various nematode parasites evince diverse egg productivity within the day and each species of adult female ascarids excretes different amounts of unembryonated eggs in the faeces [7, 53]. To our knowledge, several investigations for egg excretion in roundworms have been performed and the results varied depending on the ascarid species. The following EPG values produced by females per day at the peak

have been reported: *Toxocara canis* 980—5.700 EPG.day^{-1} [42], *Toxocara vitulorum* 52.000—168.000 EPG.day^{-1} [43], *Ascaris lumbricoides* 240.000 EPG.day^{-1} [9, 46], and *Toxocara cati* 19.000—24.000 EPG.day^{-1} [12]. The resulting egg counts per day varies significantly, as it was in our case of 900—6.800 EPG.day^{-1} with the peak output during 16:00 — 00:00 hours within the first phase of the experimental period. The same circadian rhythm of egg excretion repeated also in the post-treatment phase. These findings agree with W a t k i n s, H a r v e y [59], who reported that consequent number of excreted eggs varies depending on the timing of the female production and on feeding and defecating mode of the animal. Based on the above-mentioned facts, we can assume that the ascarids egg excretion is irregular, and cannot be predicted and is affected by many factors, whether from the parasite's side or the host's body side. This claim is supported by R i c h a r d s et al. [42]. He stated that there is no direct association between the count of the *T. canis* adult worms per host body and the eggs passed in the faeces. That means, that the fact that the finding of 2 adult females in the faecal sample after deworming may not correspond to the number of eggs. The main goal of veterinarians should be to implement early and efficient treatment, to avoid reinfection and anthelmintic resistance. The broad spectrum of anthelmintics against ascarids is available and clearly described by several authors [1, 14, 25, 26, 28, 35, 38, 51]. According to R o b e r t s [43] pyrantel is very effective against immature *T. canis* developmental stages. Studies show that its efficacy is very high and did not change since it was first introduced [8, 50]. J a c o b s [21] points out that the efficacy of pyrantel pamoate alone is 95.9 %, but on the other hand an effect of 100 % was reported by B e c s k e i et al. [7]. By using febantel-based treatment there has been claims of 94.6—100 % efficacy [20]. A comparative study of S c h e n k e r et al. [50] described the effect of orally administered milbemycin oxime- and febantel-pyrantel embonate-based tablets against *T. canis* in puppies. The combination of febantel, pyrantel embonate and praziquantel shows 84.7—98.1 % efficacy [26, 49]. In our investigation, the result of egg output was found at the bottom boundary of this margin, which was also confirmed by persistent patency after treatment. This fact just confirms that the combinations of febantel and pyrantel embonate with other drugs shows reduced effect as previously mentioned. Praziquantel has been successfully used against tapeworms in dogs [2, 48, 53] as we confirmed in our case of *D. caninum* infection in a puppy. Taking into consideration that not only monoinfection was treated, but 3 parasitic concurrent zoonotic parasites, also the course of *Toxocara* egg shedding could be changed and also physical manifestation of such animal can be different. In the case of giardiasis, the administration of metronidazole markedly changed the consistency of the faeces from watery with blood content to mild solid. Metronidazole is very efficient against cysts excretion as previously published by Z a a t et al. [62], G a r d n e r, H i l l [17], N a s h et al. [33]. As we described, it is very important to consider many factors and aspects, before the inference is deduced. Last but not least, our results are not statistically significant as only a single experimental animal had been treated, which creates the room for further investigations.

CONCLUSIONS

The companion animals are common reservoir of various illnessess with zoonotic potential, which is from the point of view of health protection very alarming. The subject of this study, a puppy, carried three parasitic zoonoses (toxocarosis, dipylidiosis, giardiasis). We specifically investigated the dynamics of *Toxocara canis* egg excretion and efficacy of Caniverm® against *Toxocara canis* and *Dipylidium caninum*. Experimental treatment using Metrobactin® against *Giardia duodenalis* was also conducted. The purpose of our study was to verify that the chosen antiparasitic therapy may not be effective in many cases, especially if animal owners use the same drugs repeatedly without previous parasitological assessment. We wanted to highlight the need of precise parasitological examination not only for general diagnostics, but also to exclude parasite presence after treatment. As advice, closer communication between the veterinary doctors and owners is needed, to explain what are the risks and disadvantages of unproper diagnostics and treatment, mainly if severe zoonosis are involved.

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USE OF BANDAGING IN THE TREATMENT OF DIGITAL DERMATITIS

Mudroň, P., Coles, L. L., Réková, P.
Clinic of Ruminants, University of Veterinary Medicine and Pharmacy in Košice
Komenského 73, 04181 Košice
Slovakia

pavol.mudron@uvlf.sk

ABSTRACT

The aim of this study was to compare two different methods for the treatment of digital dermatitis (DD) in dairy cows. Moreover, both treatment methods were tested if they could stop the recurrences of DD in the same patient. For this study, data were collected on two separate occasions across a six-month period (spring and the following autumn). During these two periods, 280 and 232 Holstein Friesian dairy cows were examined respectively in the crush. The following two treatment methods were compared:

1. **Scarification of the lesion surface and antibiotic spray;**
2. **Resection of the lesion, antibiotic spray and powder, and covering with a bandage.**

A first control 3 weeks after the initial trimming period was carried out to check the condition of the lesions. The recurrence rate of DD was assessed during the second hoof trimming (autumn). In this study, no differences between two treatment methods could be observed as all treated animals have shown a 100 % healing. The assessment of a recurrence rate showed no new cases of acute DD in the group 1 (non-bandaging) and in the group 2; however, there was a 28.6 % recurrence rate in the group 1 (non-bandaging). The material costs and treatment time were also several times higher in the bandaging group. In conclusion, the tested methods did not differ in healing success rate, however, there are other important factors like time and costs that play an important role in the decision of a farmer or manager as to which treatment to use for their dairy cows.

Key words: dairy cow; digital dermatitis, lameness; treatment

INTRODUCTION

Digital dermatitis (DD) can be described as a one of the major global problems in dairy cattle, not only through the incurred financial losses due to its main symptom of lameness but also in terms of the welfare of the animals [3, 13], therefore treatment and prevention have an important role worldwide. Described as the "leading cause of lameness in

dairy cattle in North America and Europe" [18], it develops typically as a moist, light grey-brown area with erect hairs bordering the lesion, it can be found on the plantar aspect of the foot, just between the bulbs of the heels. The characteristic lesions are most commonly found on the rear foot but can appear on the front foot and if present are found on the border of the interdigital cleft. The diagnosis of the DD lesion was based on the classification of its different stages that was formulated by [4]. Another way of classifying DD was into acute or chronic, where acute lesions were typically described as a moist, light grey-brown exuding area with erect hairs typically surrounding the lesion, when it was cleaned you may visualise the typical red, strawberry-like lesion. The chronic lesion also known as the "hairy wart" stage were neglected lesions that develop hypertrophy of the dermis and epidermis that make conical projections [15].

Treponema bacteria were thought to be a possible cause due to them being isolated from DD lesions [7]. These bacteria are spiral micro-organisms that grow best in anaerobic conditions and can be inhabitants in the GIT tract and genital tracts of both humans and other animals [6]. Treponemas were also difficult to culture mainly due to the fact that they are anaerobic bacteria and therefore are hard to isolate from lesions. The study in the UK found that 23 spirochete strains with only two of the results from two of the cows with more than one treponema was isolated from the same lesion which casted serious doubts on previous thoughts that DD was a polyspirochetal infection. The study did confirm that all the isolates were from the genus Treponema and from 3 distinctive groups [5]. Those isolated were *Treponema phagenesis*, *T. vincetti* and *T. denticola*. Most interestingly, a further study undertaken to identify Treponema's from the bovine GIT tract, using 16S rRNA gene sequences, to try to see if they are the same or similar to those isolated from DD lesions recently. This can help to try and distinguish the source of the bacteria. The study found and isolated seven treponemas from the GIT tract and compared them to those isolated from DD lesions; they found them to be different in certain clusters [5].

The management of DD and its prevention are very important and a lot can be done to help reduce the incidence of DD and other claw diseases from occurring. The infectious nature of DD results in the spread of lesions after an infected cow is introduced to the herd and is extremely difficult to eradicate once present [14]. Treatments vary on: labour involved, products available and upon the setup of the farm. Topical treatments such as the use of antibiotics in a spray that can be applied after cleaning of the DD lesions is one of the most commonly used methods on dairy farms. The use of bandaging is not a common practice worldwide for DD lesions, as less costly methods are preferred. Bandaging requires a higher labour and material input as the process takes longer than a simple spray method and requires the animals to be caught in a crush and restrained adequately for the process of bandaging to occur.

The goal of this study was to see if the bandaging of digital dermatitis treats the lesion as well as or better than a simpler technique of scarification and then an antibiotic spray. The second goal was to see if this technique stopped the recurrence of DD in the same patient.

MATERIALS AND METHODS

For this study, data were collected on two separate full hoof trimmings across a six-month period (spring and the following autumn). During these 2 periods, 280 and 232 Holstein Friesian dairy cows were examined respectively in the crush and then treated if needed. A further "control" three weeks after the initial trimming was carried out to check the progress of the lesions. The animals were kept in a free stall system with manure solid bedding and were fed a total mix ration (TMR). Diagnosis of DD occurred during the orthopaedic hoof trimming and animals with Acute DD lesion has been randomly assigned to 1 of 2 treatment groups: 1—control group; 2—bandage group. If manure presence prevented observation of the lesions, this was removed with a towel. For all the lesions, the lesion was first cleaned using a scrubbing brush and water to take away any faeces or contamination from the lesion and also for clarification of the extent of the lesion. The foot was then also dried using disposable paper and the foot was then trimmed using a grinder to standard length and any disparities were removed before forming two slopes on the inner surfaces.

For the control group, the DD lesion was initially disinfected with a simple betadine solution spray and allowed to dry before using a simple hoof knife (also disinfected beforehand) to scarify the lesion that takes away the surface. Then a chlortetracycline spray was sprayed onto the lesion and left for two minutes to dry. For the test group, the DD

lesion the top layer of skin was removed using a simple hoof knife after cleaning and disinfection of both the lesion and the knife. The lesion was then also sprayed with a chlortetracycline spray and allowed to dry before starting the bandaging. A square piece of sterile gauze was then covered with tetracycline powder and placed on the area of the lesion, in as sterile a manner as possible. Then the foot and lesion were covered in a layer of cotton wool, this layer must go above that of the lesion. Once a sufficient padded layer was encasing the foot, the wound would be dressed using a roll of wound dressing; this part of the bandaging must be kept as taught as possible and all areas of the cotton wool must be covered only leaving a ring above the top of the bandage. Black tar tape was then used to provide protection to the bandage. A control of both groups followed after 3 weeks and a transition from active to non-active stage was recorded as "good healing". The animals other legs were also checked to see if the DD had spread to any of the other legs. The treatment of the lesions, if any, could then take place. The second control was done during the next trimming session in the autumn.

The statistical analysis of the prevalence differences were performed by running a chi-squared test using the statistical software StatSoft, version 8.0. A P value of < 0.05 was considered significant. The time differences were tested by the t-test. To test a recurrence rate the patients used were divided into 4 groups, according to what trimming session they were in and what treatment was used. Groups 1 and 2 were those from the spring session, group 1 being bandaged and group 2 non-bandaged. Groups 3 and 4 were those cows from the autumn session and group 3 being bandaged and group 4 being non-bandaged.

Ethical statement

The research was approved by the UVLF Ethics Committee in accordance with applicable national and international animal welfare legislation.

The authors declare that there is no conflict of interest.

RESULTS

The occurrence of DD in this study during the two trimming sessions were not high, however the data were calculated only concerning the acute lesions (Table 1). There was a significant increase in the number of cases in the autumn. In this study, we could not demonstrate that the bandaging technique was better than the non-bandaging technique as all groups have shown a 100 % success rate (Table 2).

Table 1. Prevalence of acute digital dermatitis (DD) lesions in dairy cows

Trimming session	Total number of cows	Cows with acute DD	Acute DD [%]
Spring	280	11	3.93
Autumn	232	29*	12.50

*—Significant at $P < 0.05$

Table 2. Percentage of cows with good healing

Group	Number of cows	Treatment Type	Cows with good healing [%]
1	4	Bandaged	100
2	7	Non-bandaged	100
3	11	Bandaged	100
4	18	Non-bandaged	100

Table 3. Average cost of material per treatment per head

Treatment type	Material cost per cow [€]
Bandaging	2.03
Non-bandaging	0.03

The assessment of a recurrence rate showed no new cases of acute DD in the group 1 (bandaging) and in the group 2, however, there was a 28.6 % recurrence rate. Two cows that had been treated using the non-bandaging method of scarification and spray in the spring had new cases on a different leg in the autumn and were subsequently treated in group 3 and 4, respectively.

This study also included a calculation of the average material cost for each treatment to help in the evaluation of the treatments. The total costs as outlined in Table 3 showed a clear difference in the costs between the two treatment types. This is especially significant when the non-bandaging groups had over double the amount of patients in

Table 4. Total costs of each treatment for each treatment group

Group	Treatment type	Number of cows	Total treatment cost [€]
1	Bandaging	4	8.20
2	Non-bandaging	11	0.33
3	Bandaging	7	14.35
4	Non-bandaging	18	0.54

Table 5. Average time per treatment per cow in minutes

Treatment option	Number of treatment	Time per cow [min]
Bandaging	11	2.52 ± 0.13
Non-bandaging	29	0.33 ± 0.01*

*—Significant at $P < 0.05$

Table 6. Total times for both treatment options in all groups

Group	Treatment type	Number of cows	Total time [min]
1	Bandaging	4	10.0
2	Non-bandaging	11	3.66
3	Bandaging	7	17.5
4	Non-bandaging	18	9.0

both non-bandaging groups and where completed with the same results at a significantly smaller cost (Table 4).

As shown in Table 5, there is yet another significant difference in the time taken per cow to administer the treatment options. The non-bandaging techniques takes a third of a minute (twenty seconds), compared to the bandaging technique taking two and a half minutes.

DISCUSSION

The occurrence of DD in the herd over the two trimming sessions should ideally be below 12 % and if they were this would mean that the farmer or manager was managing the environment and dairy cows well. The incidence of DD in other countries such as the Netherlands has been studied and a percentage of 20 % and over for a herd of over 100 dairy cows was seen to be common with some herds reaching an almost 60 % incidence [9]. It is generally considered that once a herd has DD, it is extremely hard to decrease the incidence. In this study, the incidence was fairly low, however these data were calculated only with acute lesions as those with chronic lesions were not treated in this study. The incidence was low which was good and was probably achieved through good management practices by both the manager and the orthopaedic specialist, over successive years of routine foot trimmings and treatments. There was a significant increase in the number of cases in the autumn and this may be due to more humid conditions at that particular time of year and was more expected in the autumnal period.

Unfortunately, infectious claw disorders are less efficiently prevented by foot trimming than disorders affecting the claw horn. Thus, in combination with footcare/trimming, infectious lesions like digital dermatitis and foot rot preferably need a short-term and long-term biosecurity strategy. Cattle develop immunity to foot rot, but digital dermatitis can recur in the same animal over time [1]. The use of bandaging in DD lesion healing has not been widely studied as a treatment option due to the extra time and costs involved in applying the bandage compared to footbaths or a simple spray method. In a study in Germany, the use of bandaging was tested to see if it accelerated the healing of DD lesions with both an antibiotic and non-antibiotic; the results showed that by bandaging the lesion, no matter the treatment option, it helped increase the rate of healing [11]. This suggested that there was an advantage to using a bandage for treatment. In this study, no difference in the treatment success between the bandaging and the non-bandaging technique could be seen as all groups have shown a 100 % success rate. The clinical cure rate, defined as transitioning from active to nonactive stages, was used as an outcome as opposed to "healing," as the latter implies that a foot would return to an unaffected state, which is unlikely in 3 weeks [12]. Tetracyclines are commonly used as a treatment for DD by farmers, veterinarians, and hoof trimmers [16] and are often used as a positive control in DD treatment studies using various application methods with varying results. This heterogeneity in treatment regimens has resulted in clinical cure rates reaching around 70 % over 3 to 30 days [10]. In the study with the testing of two different methods, it was demonstrated that the treat-

ment of digital dermatitis with a tetracycline hydrochloride paste was as therapeutically effective as the application of tetracycline hydrochloride held in place with a bandage for two days and would eliminate the necessity of bandage removal [8]. Consequently, transition and recurrence should be considered the most relevant in terms of on-farm DD control. A significant difference in a recurrence of DD could suggest that bandaging is a good technique for ensuring less recurrence of DD, possibly due to allowing less of the aetiological agent to have the ability to spread to other legs of the cow as the bandage seals the lesion with the treatment. However, it could be argued that these particular cows may have a predisposition to the disease and as a coincidence were both treated in the non-bandaging group to start with and may have shown a recurrence even if they had originally been treated with a bandage [17].

Many dairy units worldwide are struggling with the increasing costs and the falling price of milk, therefore, every cent count when it comes to deciding what preventive strategies and treatment plans to be used for the dairy unit. In a recent study, it was calculated that the average cost of lameness caused by DD in the US is $132.96 per cow [2]. Among treated foot disorder cases over the past year, a recent study estimated that digital dermatitis was most prevalent (43.9 %) with the mean total treatment costs of $7.5 per case of digital dermatitis [3]. The average cost per cow is considerably larger for the bandaging option and therefore with the treatments showing no difference in healing potential, then there is every reason for a farmer to opt for the cheaper option of scarification and spraying. The total costs analysis demonstrated the significant difference in costs for the two treatment types. This is especially significant when the non-bandaging groups had over double the amount of patients in both non-bandaging groups; and were completed with the same results at a significantly lower cost. This is, however, not surprising when you consider how more materials are needed compared to the non-bandaging technique. It is also worth pointing out that although the non-bandaging technique requires the use of a hoof knife for the scarification of the lesion, it has not been factored into the costs as the orthopaedic specialist that undertakes the treatment will already have a hoof knife as part of their equipment. When considering the study results, there was no doubt that the non-bandaging technique would be preferable as the cost per animal is 68 times less than the alternative bandaging technique.

The time constraints of completing the two treatment types when undertaking a routine orthopaedic and foot trim of many dairy cows are limited due to cost. Therefore the more dairy cows that are able to be seen and if needed treated in one day has a positive effect on the dairy farms profit. Labour is costly to farmers and when a study was done on the costs of lameness, the treatment was one of the biggest costs for DD at 42 % and this would be a factor in the labour involved in treating these cases [2]. Therefore in this study the desired treatment would take the least amount of time possible and still provide good healing results. The results of this study demonstrate a significant difference in the time taken per cow to administer the treatment options. When the significantly shorter non—bandaging method is factored into the orthopaedic and trimming schedule for a day, there would be a much higher work rate achieved if you were using the non-bandaging option. This is also an extremely important consideration when you take into account how much less time the dairy cow has one of her legs elevated and therefore there is a decreased risk of muscle or nerve damage that can be acquired through prolonged restraint techniques.

When taking both treatments into account, the bandaging technique still has the slight advantage, in that there were no cases recurring in this study. However, when taking into account the time and cost of both treatments, there will be no doubt that farmers would prefer the non-bandaging treatment. As this also requires far less in terms of supplies, with only a hoof knife for the scarification and an antibiotic spray, this means that there is the potential for many more cases to be treated in a smaller time constraint and at a far lower cost.

It is fair to say that from this study there was no conclusive evidence to suggest that either treatment is better at healing DD lesions than the other. There are other important factors that come into the consideration when a farmer or manager brings into consideration when deciding what treatment to use for their dairy cows.

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FIELD SURVEY ON THE STATUS OF INTERNAL PARASITES IN YOUNG CALVES ON FARMS IN EASTERN SLOVAKIA

Band, N., Halán, M., Kočišová, A.

Department of Epizootiology, Parasitology and Protection of One's Health
University of Veterinary medicine and Pharmacy in Košice
Komenského 73, 041 81 Košice
Slovakia

alica.kocisova@uvlf.sk

ABSTRACT

The objective of this study was to evaluate the species composition of protozoan and helminth parasites of young calves on farms in eastern Slovakia. Faecal samples from calves were analysed using the floatation technique, McMaster Methods and stained slide method for *Cryptosporidium* spp. From 105 samples analysed, nine samples (8.6 %) were suspected to be positive for *Cryptosporidium* spp. The results gathered from this study displayed that 64.8 % of calves were infected with some species of parasite at the moment of sampling. The highest prevalence of infection was with *Eimeria* spp. with 56.2 % of calves infected being positive for this. OPG (oocysts per gram) values for *Eimeria* spp. observed in this study ranged from 100 to 75,200. The lowest prevalence was found to be from *Giardia duodenalis* and *Trichostrongylidae* family equally 0.95 %. All faecal samples had nematode egg counts below 50 EPG (eggs per gram).

Key words: helminths; prevalence; protozoa; species composition

INTRODUCTION

There are many parasites that inhabit calves, many of which are entirely harmless as parasites themselves, but can have negative effects on other aspects of health. Some species which are significant can cause disease in domestic cattle or, because of their zoonotic transmission, are a risk to humans. Parasite control in calves results in healthier calves with stronger immune systems, weight gains that provide return on investment, and cleaner pastures. Throughout their life, especially at a younger age, cattle are administered several vaccines. If an animal has a parasite infestation, it won't respond to the antigens in vaccines as much as normal, and therefore, the immune response to the vaccine is often reduced [2]. Stress is also an important factor implemented by parasites when present in cattle, and the vicious cycle continues, as a stressed animal also has a decreased immune response and less gain and progression in regards to growth and production. The effects of internal parasites also do vary with the intensity of an infection, as well as age of the animal, nutritional state, environmental conditions and quality of feeding. Generally,

younger animals and animals under stress are more likely to display signs of parasitism. Older mature cows often tend to acquire a degree of immunity to some parasites, but this immunity can be suppressed when these mature cows go through higher stress phases in their lives, like in early lactation when there is a negative energy balance [6].

Calves can harbour different parasites, but the most prevalent are protozoa. The time of year in relation to season, as well as the age of the calves plays an important role in the parasite infestation. Calves from birth begin their diet of colostrum, followed by milk, and slowly progress to concentrates and forages as they get older to allow for the development of the digestive system, especially the rumen. Therefore, there is an ever changing feed composition throughout this time, and these different feeds also play a huge role in the parasites which tend to colonise. When there is infection with parasites, not only the current health of the calves are affected, but it can also can contribute to their production, weight gain, health status, life expectancy and general appearance later in life, and all these factors are important, especially for the owners. The diagnosis of parasites from faecal samples is a key skill in the diagnosis.

Confirming a diagnosis is also important before treatment is given, as depending on the presence or absence of infection, or intensity of infection, then drug choice and duration of treatment will be chosen. The post treatment faecal samples are also important to find out if the treatment was effective, or if there was a wrong choice of treatment, too short duration of treatment, or resistance against the treatment. The control of parasites in cattle is the strategy most often taken, as eliminating them completely is nearly impossible in most cases, and trying to do so can lead to anthelmintic resistance, which is an evolving problem in cattle herds. The main goal is focused on controlling the parasites to minimise the economic implications they cause, but also allowing there to be some exposure to allow immunity to develop and reduce the build-up of resistance to treatment [11].

The aim of this study was to evaluate the species composition of protozoan and helminth parasites of young calves on farms in eastern Slovakia.

MATERIALS AND METHODS

For the study of the calf parasites on farms in Eastern Slovakia, samples were collected from three farms in the study area. One farm was situated in the Trebišov District in the village of Zemplínska Teplica, the second in Turňa nad Bodvou west of Košice, and the third in Horňa, Michalovce district. The samples were examined using the floatation technique, McMaster method and staining of slides for *Cryptosporidium* spp.

The samples were collected from 105 calves ranging from 3 days to 6 months old during the period between September 2019 and October 2020. According to anamnesis information, antibacterial treatment was applied to about a third of the calves before the collecting of faeces.

All samples were examined by the floatation technique for examination of protozoa and helminths. The positive samples from the floatation method were quantified for parasites using McMaster method [5]. Two methods for staining were carried out in this study. Initially Kinyoun stain was used to stain the slides to aid in the diagnosis of *Cryptosporidium* spp. After analysing 32 samples, the staining method was changed to carbol fuchsin. The samples were analysed under a light microscope (OLYMPUS model BX41TF) at ×40 magnification. Slides were stained with carbol fuchsin in order to allow the stained oocysts to appear as pink spheres on a blue-green background.

Ethical statement

The UVLF Ethics Committee in accordance with applicable national and international animal welfare legislation approved the research. No animals were killed for the purpose of this study.

The authors declare that there is no conflict of interest.

RESULTS

From the total of 105 faecal samples from the calves, 68 of them (64.8 %) were infected with some species of parasite, while 37 of them (35.2 %) were not. It was found that from the total number of 105 calf faeces, the highest prevalence was with *Eimeria* spp., with 59 calves (56.2 %) infected (Table 1, Fig. 1). OPG (oocysts per gram) values for *Eimeria* spp. observed in this study ranged from 100 to 75,200. All faecal samples had nematode egg counts below 50 EPG (eggs per gram). We recorded only one positive sample for *Giardia duodenalis* with 3600 cysts in gram of the faeces.

The infections with the *Giardia duodenalis* and *Tricho-*

Table 1. Number and percentage of calves infected with different parasite species from the total number of calves examined

Parasite species	Number of infected calves	Prevalence of infected calves from total number of calves examined (%)
***Eimeria* spp.**	59	56.2
Giardia duodenalis	1	0.95
***Cryptosporidium* spp.**	9	8.6
***Trichuris* spp.**	3	2.9
Strongyloides papillosus	3	2.9
***Trichostrongylidae* family**	1	0.95

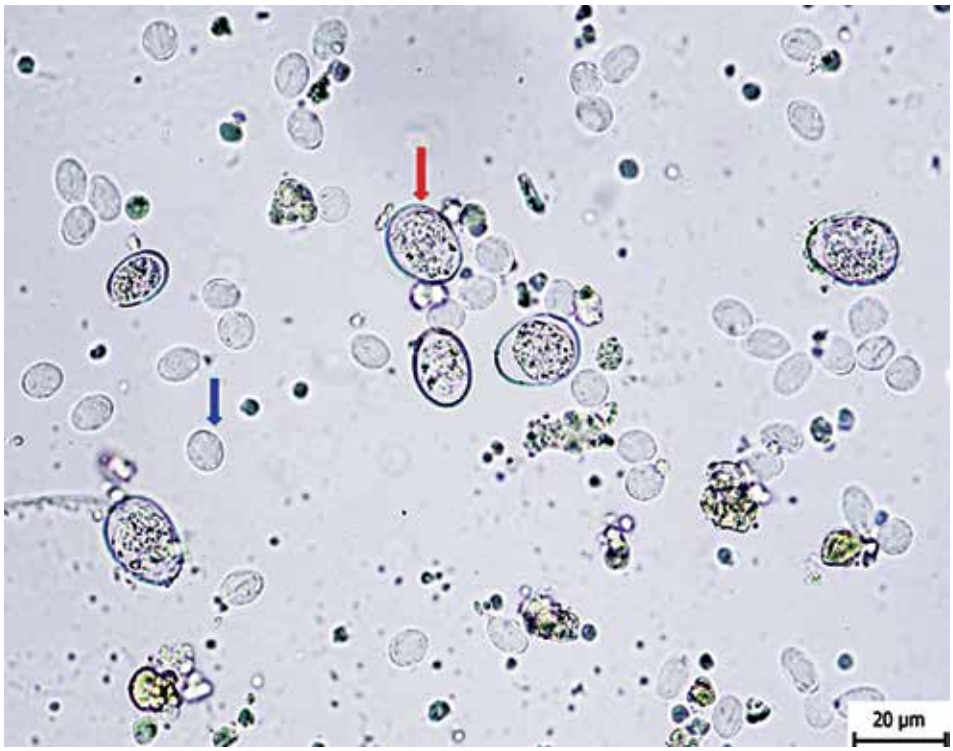

Fig. 1. Oocysts of *Eimeria* spp. (red arrow) and cysts of *Giardia duodenalis* (blue arrow) under a light microscope

strongylidae family were in the lowest prevalence equally in both cases. The results in relation to the testing samples for *Cryptosporidium* spp. were analysed after staining with carbol fuchsin. Nine calves were positive for *Cryptosporidium* spp. (Fig. 2).

The infection with *Eimeria* spp. is prevalent all year round, with infections reaching 100 % of calves in October 2020. Throughout the year, there was never less than 36 % of the calves infected with *Eimeria* spp. All other parasites found showed a much smaller occurrence, with *Strongyloides papillosus* (Fig. 3) and *Trichuris* spp. (Fig. 4) appearing to be more common in the autumn, during the months of September and October, than in other months. The *Giardia duodenalis* infections were found in a very small percentage of the calves in September and November. Infections with *Trichostrongylidae* family (Fig. 4) in only a single calve was not an aberrant result, as this was not a typical parasite found in calves of the age range analysed.

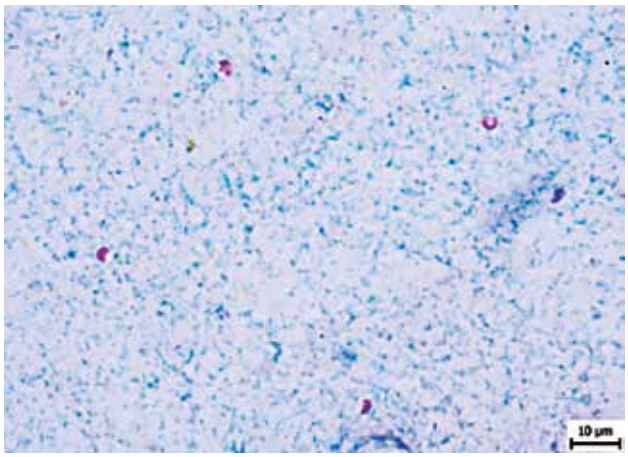

Fig. 2. Positive sample on a stained slide containing *Cryptosporidium* spp. oocysts

Fig. 4. *Trichuris* spp. and family *Trichostrongylidae* under a light microscope

Fig. 3. Eggs of *Strongyloides papillosus* under a light microscope

DISCUSSION

Analysis of faeces is carried out often to aid in the diagnosis of calf parasites worldwide. The floatation technique is a fast, simple and cheap qualitative method to determine if certain parasites are present or not. From the 105 samples analysed in our study, 64.8 % of them were infected with parasites. The calves on the farm Horňa were in close proximity to each other, meaning that transmission between an infected and uninfected individual was more likely, and the spread of faeces between calf stalls was possible, increasing the likelihood of infection. If the calves cannot come into contact with each other, the transmission of infection is much lower, resulting in lower prevalence of parasites. A similar study was carried out in Brazil with 243 faecal samples, where 46.5 % of them were negative for any protozoa, and the other 53.5 % were positive [15]. Coccidiosis is one of the most common diseases in the world, with bo-

vine coccidiosis being observed in nearly all areas where cattle are raised, especially in those younger than one year. Studies have shown that the disease is much more common in housed animals than those on pastures [6].

The results of *Eimeria* spp. infections in calves have demonstrated that in one study conducted in 2012 and 2013 in Brazil, 56.8 % of the total number of examined calves were positive and in a similar study carried out in 2018, from 400 samples analysed, 51.25 % of the calves were positive for *Eimeria* spp. also [3]. These results imply that if *Eimeria* spp. infections are present on a farm, they will affect a large population of the young stock. This, in turn, will lead to a failure of the calves to thrive and reach their optimum finishing weight or production status in later life. Therefore, farmers should try to combat the problem when it first arises, as the transmission is rapid, and can become a big problem [4]. The two stains used in this study were carbol fuchsin and Kinyoun stain. Initially the Kinyoun stain was used, but there was great difficulty in the visualisation of the oocysts, and there were reports that this stain is better for visualisation of oocysts from carnivore faeces. After the first 32 samples were analysed, the staining method was then changed to carbol fuchsin, which was much better for the visualisation of the oocysts from the faeces of ruminants. *Giardia duodenalis*, which causes infection in cattle, can be detected as early as in 14 day old calves. It can be seen at every age but it is most common in calves 2—3 months old. Mucous and fatty stool, weight loss and growth retardation are the main disease symptoms [13, 14]. The positive samples gave a prevalence of 8.6 %, whereas previous studies involving *Cryptosporidium parvum* infections on farms in Slovakia demonstrated a much higher prevalence of 31.5 % samples positive for oocysts throughout the year [10]. It has been suggested that there can be some seasonal variations in relation to *Cryptosporidium* spp. infections, but more often it is thought that the monthly variations could be related to calving periods, with infections being more prevalent around the peak calving times [7, 9]. Infections with *Trichostrongylidae* family were very low, with only 1.5 % of the calves being infected from the total amount diagnosed with parasites. These nematode eggs can survive up to 6 months in the environment, either in stables or on pastures, allowing it to be a problem on farms where the hygiene level and removal of bedding is not the best [1]. Cattle tend to develop some immunity to these worms with age, and therefore, will shed the eggs in their faces without clinical signs in some cases. This means that calves can be easily infected if they come into contact with the faeces, and this may result in negative effects [4].

Trichuris spp. infections in the calves tested during this study were relatively low. Table 1 shows that 2.9 % of the calves were infected with this parasite and the same proportion was infected with *Strongyloides papillosus*. Similar studies on the prevalence of *Trichuris* spp. gave similar results with only 2.17 % of the calves being infected and the same study also showed infections with *Strongyloides papillosus* to be 2.17 %. However, the calves analysed in this study were up to 180 days old, which is a greater age range than of the calves analysed in our study [8]. The reason that the *Trichuris* spp. infections were so low could be explained by the typical light infections these worms cause, meaning there will only be a small amount of eggs present in the faeces. Heavy infections with this whipworm are rare, so it is possible that calves may have been infected, but just no eggs were present in the faecal samples. Therefore, a more sensitive way of diagnosis would be more suitable.

Many medications can be used for the treatment of calf parasites, but without proper management, the complete resolution of a parasite will not be achieved. One of the three farms analysed in this study had calves in stalls where they were in direct contact with the next calf with both nose-to-nose contact and the same applied to faeces. This direct contact allows easy transmission of parasites. It is recommended for the housing of calves that the calf pens are separated with a space in between, the direct contact is prevented and only visual contact between the calves is allowed. On two of the three investigated farms there was used the 'hutch' system that allows such separation and the calves can move inside for shelter and warmth or outside for fresh air.

However, on one farm this was not available, and the calves were housed inside all day long. This may result in warm, humid conditions if no proper ventilation is ensured. Such environment may support longer survival of cysts, oocysts and eggs of parasites [12].

CONCLUSIONS

It was seen that from all the calves examined, 64.8 % of them were infected with parasites. The highest prev-

alence of protozoan infections in calves was recorded for *Eimeria* spp., that was most common throughout the year. Other protozoa, including *Giardia duodenalis* and *Cryptosporidium* spp. occurred in a much lower prevalence, along with *Trichuris* spp., *Strongyloides papillosus* and *Trichostrongylidae* family. None of these parasites were found to be any higher than 4.4 % from the positive samples.

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