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Faculty of Tropical AgriSciences

Survey of parasitoses in beef cattle from two geographical areas of the Czech Republic

Master's thesis

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Declaration

Hereby I declare that this thesis entitled “Survey of parasitoses in beef cattle from two geographical areas of the Czech Republic” is my own work and that all literature sources I used are listed in References.

August 2016, Prague …….

Signature

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Abstract

Research in this diploma thesis was focused on monitoring of the beef cattle parasites and periodically was done from April 2015 to November 2015 on three different farms in two different regions (Vysočina and Středočeský region) in the Czech Republic. 20 samples of fresh faeces were collected every month from each farm during morning. Processing and consequent evaluation of samples took place in parasitology laboratory at State Veterinary Institute in Jihlava. Samples were evaluated using a relatively new coprological technique FLOTAC, developed in Italy and recommended for parasitological qualitative and quantitative analysis of large farm animal eggs and oocysts. For each farm two pooled samples (10 g each) by subtracting 1 g of faeces from individual samples were used. Results were evaluated and statistically analysed by statistical software Statistica 13. There was occurrence of eggs of gastrointestinal nematodes (family Trichostrongylidae), tapeworms (*Moniezia* spp.*)* and oocysts of coccidia (*Eimeria* spp.) on all of the farms. Only on the farm 3 there was also occurrence of fluke eggs (*Paramphistomum* spp.*).* From the results it was evident, that farms that administered anthelmintic to livestock had significantly lower amounts of EPG/OPG in animal faeces. Despite of using pooled samples, method proved to be reliable and sensitive for monitoring of developing stages of livestock parasites. Even low amount of eggs or oocysts in animal faeces were detected by coprological technique FLOTAC.

Key words: FLOTAC, pasture, beef cattle, parasites, EPG, OPG

Abstrakt

Výzkum v této diplomové práci zaměřené na monitoring parazitů masného skotu byl prováděn v období od dubna 2015 do listopadu 2015 na třech různých farmách ve dvou rozdílných krajích České republiky (Vysočina a Středočeský kraj). Každý měsíc bylo sebráno 20 vzorků čerstvého trusu z každé farmy. Zpracování a následné vyhodnocení vzorků proběhlo na Státním veterinárním ústavě v Jihlavě. Vzorky byly zkoumány relativně novou koprologickou metodou FLOTAC, vyvinutou v Itálii a doporučenou pro kvalitativní a kvantitativní analýzy výskytu vajíček a oocyst v trusu hospodářských zvířat. Z 20 vzorků z každé farmy byly vytvořeny dva směsné vzorky (po 10 g), odběrem 1 g z každého vzorku. Data se sbírala každý měsíc a následně byla analyzována statistickým softwarem Statistica 13. Na všech farmách byla detekována přítomnost parazitických druhů *Eimeria* spp., gastrointestinálních nematodů a *Moniezia* spp. Na farmě č. 3 byla také detekována přítomnost rodu *Paramphistomum*. Z výsledků je patrné, že farmy, které odčervovaly, měly výrazně nižší výskyt EPG/OPG v trusu chovaného dobytka. I přes použití směsných vzorků se metoda FLOTAC prokázala jako spolehlivá a dostatečně citlivá pro monitoring vývojových stádií parazitů. Byli jsme schopni detekovat i malá množství vajíček a oocyst parazitů v testovaném trusu.

Klíčová slova: FLOTAC, pastevní chov, masný skot, EPG, OPG, parazité

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List of Abbreviations

CMSCH	Czech-Moravian Association of Breeders
EPG	Eggs per Gram
FAO	Food and Agriculture Organization
FEC	Faecal Egg Counts
FECRT	Faecal Egg Counts Reduction Test
FS	Flotation Solution
GIN	Gastrointestinal Nematodes
OPG	Oocysts per Gram
s.g.	Specific Gravity
SVI	State Veterinary Institute
TST	Targeted Selective Treatment
TT	Targeted Treatment
US	United States
US$	United States Dollar
WAAVP	World Association of the Advancement of Veterinary Parasitology

1 Introduction and Literature Review

Beef cattle breeding is important part of the agriculture in the Czech Republic. Beef meat is traditional and one of the most favourite source of proteins, vitamins and minerals. Today's consumers require safe and tasty products. It is responsibility of each farmer to produce such product and ensure good conditions and welfare for the animals in the same time. One of the problems affecting both the performance of animals as well as their welfare and health conditions are parasitoses. Parasite infections can cause diarrhoea, weight loss, low feed conversion, dehydration and in severe cases even death of the animals (Daugschies and Najdrowski, 2005). Pasture based farming of beef cattle carry high risks of parasite occurrence (Barger, 1997). In conditions of the Czech Republic, main occurring are gastro-intestinal parasites.

Occurrence and process of infection by parasites are influenced by several factors ranging from age of animals, type of pasture or climatic conditions. In past, frequent solution of how to treat infected animals was excessive use of anthelmintics (Kaplan and Vidyashankar, 2012). This lead to rise of resistant strains of nematodes that is now worldwide problem (Gasbarre, 2014). Current trend is targeted treatment or targeted selective treatment of clinically sick animals (Kenyon and Jackson, 2012). Because of this, it is very important to detect possible parasite infections as soon as possible. For detection most frequently used methods are copromicroscopic techniques (Cringoli et al., 2004). Those are methods that focus on examination of faeces and detection of presence of parasite eggs, oocysts or larvae. However, there are huge differences among the techniques, mainly in their sensitivity and accuracy. It is also important to add, that detection of parasite eggs is only positive evidence that animal is infected, however it does indicate the degree of an infection or the clinical condition of animal, because of many other factors affecting it. How to proceed with informations of animal infection and choosing correct way of treatment is up to farmer and veterinarian (Zajac et al., 2011).

1.1 Beef Cattle

The concept of cattle breeds is said to originate in Britain, under the influence of Robert Bakewell in 18th century (Porter, 1991). During this period, intensive culling and inbreeding was common in order to achieve specific breeding goals, mainly to shift from draught animals to beef producing animals. To this day, British breeds have global influence and worldwide distribution (Wiener et al., 2004). British breeds can be characterized as smaller to medium size with early maturity. Biggest populations of British breeds can be found in North America. In countries such as France, Italy, and Belgium, some of the dual purposed (milk and meat producing animals) breeds were bred specifically for meat production. By using special breeding programs, new meat breeds were created with larger body frame and later maturity (Zahrádková et al., 2009).

In comparison of beef cattle with dairy cattle and dual purpose cattle, beef cattle breeds are the most important for meat production. They have better feed conversion, higher growth intensity, higher carcass yields and better quality of meat, but are not suitable for milk production (Zahrádková et al., 2009).

1.2 Beef cattle breeds in the Czech Republic

Cattle breeding is very important part of animal agriculture in the Czech Republic. Unfortunately, due to several reasons such as current economical situations and excessive meat import from other countries, beef production is decreasing (Kvapilík a Kohoutek, 2009). According to the information from CMSCH (Czech-Moravian Association of Breeders), in year 2013 on territory of the Czech Republic there was 184 597 units of beef cattle. According to information from Ministry of Agriculture of the Czech Republic, year 1990 is said to be beginning of farming of beef cattle in the Czech Republic.

1.2.1 Aberdeen Angus

One of the most widespread breed on the world. Originally from northeast Scotland, where native breeds of cattle were crossbred with Shorthorn (Vasconcellos et al., 2003). Naturally polled with solid black or red coat. Small to medium body size frame, average weight of

cows after third calving is between 560 to 640 kg. Adult bulls can weigh between 1000 to 1100 kg. Main advantages of this breed are good mothering and calf-rearing abilities, longevity, vigorous growth from birth to harvest and high quality carcass. Meat is characterized with slight marbling, tenderness, juiciness and specific taste. In present, Aberdeen Angus is the second most spread breed of beef cattle in the Czech Republic (Zahrádková et al., 2009).

1.2.2 Belgian blue

First information about Belgian blue breed comes from 19th century. It originated in central and upper Belgium, from crossing local breeds with a Shorthorn and later with Charolaise (Zahrádková et al., 2009). In years from 1960 to 1970 breeders focused on distinctive meat production with results being that 80 to 85% of animals have distinct muscular hypertrophy: double muscling (Kambadur et al., 1997). Double muscling animals have decreased levels of collagen, implying a lower background toughness that is associated with more tender meat (Boccard, 1981). Average weight of cows is between 700 to 750 kg, adult bulls can weight up to 1250 kg. Main advantages of this breed are high carcass yield (thanks to especially development muscling), small amount of fat, great feed conversion and good maternal behaviour (Raes et al., 2001). Main disadvantage is dystocia–difficult birth thanks to double muscling and narrow birth canal resulting in frequent Caesarean sections. Another disadvantage is worse tenderness of meat thanks to low marbling (Zahrádková et al., 2009). Some of the authors reported that double muscling meat is of pale colour, less tasty and has reduced water-binding capacity (Boccard, 1981; Bailey et al., 1982). In the Czech Republic, Belgian blue breed is officially from year 1994, and currently is very popular among breeders mainly for use in crossbreeding (Zahrádková et al., 2009).

1.2.3 Blonde d'Aquitaine

This breed originates from southwest France, being result of thorough selection and combination of three local strains: Guercy, Garonnaise and Blonde des Pyrenees. Blonde d'Aquitaine is usually single coated with colours ranging from white to slightly red.

Average weight of cows is ranging from 800 to 1100 kg, average weight of adult bulls is between 1200 to 1500 kg (Zahrádková et al., 2009). Main advantages of this breed are good muscling, carcass with little amount of fat, good maternal behaviour, resistance to unfavourable weather conditions and easy parturition (Listrat et al., 2001). In the Czech Republic is Blonde d'Aquitaine officially from 1991 (Zahrádková et al., 2009).

1.2.4 Galloway

One of the oldest beef cattle from British Isles, originating from southwest part of Scotland. Herd book was established in 1881 (Decker et al., 2009). Naturally polled breed with small body frame and lower body growth intensity. Minimal average weight should be 500 kg for the cows and 640 kg for adult bulls (Zahrádková et al., 2009). Galloway breed has thick double-layered wavy or curly coat with colours ranging from black to yellow (Brenig et al., 2013). Main advantages of this breed are good adaptation to harsh weather conditions and ability to survive throughout all year on pastures, good maternal instincts and calving without problems. Meat is tender and juicy with high content of unsaturated fatty acids. In the Czech Republic is Galloway officially from 1991 (Zahrádková et al., 2009).

1.2.5 Gascon

Gascon cattle were originally bred in French Pyrenees and used as multipurpose animal for drought, meat and milk. Thanks to breeding, current Gascon cattle is focused on meat production. Average weight for cows is 660 kg and for adult bulls is 1000 kg. Colour is ranging from light grey to silver. Main advantages of this breed are great adaptability to harsh climatic conditions and ability to survive in mountain locations with steep slopes. Another advantages are ease calving, good maternal instincts and long longevity. Gascon cattle is in Czech Republic officially from 1994 (Zahrádková et al., 2009).

1.2.6 Hereford

One of the oldest and most widespread beef breed on the world, originally from Herefordshire in England. Breed is result of systematic selection of native red coated breeds focusing on muscling and fattening. Herd book was established in 1864. Suitable breed for

extensive pasture farming with minimum weight 580 kg for cows and 900 kg for adult bulls. Colour is dark red with white head and white bottom. Main advantages are easy calving, good maternal instincts, good adaptability for harsh climatic conditions, early maturing, high feed efficiency and growth and calm disposition (Zahrádková et al., 2009). In Czech Republic is officially from 1974 and currently is the third most widespread beef cattle breed (Bureš and Bartoň, 2010)

1.2.7 Charolais

One of the worldwide most widespread beef cattle breed and most widespread beef cattle breed in Europe, originating from France as result of breeding of native yellow cattle during late 18th century. Bred in central France with focus on positive selection of individuals with early maturity. Herd book was established in 1864. Average weight is 750 kg for cows and 1200 kg for adult bulls. Colouring ranges from single coated white to cream coloured. Main advantages of this breed are high intensity of growth, good muscling and low amount of fat. Often used in crossbreeding. Main disadvantage is intensive growth of calf in prenatal period resulting in complicated calving. In Czech Republic is officially since 1990 (Zahrádková et al., 2009).

1.2.8 Limousine

Originated from southwest France from Limousine region. Until first half of 20th century mainly used for drought. Thanks to selection, today Limousine is beef cattle breed with good muscling and low amount of fat (Alfredo et al., 2007). Average weight is 630 kg for cows and 1000 kg for adult bulls. Colouring ranges from single coating red to golden-red. Main advantages of this breed are high feed conversion, high fertility, good maternal instincts and easy calving. Meat is tender and juicy but with lower marbling. Often used in crossbreeding. In the Czech Republic officially since 1990 (Zahrádková et al., 2009).

1.2.9 Piedmontese

Originating from Piedmont region in northwest Italy. Originally multipurpose animal. Focusing on meat production began in beginning of 20th century. Average weight for cows

is 600 kg and for adult bulls 900 kg (Zahrádková et al., 2009). In population of Piedmontese cattle is high occurrence of double muscle animals (Kambadur et al., 1997). Colouring is single coated white. Early maturing animal with good adaptability to harsh condition and high feed conversion. High carcass yields and low amount of fat with specific taste of meat. Often used in crossbreeding. In the Czech Republic officially since 1993 (Zahrádková et al., 2009).

1.2.10 Simmental

Beef cattle breed originating from Switzerland from 18th century. Large body frame with average weight 700 kg for cows and 1100 kg for adult bulls. Colouring is red spotted. Main advantages are early maturity and good adaptability to harsh condition (Zahrádková et al., 2009). According to Gauly et al. (2001) Simmental cattle can be difficult to handle. In the Czech Republic officially since 1993 and currently is one of the most widespread beef cattle breeds (Zahrádková et al., 2009).

1.2.11 Salers

Originating from France from Cantal region. Hardy breed with good adaptability to harsh climatic conditions. Average weight is 690 kg for cows and 1050 kg for adult bulls. High intensity growth with early maturity. Colouring is mahogany red or black with whit thick coat. Main advantages are calm behaviour, easy calving and good maternal instincts. In the Czech Republic officially since 1995 (Zahrádková et al., 2009).

1.3 Biosecurity

Biosecurity is defined by FAO as: "The implementation of measures that reduces the risk of the introduction and spread of disease agents" (FAO, 2010). In the European Union health strategy for 2007-2013 is importance of biosecurity underlined with a new motto: "Prevention is better than cure" (European Commission, 2007). Biosecurity measures prevent both direct disease transmission between animals and indirect transmission between farms (Ellis-Iversen et al., 2011). According to Lin et al. (2003) disease prevention is becoming important in replacing individual animal medicine treatment. Implementation of

biosecurity includes all measures of preventing pathogens from entering a herd (external biosecurity) and reducing spread of the pathogens within a herd (internal biosecurity) (Sarrazin et al., 2014).

In farming of beef cattle, biosecurity is directly dependent on farming system. Pastoral or grazing system offers opportunity to reduce input costs during the grazing season and increases animal welfare (Waller, 2006). Generally, animals are kept on pasture during vegetation season and in confinement during winter season. Basic measures of biosecurity are easier to kept in confinement, during grazing upholding of those measures is more complicated and animals are exposed to pasture borne parasites (Stromberg and Averbeck, 1999).

Biosecurity is influenced by several factors: animal, human, environment and occurrence of pathogens. In case of animals, biggest danger is inclusion of new animals. Preventive measure is thorough health inspection prior buying, and obtaining maximum information about health situation within herd and region from which animals are bought. Precautions should relate also on transport of animals, feeds and even water. All transporting vehicles should be cleaned and disinfected. On pasture, health condition of animals should be checked every day and all unusual signs should be noted (strange behavior, sudden deaths, large amount of sick animals). Human factor is also very important. During contact with animals, personnel should follow basic hygienic procedures. During grazing season, problem can be entry of persons on pasture if touristic or cycle trails lead through them. Problem of environment is also entry of domestic or wild animals (dogs, cats, deer, birds, insects etc.) Environmental factor also includes weather conditions such as temperature, rain, humidity, sunlight. Pasture is the site of egg deposition, hatching, larval development and ingestion of infective larvae by the definitive host (Stromberg, 1997; Stromberg and Averbeck, 1999; Waller, 2006; Sahlstrom et al., 2013; Sarrazin et al., 2014; O'Mahony, 2015).

One of the possibilities of how to increase biosecurity is control by management. This means role of grazing management in reducing anthelminthic use and improving helminth control. Control by management include several strategies designed to reduce or affect the

numbers of parasites in animal production systems. These strategies are classified by Michel (1985) into three categories: preventive, evasive, diluting.

Preventive strategies, can be specified as those that rely on putting healthy (worm free) animals on a clean pasture or by using anthelminthic treatment in early part of grazing season for suppressing the egg output. Most common example of preventive strategy is regime using short intensive treatment on the first half of the grazing season for calves in their first year (Barger, 1997). Major drawback of this strategy is increasing anthelminthic resistance, as reported by Rose et al. (2015). Another form of preventive strategy is the alternation of different host species – most commonly sheep and cattle over the same pasture. Prevention of contamination is achieved by host specifity. In general, parasite species that are highly pathogenic in one host species are less pathogenic in an alternate host. Of course, this does not function every time (Barger, 1997).

Evasive strategies rely on removal of existing infection by anthelminthic treatment together with a movement of a herd to safer pasture, just before the population of infective larvae rise to dangerously high concentration (Barger, 1997). Example of the evasive strategy can be popular rotational grazing. During rotation, pasture growth is optimized, resulting in increased carrying capacity of the pasture. Rotational grazing involves moving of cattle to new segments of the pasture, and remaining there from 1 day up to several weeks. Longer periods result in consumption of almost all available forage by cattle. This forces animals to graze closer to the ground and closer to the faeces, increasing chance of ingesting infective larvae found on herbage close to the faeces (Stromberg and Averbeck, 1999). Biggest problem of rotational grazing is long survival times of infective larvae on pasture with short rotational periods. This is of course species specific (Gibson, 1973).

Diluting strategies can be described as an exploiting of grazing of susceptible animals with a greater population of less susceptible animals of the same or different species. The principle is that average rate of contamination on the pasture will be reduced over what it would have been on pasture stocked with more susceptible animals alone (Barger, 1997). According to Jordan et al. (1988) who examined the effect of mixed grazing of ewes and

cows, results indicated decreased parasitism and increased productivity of lambs in ewes however the opposite in cows.

Loosely connected to biosecurity is biological control, as a potential part of control strategy against parasites in grazing livestock. In cattle, biological control can implement a concept of fungi destroying nematodes. This concept is not new, first attempts are dated in 1930s (Caswell and Apt, 1989). Potential control agents are nematode-destroying fungi or nematophagous fungi, majority belonging to Deuteromycetes. These fungi can be divided into two groups: predacious fungi that trap nematodes on the growing hyphae and endoparasitic fungi that produce spores, which lodge in the oesophagus of the feeding nematode (Larsen et al., 1997).

1.4 Often occurring of parasites species

Parasites are organisms that lives on or in a host organism and gets their food from or at the expense of their host. Parasites can be divided into two main classes: endoparasites and ectoparasites. Endoparasites are parasites that live inside their hosts and ectoparasites are parasites that lives on their host, for example attached to their skin. (Zajac et al., 2011; Prantlová Rašková and Wagnerová, 2013). Research in this thesis is focusing on endoparasites. Following information are focused on some of the often occurring parasite species on pastures of the Czech Republic.

1.4.1 *Eimeria* spp.

The genus *Eimeria* belong to the phylum Apicomplexa. It can also be known under its common name Coccidia. It is worldwide parasite which infect the intestinal tract of domestic or wild ruminants and camelids (Rommel et al., 2000). Faecal oocysts sporulate in the environment in 4-6 days (sporulated oocysts contain four sporocysts each with two sporozoits) and infect intestinal cells following ingestion. Further development consisting of asexual multiplication, gamogony and sexual stage takes place in small and large intestines, after which oocysts are produced and exit the host in faeces (Daugschies and Najdrowski, 2005). In favourable environmental conditions, sporulated oocysts can survive for long periods of time (Zajac et al., 2011; Prantlová Rašková and Wagnerová, 2013).

Following **Table 1** shows the main species of *Eimeria* spp.

Table 1. Main species of *Eimeria* spp.

Species	Discovery
E. bovis	Zublin, 1928
E. zuernii	Rivolta, 1878
E. subspherica	Christensen, 1941
E. alabamensis	Christensen, 1941
E. ellpsoidalisi	Becker a Frye, 1929
E. cylindrica	Wilson, 1931
E. cadanensis	Bruce, 1921
E. auburnensis	Christensen a Porter, 1939
E. bukidnonensis	Tubangui, 1931
E. pellita	Supperer, 1952
E. illinoisensis	Levine et Ivens, 1967
E. wyominensis	Huizing et Winger, 1942
E. brassiliensis	Torres et Ramos, 1939

Most of the *Eimeria* spp. cause subclinical coccidiosis or diarrhoea, but in most cases it ceases after completion of intestinal reproduction. These species vary in pathogenicity with only *E. bovis* and *E. zuernii* causing serious clinical disease, with symptoms like

haemorrhagic diarrhoea, intestinal lesions, cachexia, exhaustion, and sometimes fatal results especially in young calves. (Chroust et al., 1998; Munya and Nhotho, 1990; Daugschies and Najdrowski, 2005; Prantlová Rašková and Wagnerová, 2013). However according to Fitzgerald (1980), subclinical coccidiosis exceeds the clinical in monetary losses, because of much more frequent occurrence and possible disruption of feed conversion and growth of animals.

Prevalence of *Eimeria* spp. infection in cattle is relatively high and in calves can reach up to 100%, with most susceptible being calves in age of 3 weeks to 6 months (Taylor and Catchpole, 1994). Infection of *Eimeria spp.* can be diagnosed by simple faecal flotation exams, more complicated PCR examinations, or by microscopic examination of intestine during autopsy (Zajac et al., 2011; Prantlová Rašková and Wagnerová, 2013). Therapy and prevention depends on early diagnosis and for curing sulfonamids are used (Chroust et al. 1998).

1.4.2 *Paramphistomum* spp.

Paramphistomum spp. belong to the class Trematode. It is also known under its common name rumen fluke. Definitive hosts being ruminants, mainly cattle and sheep and also camelids. Adult flukes are located in the rumen of host. After releasing with faeces, eggs hatch in water into miracidia. For a full life cycle, *Paramphistomum* spp. needs an intermediate hosts, these hosts are aquatic snails of genera *Bulinus, Planorbis, Physa*. After development in snails, cercariae are released and encyst on vegetation. Definitive hosts are infected by ingesting fluke metacercariae during grazing (Zajac et al., 2011; Prantlová Rašková and Wagnerová, 2013). Following **Table 2** shows main species of *Paramphistomum* spp. affecting cattle (Mage et al., 2002).

Table 2. Main species of *Paramphistomum* spp.

Species	Discovery
P. cervi	Zeder, 1970
P. microbothrium	Fischoeder, 1901
P. daubneyi	Dinnik, 1962
P. ichikawai	Fukui, 1922

Paramphistomum spp. is causing disease called paramphistomosis. It is a worldwide illness, although the highest frequency has been registered in tropical and subtropical regions. Infection of *Paramphistomum* spp. affects production: low feed conversion, loss of weight, and decrease of milk production in dairy cattle (Ragel-Ruiz et al., 2003). In heavy infections, they cause enteritis, characterized by oedema, ulceration and haemorrhages (Ghosh et al., 2013). According to Panda (1985), immature flukes cause high degree of morbidity and mortality.

Infection of *Paramphistomum* spp. can be diagnosed using sedimentation or flotation methods for eggs and autopsy of rumen for adult flukes

1.4.3 *Fasciola* spp.

Genus *Fasciola* belongs to class Trematode. Under common name is known as liver fluke. Main species are *Fasciola hepatica* (temperate liver fluke) and *Fasciola gigantica* (tropical liver fluke). Definitive hosts are ruminants, camelids and variety of other animals, including dogs, horses and even humans. Intermediate hosts are snails, in the Czech Republic species *Galba trucantula*. Adult flukes are located in bile ducts. Life cycle begins when miracidia hatch from the eggs and invade appropriate snail host. Cercariae emerge from the snail and encyst on vegetation until they are ingested by host animals. Larvae then

leave the gastrointestinal tract and travel through livers until they reach the bile ducts (Zajac et al., 2011; Prantlová Rašková and Wagnerová, 2013). Accordind to Happich and Boray (1969), *Fasciola hepatica* is capable of high reproduction, reaching 25 000 eggs per fluke per day in sheeps.

Fasciola spp. causes disease called fasciolosis. It is estimated that worldwide fasciolosis affect more than 600 million animals and annual loss is more than 3 billion US$ through production losses, mortality etc. (Mahana et al., 2015). Main symptoms are fever, gastrointestinal symptoms, anemia, and abdominal pain; in chronic cases inflammation of bile ducts, gall bladder and apathy (Prantlová Rašková and Wagnerová, 2013).

Eggs of *Fasciola* spp. can be detected using sedimentation, but can be hard to detect. Adult flukes can be detected during autopsy (Zajac et al., 2011).

1.4.4 Moniezia spp.

Genus *Moniezia* spp. belong to class Cestoda. It can also be known under common name Tapeworm (Zajac et al., 2011). Worldwide parasite with definitive hosts ruminants and camelids. For completing of life cycle it requires mite of family *Oribatidae*. Matured eggs are shed in segments from the host and ingested by the mite. Inside mite the eggs continue to grow into an invasive cysticercoid larva. Full formation of developed cysticercoids takes 15 to 18 weeks. The *Oribatid* mite is then consumed by the definitive host, where further development into adult occurs in the intestine (Barriga, 1994). Following **Table 3** shows main species of *Moniezia* spp.

Table 3. Main species of *Moniezia* spp.

Species	Discovery
M. expansa	Rudolphi, 1980
M. benedeni	Moniez, 1879
M. autumnalis	Kuznetsov, 1967
M. monardi	Fuhrmann, 1931
M. baeri	Skrjabin, 1931

According to Dever et al. (2015) infection of *Moniezia* spp. is very common. Generally, it has low clinical importance, only in case of large infection can cause diarrhea, lower body growth or colic pains (Gomez-Puerta et al., 2008).

Infection of *Moniezia* spp. can be detected macroscopically by seeing segments of body in faeces, or by flotation for eggs and autopsy of intestine for adults (Prantlová Rašková and Wagnerová, 2013).

1.4.5 *Nematodirus* spp.

Genus Nematodirus belong to phylum Nematoda. Under common name is known as thread neck worm (Zajac et al., 2011). Worldwide parasite of order Strongylida infect small intestine of ruminants and camelids. Larvae develop to the infective stage within the egg (this makes them resistant to cold and dryness) and hosts are infected after ingestion of the hatched infective larvae (Zajac et al., 2011). Following **Table 4** show main species of *Nematodirus* spp.

Table 4. Main species of *Nematodirus* spp.

Species	Discovery
N. abnormalis	May, 1920
N. battus	Crofton and Thomas, 1951
N. spathiger	Railliet, 1896
N helvetianus	May, 1920
N. filicollis	Rudolphi, 1802

Infection of *Nematodirus* spp. is not generally very harmful. Relatively high pathogenicity shows species *Nematodirus battus* and is dangerous especially for lambs. It can cause diarrhea and dehydration. For calves, most dangerous species are *Nematodirus helvetianus* and *Nematodirus spathiger*. *Nematodirus* spp. can be diagnosed with coprological examinations (Prantlová Rašková and Wagnerová, 2013).

1.4.6 *Cooperia* spp.

Genus Cooperia belongs to phylum Nematoda. Worldwide parasite with hosts being ruminants and deer. Under common name known as small intestinal roundworms. *Cooperia* spp. life cycle is typical for family *Trichostrongyloidea*, with exception that they do not feed on blood. After ingestion larvae penetrate mucosa of small intestine. Adult worms in small intestine produce eggs that develop in manure in the environment (Zajac et al., 2011). Following **Table 5** show main species of *Cooperia* spp.

Table 5. Main species of *Cooperia* spp.

Species	Discovery
C. curticei	Railliet, 1893
C. oncophora	Railliet, 1898
C. pectinate	Ransom, 1907
C. punctata	von Listow, 1907
C. surnabada	Antipin, 1931

Cooperia spp. is one of the most prevalent cattle parasite in temperate climate, but is considerate as mild pathogens, with greater veterinary importance only being *Cooperia punctata* and *Cooperia pectinata* (Van Meulder et al., 2015). Mixed infections with species *Ostertagia ostertagi* can result in significant economic losses (Charlier et al., 2014). Main symptoms of *Cooperia* infection are diarrhoea, dehydration, lower body weight gain. Adults burrow into the wall of duodenum ad harm the tissues and blood vessels. Massive infection is harmful especially for young animals (Prantlová Rašková and Wagnerová, 2013).

Eggs of *Cooperia* spp. can be detected with flotation based FEC techniques, larvae with coprocultivation and adults during autopsy in small intestine (Prantlová Rašková and Wagnerová, 2013).

1.4.7 *Ostertagia* spp.

Genus Ostertagia belongs to phylum Nematoda and comprise of several species including *Ostertgia ostertagi* and Ostertgia *lyrata*. Under common name known as brown stomach worms. Hosts are domestic and wild ruminants. Worldwide parasites, with direct life cycle and no intermediate hosts. Adult females lay eggs in stomach or intestine of the host that

are shed with the faeces. Infective larvae are capable of surviving on pasture for up to 14 months. Hosts become infected after ingesting infective larvae on pasture (Zajac et al., 2011).

Ostertagia spp. is considered as the most damaging worms of cattle (Bloemhoff et al., 2014). There are two clinical developments, type I ostertagiosis and type II ostertagiosis. Type I affects calves during first grazing season when they become infected for the first time. Type II affects adult cattle when larvae resume development during winter and spring. Most damaging are larvae. They burrow into cells of stomach, subsequently cells of stomach proliferate trying to heal, being unable to produce acid. Consequence of this is rising of pH of the abomasum. All of this results in inability of pepsinogen to transform into pepsin, hindering protein denaturation and subsequent digestion in the intestine. Symptoms of *Ostertagia* spp. infection is diarrhoea, losing of appetite, gastroenteritis, dehydration. Massive infection can lead to high mortality (Prantlová Rašková and Wagnerová, 2013).

Eggs of *Ostertagia* can be detected with flotation based FEC techniques adults during autopsy in stomach. Possibility is to measure pH of rumen liquid (Prantlová Rašková and Wagnerová, 2013).

1.5 Faecal egg counting techniques

Faecal egg counting (FEC) techniques are one of the most common diagnosis of gastro-intestinal parasitic infections. They are non-invasive and relatively inexpensive flotation based examinations that can reveal presence of parasites in body system of animal. Parasites inhabiting the digestive system produce eggs, larvae or cysts that leave the body of the host by the way of the faeces (Zajac et al., 2011), which are subsequently identified and quantified. Egg counting techniques are recommended primarily for estimation of the extent of parasite egg contamination and determination of the efficacy of drug treatment. Parasite eggs, larvae or cysts are positive evidence that an animal is infected, however does not indicate the degree of an infection and the clinical condition of animal because of many factors affecting it, such as egg production of parasitic species, individual host immunity

and stage of infection. Generally, it is believed that there is no correlation between the numbers of eggs, larvae or cysts per gram of faeces and the number of adult parasite present in animals (Zajac et al., 2011; FAO, 2015).

There are various factors that can limit the accuracy and significance of faecal egg count. There can be fairly regular fluctuation in faecal egg output and eggs are also not evenly distributed throughout the faeces. The egg output is influenced by the season of the year. Without the knowledge of characteristics of parasite eggs to recognize and determine the species, the egg count only show total number of eggs of a mixture of species which can differ in their biotic potential and their pathogenicity. Some techniques have low sensitivity and do not detect low numbers of eggs (FAO, 2015).

1.5.1 Faecal flotation

The simplest procedure of flotation based techniques. Involves mixing a small amount of faeces with flotation solution in a cylinder (or centrifuge tube) and then adding the solution until the cylinder is nearly full (Dryden et al., 2005). After mixing and waiting, the less dense material floats to the top and sample can be removed from the top to a microscope slide using a wire loop, straw, needle hub or glass rod. Possible modification is filling the cylinder until a slight positive meniscus is formed and placing glass coverslip over it (Zajac et al., 2011). Further modification involves centrifugation to spin down the debris and dirt and to allow the eggs to float to the top (Dryden et al., 2005). Very important part of faecal flotation is choosing right flotation solution. The higher the specific gravity of the flotation solution, the greater the variety of parasite eggs that can be found floating on top. However, with higher specific gravity more debris will float as well (Zajac et al., 2011).

1.5.2 Faecal sedimentation

Procedure used for isolation of eggs of flukes, acanthocephalans and tapeworms. Simple sedimentation uses tap water mixed with faeces and subsequent settling before the supernatant is removed. Simple sedimentation test has only limited concentrating ability. Centrifugal sedimentation test can also be used, using ethyl acetate. With centrifugal sedimentation test fat and mucus can be removed from faeces sample (Zajac et al., 2011).

1.5.3 McMaster technique

Traditional faecal egg counting technique. Developed in Australia in 1939 by a laboratory assistant for easier routine egg counts in sheep faeces (Gorden and Whitlock, 1939). In world, many variations of McMaster technique can be found and many scientists still continue to introduce new modifications. These variations differ in use of various weight of faeces examined, volumes and types of flotation solution, sample dilutions, flotation times, applications of additional centrifugation, durations and speeds of additional centrifugation, numbers of sections of the McMaster slide counted and different coefficients for interpretation (Vadlejch et al., 2011). Problems of McMaster technique is lack of sensitivity at particularly low egg counts. According to Coles et al. (1992) McMaster is accurate from 50 EPG. In some cases of special modification of McMaster method, the sensitivity and accuracy can be from 10 EPG (Cringoli et al., 2010). Another problem is using small amount of faeces to determination of faecal egg count, because of following extrapolation to one gram of faeces, which renders the estimation of eggs per gram (EPG) less precise (Mes, 2003). According to Duthaler et al. (2010), McMaster is not suitable for detecting of parasites such as flukes and for situations where sensitive egg counts are required, otherwise in most cases this method is adequate.

1.5.4 FECPAK

A commercial kit FECPAK can essentially be described as a larger version of McMaster. It is a modified McMaster approach without the need of centrifuge and with minimum detection limit of 30 EPG (Coles et al., 2006) and larger starter aliquot – 20 g. The principle of this method is mixing samples with flotation solutions and subsequent placing under a slide with two gridded chambers. Eggs float to the surface and the gridded slide can be examined under microscope (Goldber et al., 2014). According to Presland et al. (2005), in horses, FECPAK is simpler to use than McMaster.

1.5.5 Kato-Katz

Kato-Katz is a quantitative method for preparing faecal samples mainly used in human samples. Faeces is pressed through sieve and faecal sample (20-50 mg measured by

template) is transferred to a slide. A piece of cellophane is soaked in glycerine and pressed on the faeces resulting in glycerine clearing debris and enabling to see parasite eggs. The results allow an estimation of intensity of infection (Katz et al., 1972). There are some arguments for rejecting the Kato-Katz technique: high risk of infection for the technician and low sensitivity (Kongs et al., 2001) Arguments for recommending the technique are that is cheap, easy to learn and that sensitivity can be increased using double Kato-Katz technique (Ebrahim et al., 1997). Analytic sensitivity of Kato-Katz is 24 EPG (Rinaldi et al., 2011)

1.5.6 FLOTAC

FLOTAC is relatively new method, developed in 2009 by team of professor Cringoli from University of Naples Federico II. This method uses special FLOTAC apparatus and requires several centrifugations. The exact process of using FLOTAC is described in chapter Material and methods. FLOTAC method can be used in three different modifications. First is FLOTAC basic technique which uses a single flotation solution, and is recommended for diagnoses of faecal samples containing very low number of parasite eggs from a single parasite species. The analytic sensitivity of FLOTAC basic technique is 1 EPG. Second is FLOTAC dual technique, based on the use of two different flotation solutions and are used parallel on the same faecal sample. Recommendation of this technique is for epidemiological surveys and routine diagnosis in order to perform a wide-ranged parasitological screening. The analytic sensitivity of FLOTAC dual technique is two EPG. Last is FLOTAC double technique, based on the simultaneous examination of two different faecal samples from two different hosts using a single FLOTAC apparatus. The analytic sensitivity of FLOTAC double technique is 2 EPG (Cringoli et al., 2010).

1.6 Anthelminthic resistance

As was mentioned in previous chapters, anthelminthic resistance in cattle is growing problem and can no longer be ignored (Kaplan and Vidyashankar, 2012). Previously, anthelminthic resistance that limited effective nematode control has been issue mainly for sheep and goat breeders (Besier and Love, 2003). With pasture based farming, cattle are

frequently exposed to parasite, and with anthelminthic being essential in optimizing performance and health condition, possible reduction of their efficacy can lead to reduced animal performance and worsen health condition (O'Shaughnessy et al., 2015). In recent years, anthelminthic resistance in nematodes of cattle has been reported from number of countries as suggest reports from Sutherland and Leatwick (2011), Kaplan and Vidyashankar (2012), Gasbarre (2014) or Waghorn et al. (2006). Consequently, anthelminthic resistance is important problem redefining how parasite control should be practiced and how anthelminthic should be used within the context of parasite control programs (Kaplan and Vidyashankar, 2012). Early detection of resistance to all types of anthelminthic is important, so that necessary changes in management can be made (Rinaldi et al., 2010). It is important to establish sustainable nematode control practices, which would protect the future use of current anthelminthic families (van Wyk et al., 2006) In 1995, Craig and Wikse (1995) stated: "Twenty years ago, anthelminthics in cattle were used to salvage clinically sick animals. Today, they are used to maximize profit". According to Ramos et al. (2016) in Brazil anthelminthics are used "at will" with no restriction to access to commercially available drugs and no assistance from veterinarians, resulting in inadequate use of anthelminthics. Similar problems have been reported also from other South American countries (Delgado et al., 2009; Zanetti Lopes et al., 2013).

1.6.1 Methods of detecting anthelminthic resistance

According to Wood et al. (1995) the definitive test for measuring of effectiveness of anthelminthic drug against any species of nematode parasite is treatment of the host with anthelminthics and subsequent killing of the host and recovery, enumeration and speciation of the nematodes surviving treatment. Comparison with control group (untreated animals) will allow direct measure of the effectiveness of the drug when administered according to approved protocol. Such testing is required for licensing of the new anthelmintic drug. Major drawback of this test is of course that for statistically significant results, sufficient number of animals must be killed in both groups. As Gassbare (2014) pointed out, nematode distribution in animals is arranged in "negative binomial" distribution. This distribution is characterized by the standard deviation exceeding the mean of the group.

Meaning that majority of animals will have relatively low numbers of parasites, while few animals will have large numbers of animals. This means, that testing and control group need to include some of the "high parasite" animals. According to World Association of the Advancement of Veterinary Parasitology (WAAVP) sufficient amount of animals is six per each group (Powers et al., 1982).

For obvious reasons, previous method is not preferred method in field studies focusing on anthelminthic resistance. As such, these studies must rely on less precise methods. These methods can be divided into two groups: the first is in vitro method, second is method measuring egg output in faeces (Gassbare, 2014).

In vitro method were used firstly in small ruminants and have limited range of targets. In cattle in US, according to Ciordia (1973) 25 different species can be tested by in vitro method. In addition to small amount of targets, some of the most economically dangerous parasites such as *Ostertagia ostertagi* are difficult to maintain in vitro for longer time periods. According to Demeler et al (2010) there has been advances in Europe with in vitro method, but still only few laboratories offer such testing.

Second method, focusing on use of faecal egg reduction count test (FECRT). According to guidelines of WAAVP in cattle, recommended amount is 15 animals in each group with minimum individual count of 100 EPG. The timing between treatments FECRT test is different for each group of anthelmnithics – for Levamisole 3-7 days, for benzimidazole 8-10 days and for macrocyclic lactones 28 days. For testing method, more sensitive methods than McMaster are recommended (Coles et al., 2006).

1.6.2 Current trends of sustainable use of anthelminthic

As was written before, to protect current anthelminthic families from resistance, sustainable use is need to be introduced. Kenyon and Jackson (2012) introduced two possible concepts: targeted treatment (TT where the whole herd is treated based on knowledge of risk or parameters that quantify the severity of infection; and targeted selective treatment (TST) where only individuals are treated. The aim of these concepts is to effectively control nematode prevalence while preserving the efficacy of the anthelminthic drugs by allowing

pool of untreated parasites that can complete their life cycle and pass on susceptibility associated genes to the next generations.

According to Morgan et al. (2014) in cattle TT and TST are appropriate for all ages, however the means of application are different. In first season grazing cattle, indicators to support TT are mean faecal egg counts after 4 to 8 weeks and mean serum pepsinogen level at the end of grazing season. Serum pepsinogen levels are good parameter to assess the pathology induced by *Ostertagia ostertagi*. Serum pepsinogen concentrations can be used retrospectively for future first season grazing cattle to change parasite management, while faecal egg counts can be used to change parasite management in the same season. According to Areskog et al. (2013) who performed study focus on FEC. An average amount of 100 EPG was suggested as a threshold for the anthelminthic treatment. With knowledge gain from first year of field trials, next year use of anthelmithics was reduced while maintaining same amount of infected animals. The TST approach, which investigated correlation between FEC and daily weight gain successfully reduced use of anthelminthic by 92% (Greer et al., 2010). In adult beef cattle no research concerning TT or TST approach has been done.

2 Aims of the thesis

The general aim of the thesis was to monitor and evaluate parasite occurrence (amount of EPG/OPG) in beef cattle fed on pastures with FLOTAC method. Specific aim was to evaluate and analyse amount of eggs and oocysts in faeces of the animals.

We established two hypotheses.

H1: Farms using anthelminthic will have lower amounts of EPG/OPG in the faeces of the animals than the farm that did not use anthelminthic.

H2: We do not expect presence of oocysts of *Eimeria* spp. in adult cattle.

3 Materials and methods

Samples were collected from three different farms in two different regions in the Czech Republic. As promised to the owners of farms, exact locations of farms will not be revealed to protect their anonymity, instead only regions and numbers will be used. Two farms are located in Vysočina region (farms 1 and 2) and one in Středočeský (farm 3) region. Samples were collected in cooperation of MVDr. Libor Borkovec, a technical manager for livestock from Zoetis Czech Republic. Samples were collected every month from April 2015 to November 2015. Each month was collected 20 samples of fresh faeces per every farm during morning. On farm 2 and 3 were samples collected directly on pasture. On farm 1 were samples collected in holding box where animals were brought. Samples were collected into plastic box, marked, put into cooling box and transported to State Veterinary Institute (SVI) in Jihlava.

Table 6. Information about farms in Vysočina and Středočeský region

Farm	No. of cows	No. of calves	No. of bulls	Breed	Deworming	Used anthelmintic	Pasture season
Farm 1	37	35	1	Aberdeen Angus	Yes	Levatum super	May - November
Farm 2	100	100	5	Aberdeen Angus	Yes	Levatum super	April - November
Farm 3	29	28	1	Aberdeen Angus	No		April - November

According to information from the owners of the farms, only two farms (Farm 1 and 2) used anthelminthic during their pasture season. In case of Farm 1 they used Levatum Super® (Zoetis Czech Republic), anthelmintic with active substances ivermectin (10 mg per 1 ml) and clorsulon (100 mg per 1 ml). Drug administration of anthelminthic was done in May, at the beginning of the pasture season on Farm 1. Farm 2 also used Levatum Super® (Zoetis Czech Republic), but to the animals was given twice a year: in June and November. Farm 3 did not use any anthelminthic treatment.

3.1 Laboratory evaluation of samples

Processing and consequent evaluation of samples took place in laboratory at State Veterinary Institute (SVI) in Jihlava under supervision of MVDr. Karol Račka, a head of Department of Parasitology. For laboratory evaluation of samples was used relatively new method FLOTAC, developed in Italy and recommended for qualitative and quantitative analysis of large farm animals. This method has recommendation of *World Association for the Advancement of Veterinary Parasitology* (WAAVP).

3.2 FLOTAC

FLOTAC is highly accurate and sensitive flotation method intended for diagnosis of parasite eggs or oocysts. This method was developed by professor Cringoli and his team from University of Naples Federico II. The exact modification was FLOTAC dual technique based on parallel testing of one sample with two different flotation solutions. During the research we followed the protocol by professor Cringoli and MVDr. Karol Račka, a supervisor of this research, gained experiences directly from University of Naples Federico II.

From practical point of view, SVI Jihlava used modification of previously described FLOTAC dual technique, when there were prepared two pooled samples (10 g each) by subtracting 1 g of faeces from individual samples. These pooled samples were diluted in tap water (dilution ratio 1:10). Sample was homogenized firstly by mixing it with glass rod and then by using hand blender. After that, suspension was filtrated through a wire mesh (aperture 250 μm). Filtered suspension was transferred into two conic tubes. Total volume of each conic tube was 6 ml. Each of two flotation chambers of the FLOTAC apparatus require 5 ml, spare 1 ml is necessary to easily fill each flotation chamber. At this time, from two pooled samples were prepared four conic tubes. Tubes were put in centrifuge (Eppendorf 5810 R) for 3 minutes at 170g at room temperature.

After centrifugation, supernatant was discarded, leaving only sediments in tubes. Tubes were filled with flotation solutions to the previous 6 ml level. Two flotation solutions at SVI Jihlava were used: flotation solution 2 (FS2) and flotation solution 7 (FS7). FS2 is

saturated sodium chloride (NaCl) of specific gravity 1.20. FS7 is zinc sulfate ($ZnSO_4 \cdot 7H_2O$) of specific gravity 1.35. Preparation of flotation solutions can be seen in appendix.

Next step is thorough homogenizing of samples (before and between fillings) and filling the two flotation chambers of the FLOTAC apparatus with two faecal suspensions: chamber 1. with suspension in FS2 and chamber 2. with suspension in FS7. The same way was filled second FLOTAC apparatus. Before filling it was necessary to assemble the FLOTAC apparatus (see in the Appendix). FLOTAC apparatus consists of seven parts and assembling takes less than one minute. It was used FLOTAC-100, FLOTAC apparatus that permits magnification of ×100. After filling, FLOTAC apparatus is closed and put in centrifuge for 5 minutes at 120g at room temperature.

After centrifugation, top parts of FLOTAC apparatus were removed (see Appendix) and examined under a microscope. Due to use of two different flotation solutions, we were able to observe different parasites in each flotation chamber. FS2 is suitable for observing eggs of gastrointestinal strongyles and *Moniezia* spp. or oocysts of *Eimeria* spp. FS7 is suitable for observing eggs of flukes, such as *Fasciola hepatica* or *Dicrocoelium dendriticum.* Under microscope we detected different parasites and numbers of eggs/oocysts were noted in laboratory protocol. Used magnification was of ×100.

3.3 Statistical analysis

Acquired data were statistically and graphically processed in PC software Statistica 13 (DELL,inc.). Calculated were basic statistics, regression analysis (polynomial regression of 3rd degree), one-way ANOVA and factorial ANOVA. Statistical analysis was followed by post-hoc test (Fischer LSD test). For all calculations significance level $\alpha = 0.05$ was established.

3.4 Weather information

Following **Figures 1** and **2** were prepared from freely accessible source of CHMI (Czech Hydrometeorological Institute). These figures show average monthly temperature and monthly precipitation in specific regions: Vysočina (where Farm 1 and 2 were located) and Středočeský region (where Farm 3 is located).

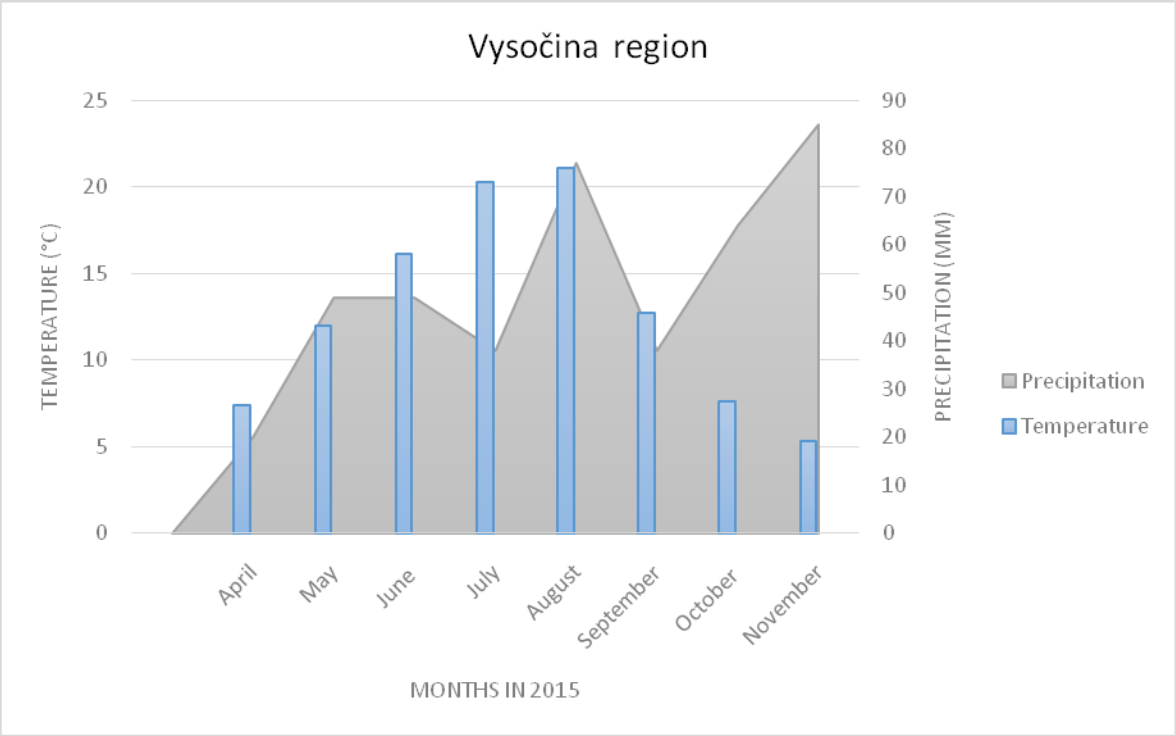

Figure 1. Average monthly temperature and monthly precipitation in Vysočina region

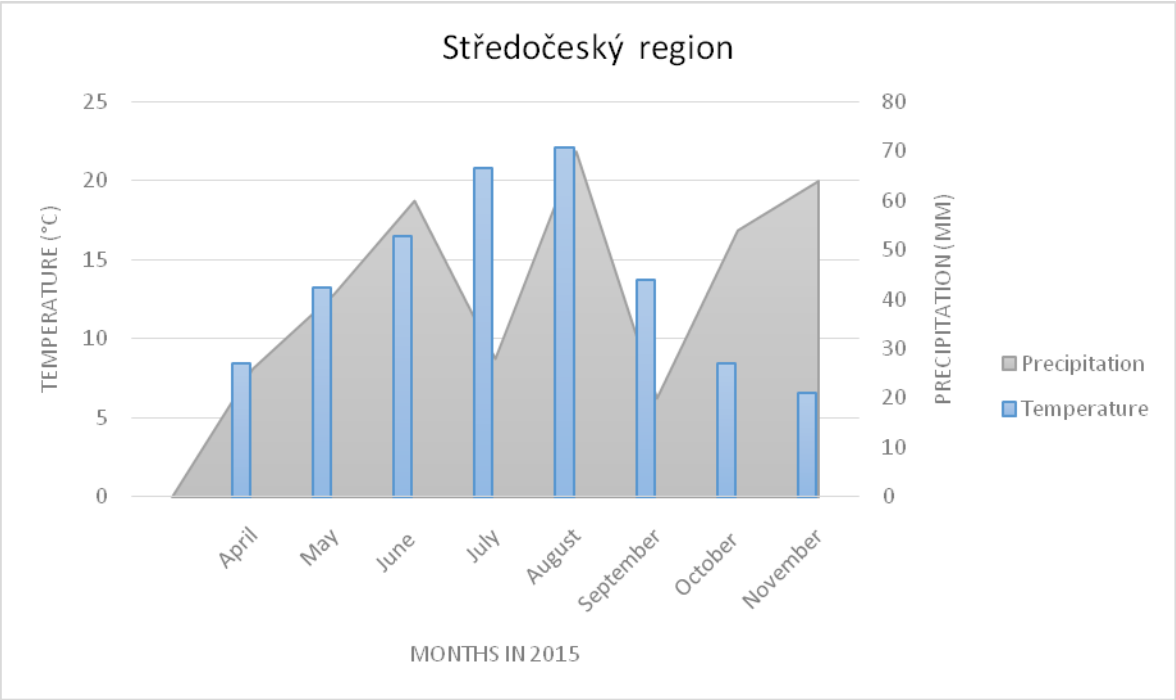

Figure 2. Average monthly temperature and monthly precipitation in Středočeský region

4 Results

Following results are showing information we obtained from our research focused on parasites of beef cattle.

4.1 Collection of samples

In total, 23 collection of samples was carried out (during pasture season from April 2015 to November 2015) during which was collected 460 samples of fresh faeces of pasture farmed cattle. On Farm 2 we collected samples separately from adult animals and first grazing calves (FGC) due to presence of holding box.

4.2 Evaluation of samples with FLOTAC method

From higher mentioned number of samples were prepared 62 pooled samples. Following table and figures shows number of EPG/OPG (eggs per gram/oocysts per gram) on each farm during pasture season from April 2015 to November 2015. Following **Table 7** is showing exact results of evaluation.

Table 7. Results of evaluation of samples with FLOTAC dual technique method showing occurrence of EPG/OPG in faeces

Farm	Parasites	EPG/OPG							
		April	May	June	July	August	September	October	November
1	*Eimeria* spp.		0	0	0	0	64	14	38
	GIN		16	18	8	0	6	22	10
	Moniezia spp.		2	0	8	8	0	0	8
2 (Adult cattle)	*Eimeria* spp.	10	2	0	0	0	0	0	70
	GIN	2	18	14	8	28	4	4	6
	Moniezia spp.	0	2	2	0	0	2	0	6
2 (First grazing calves)	*Eimeria* spp.	120	6	8	2	20	78	31	0
	GIN	0	0	34	0	26	18	4	0
	Moniezia spp.	0	0	2	0	0	2	0	0
3	*Eimeria* spp.	24	10	42	0	14	10	42	124
	GIN	2	16	102	36	20	30	106	110
	Moniezia spp.	8	8	16	28	8	6	0	72
	Paramphistomum spp.	24	4	26	82	26	64	6	38

(GIN – gastrointestinal nematodes)

Following **Figure 3** shows number of EPG/OPG found in faeces collected on **Farm 1.** From this figure can be read that there were large fluctuations in number of OPG found in

Eimeria spp. From beginning to middle of pasture season (May to August 2015), beef cattle on this farm was not infected by *Eimeria* spp. During September there was huge increase of found oocysts in faeces of cattle, following decrease in October and again increase in November. In case of gastrointestinal nematodes (GIN), were observed relatively low amounts of found eggs in faeces. Increase of EPG was in May and June, following by gradual decrease until August, followed by gradual increase until October, after which followed decrease in November. Amount of found EPG *Moniezia* spp. in faeces was very small, not exceeding 10 EPG. Higher numbers were found from June until July and then again in November.

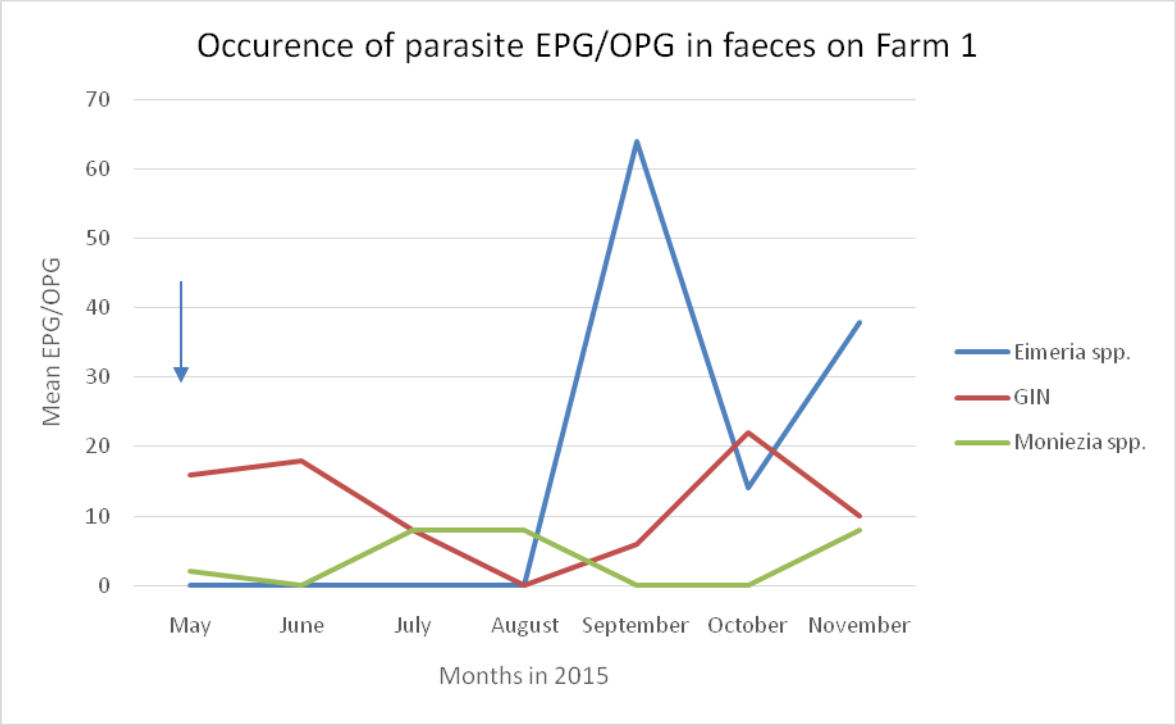

Figure 3. Number of EPG/OPG found on farm 1. GIN implements all generas of gastrointestinal nematodes. Blue arrow symbolizes when the anthelmintic were administered

On Farm 2 we tested separately adult animals and FGC (first grazing calves). Following **Figure 4** is showing EPG/OPG findings in adult animals. Oocysts of *Eimeria* spp. were found only in April and then again in November, in highest number of all findings on farm

3. Situation of gastrointestinal nematodes can be characterized with several peaks throughout the pasture season. Increase is visible in May and then again in August, followed by huge decrease in autumn months. *Moniezia* spp. was found only sporadically and in low numbers of EPG. Increase was in May and June and then again in September and November.

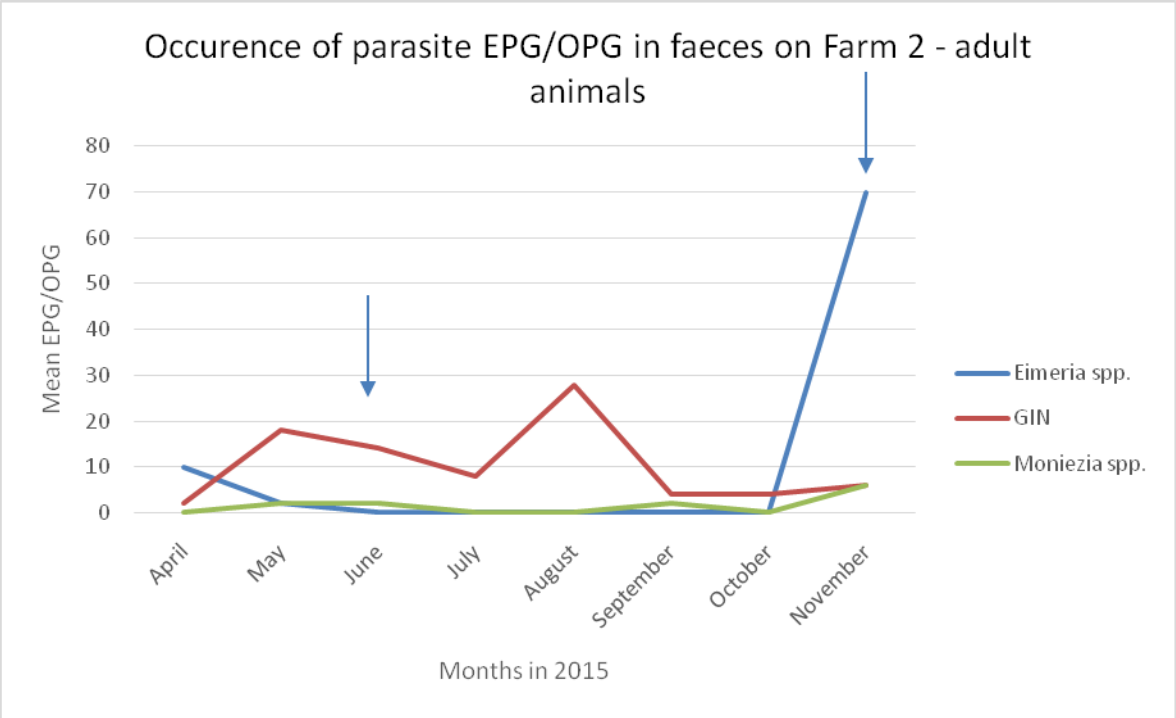

Figure 4. Number of EPG/OPG found on Farm 2. Situation depicts findings in faeces of adult animals. GIN implements all generas of gastrointestinal nematodes. Blue arrows symbolize when the anthelmintic were administered

In our second hypotheses H2, presence of oocysts of *Eimeria* spp. in adult cattle was not expected. **We can reject hypotheses H2 as incorrect.**

Situation of first grazing calves on Farm 2 is depicted in the following **Figure 5.** Largest findings were in case of *Eimeria* spp. with two major peaks in April (exceeding 100 OPG) and in September. Besides those two months, findings were relatively low. Gastrointestinal nematodes followed increase in June and in August, otherwise situation was similar as in adult animals. In case of *Moniezia* spp. the situation was basically same as in adult animals.

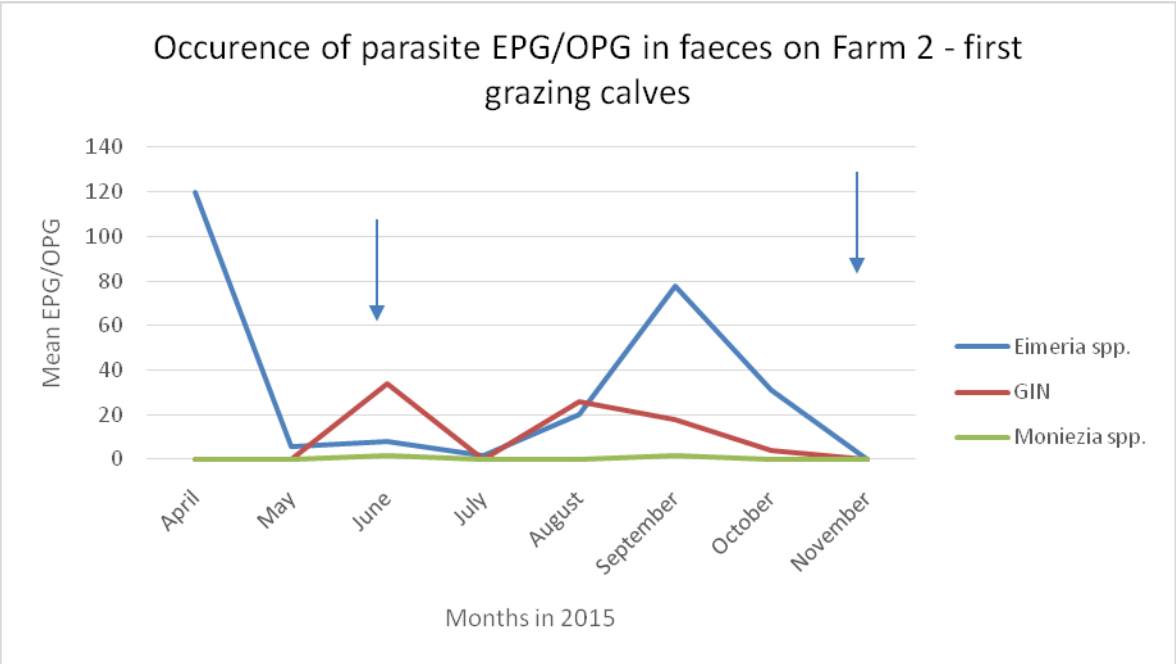

Figure 5. Number of EPG/OPG found on farm 3. Situation depicts findings in faeces of first grazing calves. GIN implements all generas of gastrointestinal nematodes. Blue arrow symbolizes when the anthelmintic were administered

On farm 3, the occurrence of parasite was much more varied compared to farm 1 and 2. From following **Figure 6**, huge fluctuations are visible in found EPG/OPG. In case of *Eimeria* spp., the increase of OPG was in period from May until July and the again in period from September to November, in October and November exceeding 100 EPG. Gastrointestinal nematodes had two major peaks in occurrence of their egg in faeces. First was in June, second in October. Both of these peaks exceeding 100 EPG. Occurrence of *Moniezia* spp. was higher than in farm 1, however we still found relatively low amounts of EPG, with major peak being in November. *Paramphistomum* spp. the increase was of EPG was from June to July, followed by decrease in August and again increase in September, decrease in October and final increase in November. *Nematodirus* spp. was not included in GIN, and was detected only in October and November.

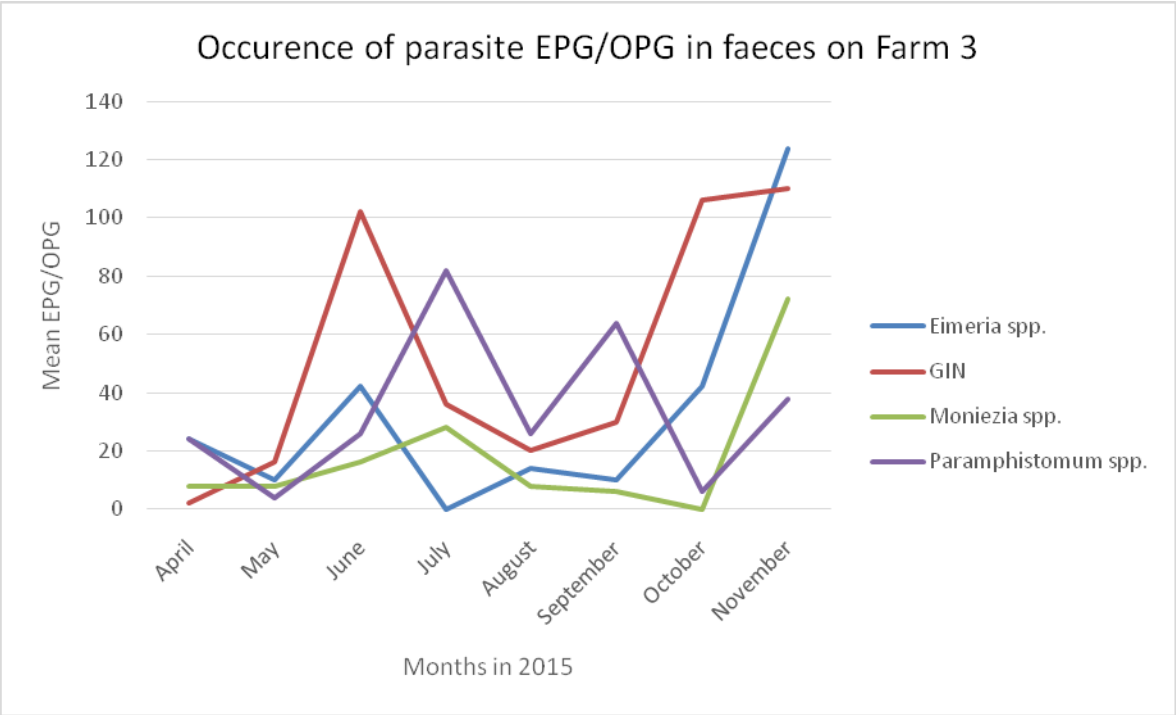

Figure 6. Number of EPG/OPG found on Farm 3. GIN implements all generas of gastrointestinal nematodes

4.3 Statistical analysis

In statistical analysis we excluded *Paramphistomum* spp. as it is occurring only on farm 3. This means, that in analysis we included *Eimeria* spp., GIN and *Moniezia* spp.

In following **Figure 7**, we tested average amount of all founded EPG/OPG on different farms. The highest average amount of founded EPG/OPG was on Farm 3 – 104 EPG/OPG. On Farm 1, the average amount was 28 EPG/OPG, and on Farm 2 the average amount was 22 EPG/OPG. Statistically **significant difference ($P \leq 0.05$) was founded between Farm 3 and Farm 1 and between Farm 3 and Farm 2.** Between farms 1 and 2 there was no significant difference in average amount of EPG/OPG. The tables showing descriptive statistics and results of post-hoc Fischer LSD test can be found in Appendices 3.

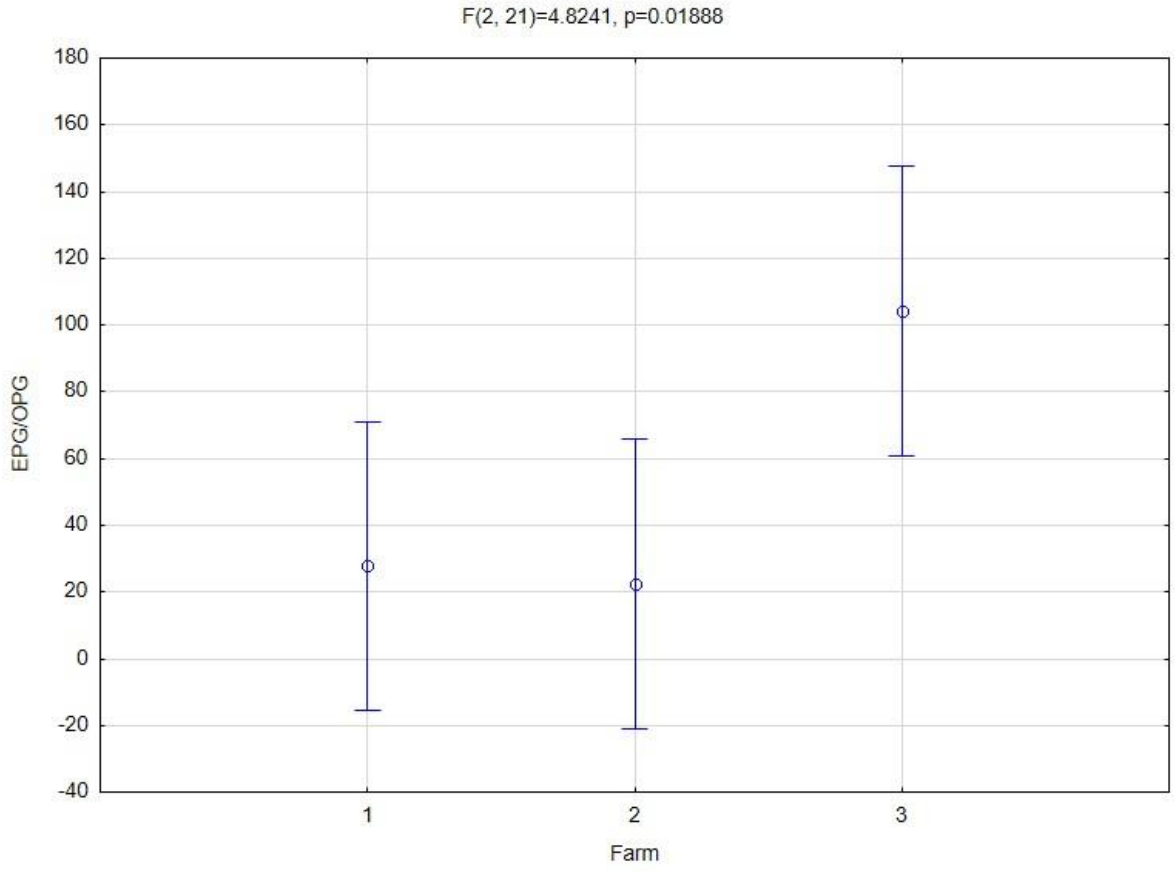

Figure 7. Average number of all founded EPG/OPG on different farms. Vertical bars denote 0.95 confidence intervals

Following **Figure 8** is showing average number of selected parasite species (*Eimeria* spp., GIN, *Moniezia* spp.) on different farms.

Farm 3 showed highest amount of oocysts of species *Eimeria* spp., the result being 33 OPG in comparison with 15 OPG (Farm 1) and 10 OPG (Farm 2). According to Fischer LSD post-hoc test, **the difference is not significant (P≤ 0.05) between any of the farms**.

In case of GIN, highest average amount of eggs was found on Farm 3, with the result 53 EPG, in comparison with 10 EPG (Farm1) and 11 EPG (Farm 2). According to post-hoc test **there was a significant difference (P≤ 0.05) between Farm 3 and Farm 1 and also between Farm 3 and Farm 1**. There was no significant difference (P≤ 0.05) between farms 1 and 2.

Last species was *Moniezia* spp. Highest average amount of eggs was found again on Farm 3 (18 EPG). Result on Farm 1 was three EPG and in Farm 2 two EPG. According to post-hoc test, **there was a significant difference (P≤ 0.05) between Farm 3 and Farm 1 and also between Farm 3 and Farm 2**. There was no significant difference (P≤ 0.05) between farms 1 and 2. The tables showing descriptive statistics and results of post-hoc Fischer LSD test can be found in Appendices 4.

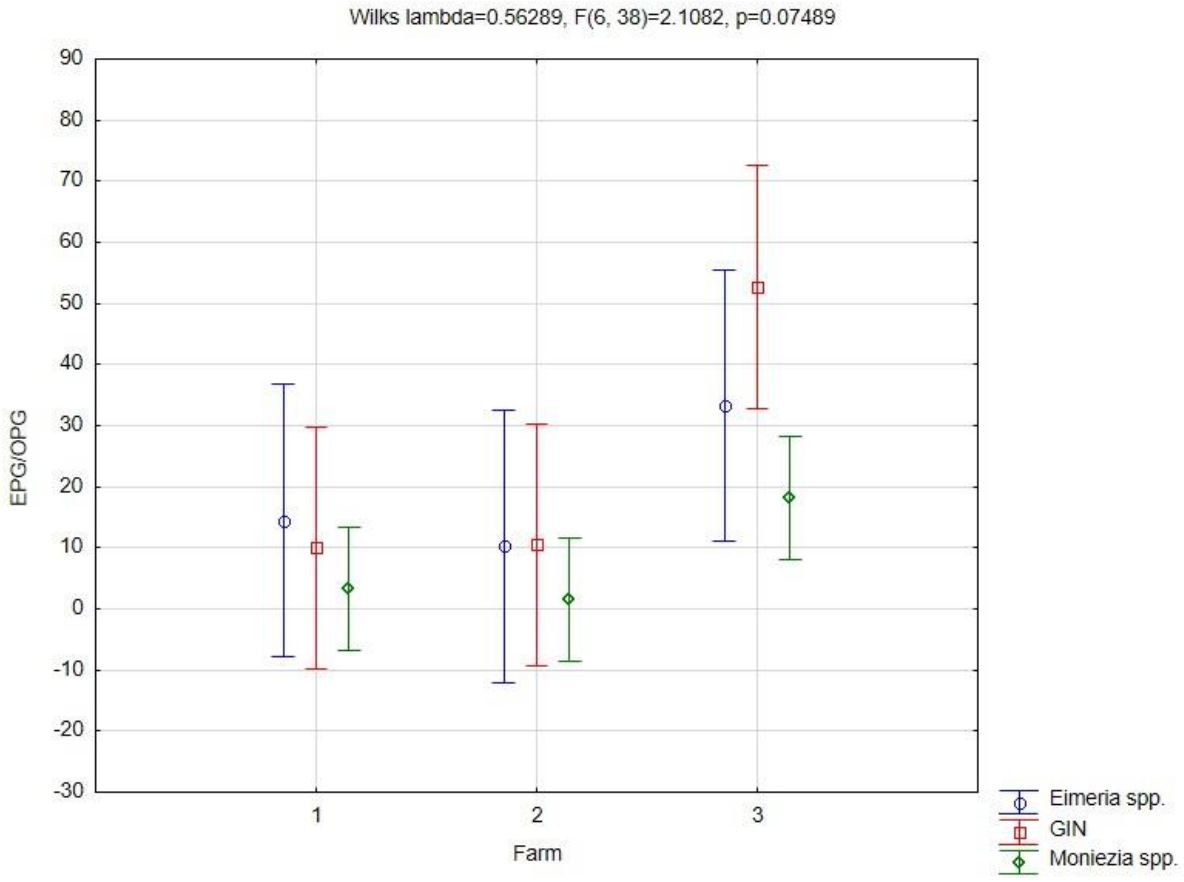

Figure 8. Average number of selected parasite species (*Eimeria* spp., GIN, *Moniezia* spp.) on different farms. Vertical bars denote 0.95 confidence intervals

Following **Figure 9** is showing average number of EPG/OPG in different farms in dependence on season of the year. As pasture season is from spring to autumn, winter is excluded. Fischer LSD post-hoc test showed that on Farm **3 there is a significant difference (P≤ 0.05) in comparison with Farms 1 and 2 in Autumn season**, when there

is a significant increase of number of parasites eggs and oocysts. The tables showing descriptive statistics and results of post-hoc Fischer LSD test can be found in Appendices 5.

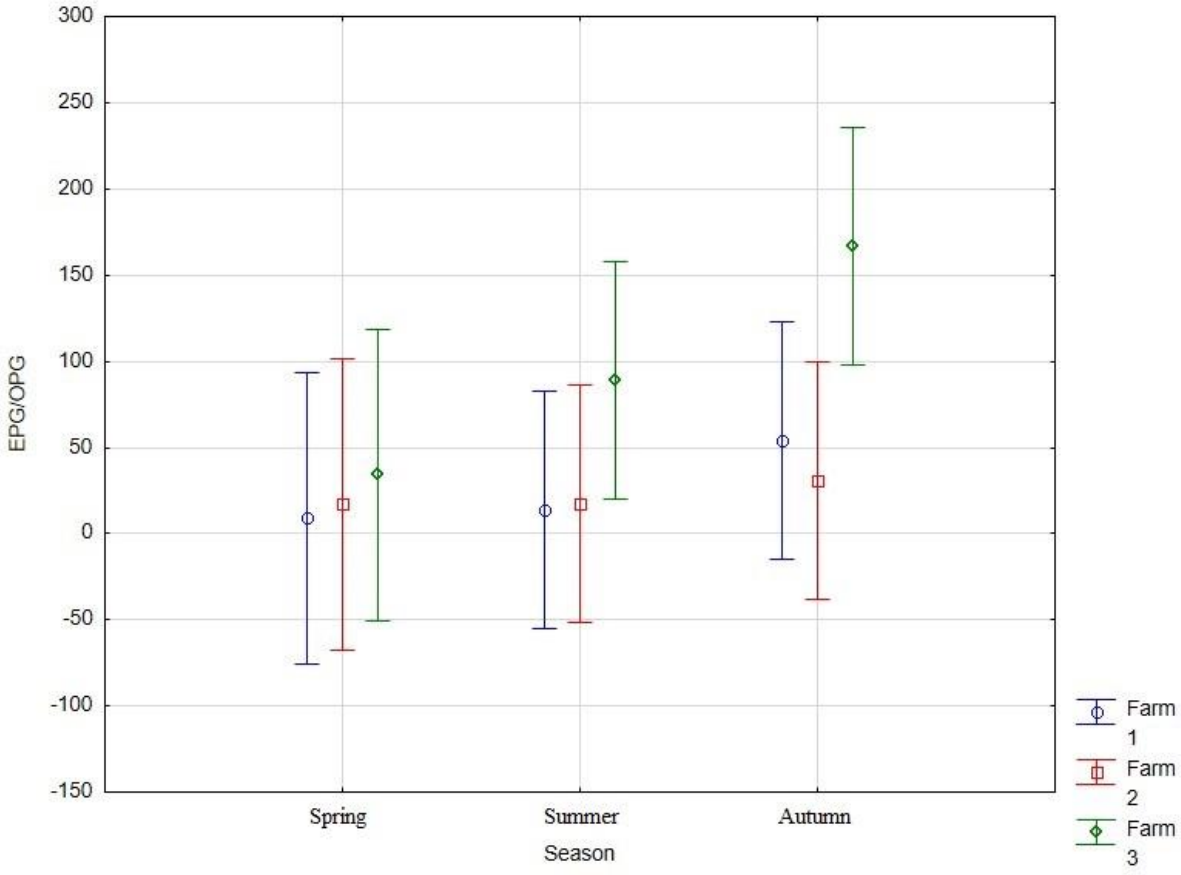

Figure 9. Average amount of all parasite species on different farms in dependence on season of the year. Vertical bars denote 0.95 confidence intervals

Following **Figure 10** is showing occurrence of parasite species on different farms in dependence on months of the year. This figure supports previous results from Figure 9, as the trend on Farm 3 is different from Farms 1 and 2.

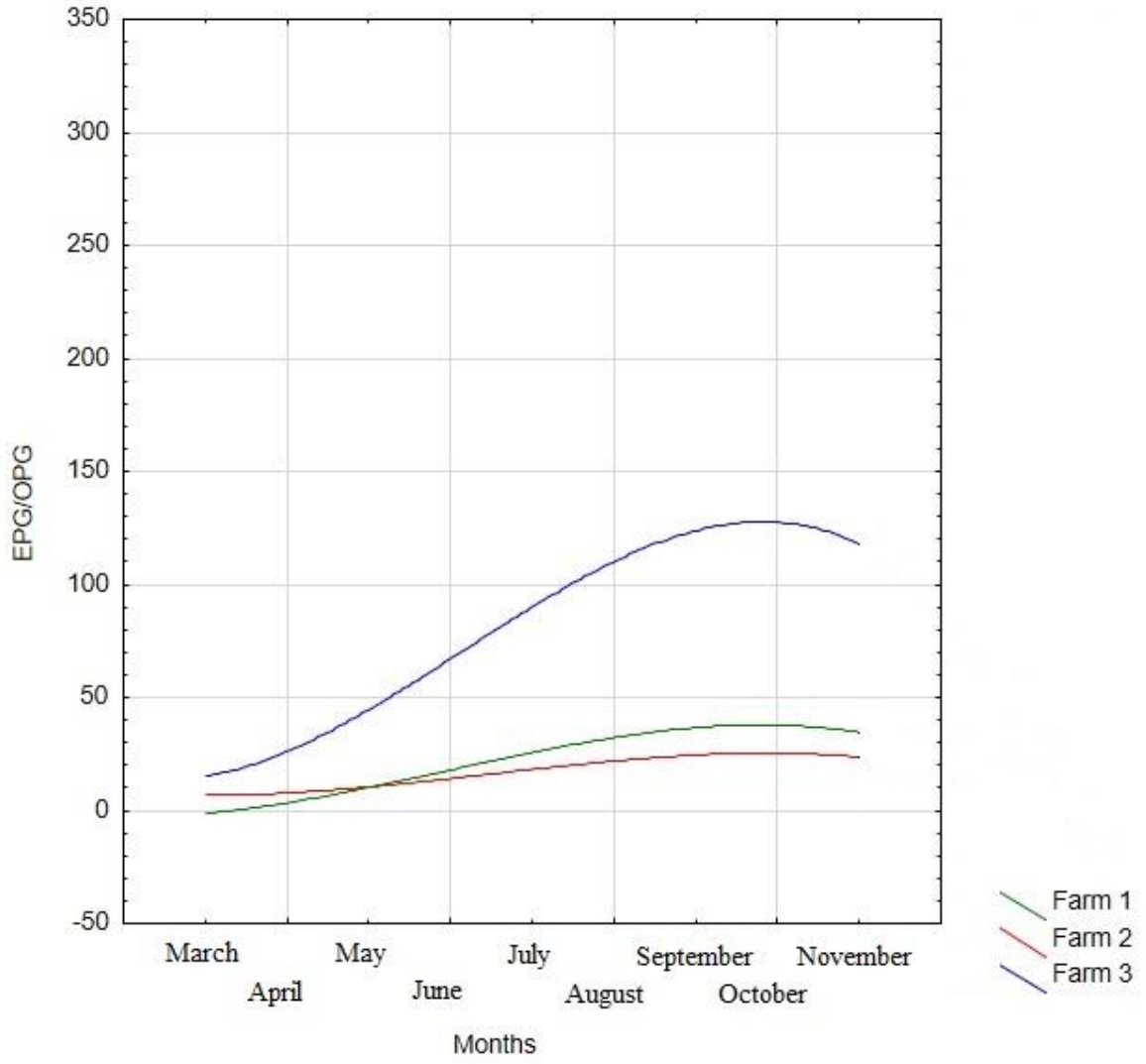

Figure 10. Occurrence of parasite species on different farms in dependence on months of the year

In our first hypothesis H1, we expected that farms that administered anthelmintic treatment will have lower amounts of EPG/OPG in faeces of animals than farm, that did not administer anthelmintic. According to statistical analysis, where were shown significant differences between Farm 3 (did not administer anthelmintic treatment) and Farms 1 and 2 (administered anthelminthic treatment) we can **accept H1 as correct**.

5 Discussion

Research in this diploma thesis was part of project focused on monitoring of parasitoses of beef cattle and subsequent testing of anthelminthic efficacy. Research is led by MVDr. Karol Račka, a head of parasitological department at SVI Jihlava. Whole research runs in cooperation with Zoetis Czech Republic. So far, the research is in its pilot year, focused only on parasitological monitoring. Main goal of this monitoring is to establish species occurrence of parasite in faeces of beef cattle using coprological methods, determine their seasonal dynamics and selection of suitable localities for next stage of project. In the following year, serological examinations take place, mainly use of ELISA method, for determination of antidotes against *Ostertagia ostertagi, Fasciola hepatica* and *Dictyocalus viviparus*. Output from coprological and serological methods will be suggesting most suitable method for control/monitoring of parasitoses in beef cattle, and determining dependence of levels of antidotes against *Ostertagia ostertagi* and lowered performance. Second stage of project will focus on coprocultivation of larvae and their subsequent isolation and species identification. On those larvaes, the test for determining of anthelmintic efficacy takes place, by using Faecal Egg Count Reduction Test (FECRT). Final output of research will be establishment of anthelmintic efficacy and determining of resistant parasitic strains in the Czech Republic.

Samples collection on Farms 1 and 3 took place directly on the pasture among animals. From this reason it was unable to determine to which animal which sample belongs. Therefore, there was possibility of multiple collection of samples belonging to same animal. Farms 1 and 3 also have small amount of kept animals, thanks to which the possibility of multiple sample collection belonging to same animal increased. Original intention was to collect 15 samples from adult animals and 5 samples from first grazing calves (FGC). From previously written reasons, this intention was abandoned and was collected 20 fresh samples of faeces instead. Only on farm 2 it was possible to directly obtain samples from rectum thanks to presence of holding box. From this reason, we were able to get samples from adult animals as well as FGC.

Possible problem, from scientific point of view, was rotational grazing, present on all three farms. As was written by Barger (1997), this method of farming of animals has several advantages: pasture can "rest" and optimization of plant growth can take place, resulting in increase of pasture carrying capacity. If used correctly, rotational grazing can also be effective form of biosecurity. We were also not able to get any information about time management of rotational pasture on each farm. From those reasons, results of FLOTAC examinations may not fully correspond with actual situation of parasite occurrence on pastures.

As was written by many authors (Kalis et al., 2000; Cringoli et al., 2010; Zajac et al., 2011; Demeler et al., 2013) copromicroscopic methods and faecal egg counting methods (FEC) are most used examinations for detection of parasitological infections. With this is related using of FECRT method for determining of anthelmintic resistance as most wildly used method as was written by Coles et al. (1992, 2006). According to Rinaldi et al. (2014), biggest problem of these methods is large time consumption of preparation and examinations of individual samples. For research in this diploma thesis we used FLOTAC method, developed by team of professor Cringoli at university of Naples Federico II (Cringoli et al., 2010). From my own experience I can say that this method can be time consuming, especially during examination of large quantity of individual faecal samples. This was reason, why we used composite (pooled) samples for examinations. Mixing of several samples of same weight can be used for determining average faecal egg count (FEC) for group, as was written by Baldock et al. (1990) Nicholls and Obendorf (1994), Ward et al. (1997), Eysker et al. (2008) and Musella et al. (2011). Using composite samples is faster and more economical alternative. As was written by Gregory and Woolhouse (1993), incorrect use (e.g. too many mixed samples) can result in wrong interpretation of results. Also as was written by Cabaret and Berrag (2004) and Torgerson et al. (2005), composite samples are not suitable for testing of anthelmintic resistance. This is in contrast with study performed by Calvete and Uriarte (2013), which stated that composite samples do not have any effect on determining of anthelmintic resistance.

Baldock et al. (1990) and Morgan et al. (2005) focused on determining of correct number of samples suitable for preparing composite samples. For their research they used McMaster method and samples of faeces from sheep. Results are different, with suitable number of samples being 3 (Baldock et al., 1990) and 10 (Morgan et al., 2005). Similar research performed Rinaldi et al. (2014), in this case they used FLOTAC method. According to their results, it is safe to use 5, 10 or even 20 individual samples of faeces for preparing of one composite sample. However, all authors mentioned higher accuracy of individual samples compared with composite samples and held opinion that more research is necessary. In research for this diploma thesis we used mixing of 10 samples for preparation of one composite sample. With consideration of reasons written higher it is possible that results may not full correspond with actual situation. Main disadvantage of using composite samples is lack of applicable statistical analysis for evaluation of samples (e.g. impossibility of determining prevalence, which is count as ratio of infected samples and total number of examined samples).

According to Cringoli et al. (2010) FLOTAC method has analytic sensitivity of 1 EPG and is the most accurate and sensitive of all FEC and coprological methods. Knopp et al. (2009) focused on comparing of FLOTAC method with Kato-Katz. According to their results, sensitivity of FLOTAC method was in range of 82.8% (for *A. lumbricoides*) and 88.7% (for *T. trichura*). Sensitivity of Kato-Katz was lower with range of 46.0% (for *A. lumbricoides*) and 71.8% (for *T. trichura*). Similar research was performed by Gliz et al. (2010), with similar results, showing higher sensitivity of FLOTAC method in comparison with Kato-Katz. Speich et al. (2010) focused on comparison of economic costs and time consumption of each method. Results showed that faster method is Kato-Katz with average time 20 minutes and 34 seconds. Average time of performing FLOTAC dual technique was 27 minutes and 21 seconds and FLOTAC double technique 28 minutes and 34 seconds. From economic point of view, cheapest method was Kato-Katz with average costs 1.73 US$. Cost of FLOTAC dual technique were 2.35 US$ and FLOTAC double technique 2.83 US$. Biggest part of costs were salaries of employees. Rinaldi et al. (2010) compared FLOTAC method with McMaster method. In their research they focused on several species of parasites: *Dicrocoelium dendriticum*, *Moniezia expansa* and gastrointestinal strongyles. In

all cases of examinations, FLOTAC showed higher sensitivity and accuracy than McMaster method. Also, their results showed that with FLOTAC method there is lower chance of getting negative results. Similar research was performed by Silva et al. (2013), with same results. Also they wrote, that detection of eggs/oocysts is influenced by choice of selection of reading area. Reason for this is absence of guidelines, this problem is eliminated in FLOTAC method with presence of guidelines. Problems of wrong interpretation of data due to choice of reading area in McMaster method was also reported by Cringoli et al. (2004). Godber et al. (2014) compared FLOTAC method with FECPAK method. From their results is evident, that FLOTAC has higher accuracy and sensitivity than FECPAK. They also found, that in FECPAK there was possibility of underestimation of real amount of EPG. Duthaler et al. (2010) performed research focused on comparison of FLOTAC with sedimentation method. Their results were higher sensitivity and accuracy of FLOTAC method. Another result was lower time consumption compared with performing of multiple sedimentations. According to their results, sedimentation is easier to use and more suitable in field conditions, while FLOTAC is more suitable in laboratories.

Also, high sensitivity of FLOTAC is apparent from results in this diploma thesis, when we were able to detect even low amounts of EPG/OPG. FLOTAC method proved to be reliable and thanks to usage of pooled samples also relatively fast. Biggest problem of using of pooled samples are statistical analysis. At the end, the number of evaluated samples was relatively low, and did not allow in-depth scientific processing. For example, using of pooled samples did not allow calculating of parasitic prevalence. However, it is useful mainly for veterinary monitoring of parasite occurrence in field conditions.

From evaluated results, there was apparent the difference in number of found EPG/OPG between farms that used anthelminthic and farm that did not administered anthelminthic as we expected in our hypothesis H1, which we accepted as correct. Farm 3, that did not administer anthelminthic had significantly higher amount of found parasite eggs and oocysts. We can assume that given anthelminthic treatment had positive effect on occurrence of parasites. Farm 1 used anthelminthic only in May, at the beginning of the pasture season, in contrast with Farm 2 that used anthelminthic twice a year: in June and in

November. However, according to statistical analysis, there was no significant difference between average amounts of found EPG/OPG. Lower amounts of found eggs and oocysts can also be caused by different geographical location. Farms 1 and 2, were located in Vysočina region and Farm 3 in Středočeský region. However, all farms were located is similar altitude: all three were located between 400 to 550 m above sea level. Also as is visible from Figures 1 and 2 in chapter Materials and methods depicting average monthly temperature and average monthly precipitation, weather conditions were similar.

As was written by several authors (Munya and Nhotho, 1990; Daugschies and Najdrowski, 2005; Koutny et al., 2011) *Eimeria* spp. is most prevalent in young calves, and prevalence decreases with age. In our hypothesis we assumed, that there will be no occurrence of *Eimeria* spp. in faeces of adult cattle. However, we were able to find relatively high amounts of oocysts of *Eimeria* spp. in adult cattle kept on Farm 2. From this reason we rejected our hypothesis H2.

Similar research focused on dynamics of parasitoses in beef cattle was performed in years 1997-2000 by prof. MVDr. Karel Chroust, DrSc. (Chroust, 2006). From his results is apparent similar composition of occurring parasites. Most often found were gastrointestinal nematodes, *Eimeria* spp. and *Moniezia* spp. This corresponds with our research. What is different is seasonal dynamics, mainly of gastrointestinal nematodes. According to results of prof. Chroust, gastrointestinal nematodes had continuous increase of EPG from April through June with maximal peak in August, followed by continuous decrease. This is in contrast with results in this diploma thesis, which showed two peaks: end of spring/beginning of summer and end of summer/beginning of autumn. Another differences were occurrence of eggs of *Paramphistomum* spp., which we detected in relatively high amount on farm 2 compared no detection in study of prof. Chroust. Possible reason is different geographic location of farms: research from 1997-2000 was performed on farms in Moravia and our research was done in area of central Bohemia and Vysočina region.

As was written by Lass and Ebert (2005), seasonal dynamics is common in nature, but exact reasons why are often unknown. Reasons can be following: external (environmental) conditions (May and Anderson, 1979; Grenfell and Bjornstad 2005), climatic conditions

(Kelly et al., 2002), influence of pastures and feeding (Yan and Larsson, 1988), host behavior (Hosseini et al., 2004), influence of host immunity and population of parasites (Anderson and May, 1985; Woolhouse, 1998;Hosseini et al., 2004; Catttadori et al., 2005; Grassly et al., 2005), parasitic virulence (Ebert et al., 2000), transfer of parasites (Lipsitch et al., 1995) or influence of age of host (Anderson and Gordon, 1982; Pascal and Dobson, 1988). All of these are possible causes of seasonal dynamics. All of these are also possible reasons why our and prof. Chroust's results differ.

In regards of occurrence of parasites, in our research we found out that largest occurrence of *Eimeria* spp. was in late summer and in autumn. These results correspond with study done by Wacker et al. (1999). Because of use of composite samples we were unable to count prevalence, however many scientific studies do have different results of prevalence of *Eimeria* spp. in beef cattle: Kemper and Henze (2009) reported prevalence of 29.5%, Wacker et al. (1999) reported up to 48%, Chroust (1964) reported prevalence of 64% and Ernst et al. (1984) reported prevalence of 72.5%. As was written by Lípová (1985) younger animals and mainly calves are more susceptible to infection by *Eimeria* spp. This results also reported Cornelissen et al. (1995), Daugschies and Najdrowski (2005) and Lassen et al. (2009).

Moniezia spp. was in our research detected in all farms, however in small amounts, which partially corresponds with Chroust et al. (1998) and Chroust (1999), however in these studies occurrence of *Moniezia* spp. was higher.

Gastrointestinal nematodes in our research were not species determined (with exception of *Nematodirus* spp.). In comparison with Chroust (2000), total findings of amounts of EPG were lower. This might be due to geographic location and/or due to using of composite samples. Our results showed two main peaks of gastrointestinal occurrence in seasonal dynamics (end of spring/beginning of summer and end of summer/beginning of autumm). These results are similar with results in study performed by Couvillion et al. (1996), who reported main peaks in May and in September. Similar results also reported Malczewski et al. (1996), who reported main peaks in June and in November. Generally, we found high amounts of EPG of gastrointestinal nematodes. According to literature, most frequent

member of GIN is *Ostertagia* spp. (Agneessens et al., 1996; Corwin, 1997; Gasbarre, 1997; Shaw et al., 1997. Agneessens et al. (1996) also reported up to 90% prevalence of *Ostertagia* spp. in calves. Another frequent member of GIN is *Cooperia* spp. (Yazwinski and Gibbs, 1975; Malczewski et al., 1996; Almeía and Uriarte 1999; Chollet et al., 2000). Third most frequent member of GIN according to literature is *Trichostrongylus* spp. (Suarez et al., 1991; Agneessens et al., 2000).

No owner of the farm reported any serious problem with health of the animals due to parasitic infection, and during the collection of samples we did not observed any serious diseases or seriously weak animals on either farm.

6 Conclusions

Occurrence of parasites was monitored during pasture season (from April 2015 to November 2015) in three farms located in the Czech Republic. For our research we used faecal egg counting method FLOTAC dual technique, which proved to be accurate and highly sensitive, when we were able to detect even small amounts of eggs or oocysts. Using of pooled samples is more suitable for veterinary monitoring of parasite occurrence, due to its simplicity and speed of examination. For scientific research it is more suitable to use individual samples as they allow better in-depth processing of results, establishing seasonal dynamics or anthelminthic resistance. Most often detected parasites were: *Eimeria* spp., gastrointestinal nematodes and *Moniezia* spp. that were observed on all three farms. However, on farm 3, that did not administer anthelminthic treatment we were able to detect significantly higher amounts of detected parasites. Implementing of anthelmintic treatment is recommended.

Research in this diploma thesis was part of project focused on monitoring of parasitoses of beef cattle and determining of anthelmintic resistance. Monitoring of parasitoses is important, due to health problems that could affect animals if there would be parasitic outbreak. Project is successful so far and should continue in following years.

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List of Appendices

Appendices 1

Flotation solutions recommended for FLOTAC and their preparation (Cringoli et al., 2010)

FS1: Sheather's sugar solution (s.g., 1.20)

Preparation: Add 454 g of sucrose to 355 ml of tap water (corn syrup and dextrose are not suitable substitutes). Dissolve sugar in water by stirring on a magnetic stirrer over low or indirect heat (e.g., the top half of a double boiler). Once sugar is dissolved and the solution has cooled down to room temperature, add 6 ml of formaldehyde (40%) USP to prevent microbial growth. Check the s.g. with a hydrometer.

FS2: Saturated sodium chloride (NaCl) (s.g., 1.20)

Add NaCl to 1 liter of warm water (40–50 °C) until no more salt goes into solution (~500 g) and the excess settles on the bottom of the container. Dissolve by stirring on amagnetic stirrer. To ensure that the solution is fully saturated, it should be allowed to stand overnight at room temperature. Check the s.g. with a hydrometer, recognizing that the s.g. of the saturated solution will vary slightly depending on ambient temperature.

FS3: Zinc sulfate ($ZnSO4 \cdot 7H2O$) (s.g., 1.20)

Add 330 g of zinc sulfate heptahydrate to 500 ml of tap water. Dissolve zinc sulfate in water with a magnetic stirrer. Add tap water to reach a final volume of 1 liter. Check the s.g. using a hydrometer.

FS4: Sodium nitrate ($NaNO3$) (s.g., 1.20)

Add 315 g of sodium nitrate to 500 ml of tap water. Dissolve sodium nitrate in water with a magnetic stirrer. Add tap water to reach a final volume of 1 liter. Check the s.g. with a hydrometer.

FS5: Sucrose and potassium iodomercurate (s.g., 1.25)

Add 600 g of sucrose to 600 ml of tap water. Dissolve sugar in water with a magnetic stirrer over low or indirect heat (e.g., the top half of a double boiler). Once sugar has

dissolved and the solution has cooled down to room temperature, add 20 ml of solution B (see below). Check the s.g. with a hydrometer. Solution B: Add 100 g of mercury (II) iodide to 63 ml of tap water. Stir vigorously. Add 78 g of potassium iodide and stir again.

FS6: Magnesium sulfate (MgSO4) (s.g., 1.28)

Add 350 g of magnesium sulfate to 500 ml of tap water. Dissolve magnesium sulfate in water with a magnetic stirrer. Add tap water toreach a final volume of 1 liter. Check the s.g. with a hydrometer.

FS7: Zinc sulfate ($ZnSO4 \cdot 7H_2O$) (s.g., 1.35)

Add 685 g of zinc sulfate heptahydrate to 685 ml of tap water. Dissolve zinc sulfate in water by stirring on a magnetic stirrer. Check the s.g. with a hydrometer.

FS8: Potassiumiodomercurate (s.g., 1.44)

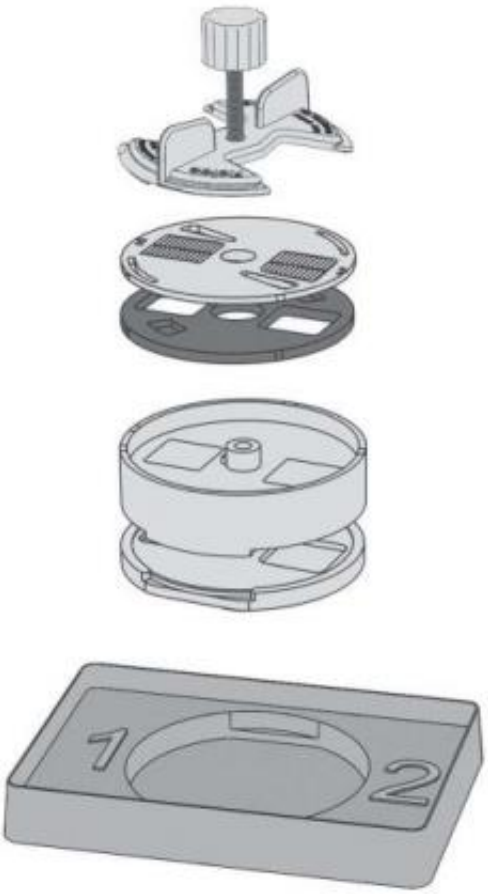
Add 150 g of mercury (II) iodide to 399 ml of tap water. Stir vigorously. Add 111 g of potassium iodide and stir again. Check the s.g. with a hydrometer.

FS9: Zinc sulfate and potassium iodomercurate (s.g., 1.45)

Add 600 g of zinc sulfate heptahydrate ($ZnSO4 \cdot 7H_2O$) to 600 ml of tap water. Dissolve zinc sulfate in water by stirring on a magnetic stirrer. Once zinc sulfate has been dissolved, add solution B (see below). Check the s.g. with a hydrometer. Solution B: Add 100 g of mercuryiodide to 63 ml of tap water. Stir vigorously. Add 78 g of potassium iodide and stir again.

Appendices 2

FLOTAC apparatus assembly process (Cringoli et al., 2010)



Appendices 3

Post-hoc Fischer LSD test for Figure 7

Cell No.	Farm	{1} 27,750	{2} 22,250	{3} 104,25
	LSD test; variable Total (List1 in Sumar) Probabilities for Post Hoc Tests Error: Between MS = 3484,3, df = 21,000			
1	1		0,853957	0,017012
2	2	0,853957		0,011263
3	3	0,017012	0,011263	

Descriptive statistics for Figure 7

Effect	Level of Factor	N	Total Mean	Total Std.Dev.	Total Std.Err	Total -95,00%	Total +95,00%
	Descriptive Statistics (List1 in Sumar)						
Total		24	51,4167	68,13919	13,90885	22,64401	80,1893
Farm	1	8	27,7500	24,31784	8,59765	7,41978	48,0802
Farm	2	8	22,2500	25,48809	9,01140	0,94142	43,5586
Farm	3	8	104,2500	95,97879	33,93363	24,00972	184,4903

Appendices 4

Post-hoc Fischer LSD test for Figure 8 – variable *Eimeria* spp.

Cell No.	Farm	{1} 14,500	{2} 10,250	{3} 33,250
LSD test; variable Eimeria spp. (List1 in Sumar) Probabilities for Post Hoc Tests Error: Between MS = 916,62, df = 21,000				
1	1		0,781647	0,229155
2	2	0,781647		0,143583
3	3	0,229155	0,143583	

Post-hoc Fischer LSD test for Figure 8 – variable GIN

Cell No.	Farm	{1} 10,000	{2} 10,500	{3} 52,750
LSD test; variable GIN (List1 in Sumar) Probabilities for Post Hoc Tests Error: Between MS = 731,31, df = 21,000				
1	1		0,970851	0,004703
2	2	0,970851		0,005123
3	3	0,004703	0,005123	

Post-hoc Fischer LSD test for Figure 8 – variable *Moniezia* spp.

Cell No.	Farm	{1} 3,2500	{2} 1,5000	{3} 18,250
LSD test; variable Moniezia spp. (List1 in Sumar) Probabilities for Post Hoc Tests Error: Between MS = 187,10, df = 21,000				
1	1		0,800533	0,039686
2	2	0,800533		0,023182
3	3	0,039686	0,023182	

Descriptive statistics for Figure 8

Effect	N	Eimeria spp. Mean	Eimeria spp. Std.Dev.	GIN Mean	GIN Std.Dev.	Moniezia spp. Mean
Descriptive Statistics (List1 in Sumar)						
Total	24	19,33333	30,67738	24,41667	32,96364	7,66667
Farm	8	14,50000	24,06539	10,00000	8,14160	3,25000
Farm	8	10,25000	24,38823	10,50000	8,92829	1,50000
Farm	8	33,25000	39,69797	52,75000	45,25404	18,25000

Appendices 5

Post-hoc Fischer LSD test for Figure 9

	LSD test; variable Total (List1 in Sumar) Probabilities for Post Hoc Tests Error: Between MS = 3147,1, df = 15,000									
Cell No.	Farm	Season	{1} 9,0000	{2} 14,000	{3} 54,000	{4} 17,000	{5} 17,333	{6} 30,667	{7} 34,000	{8} 88,667	{9} 166,67
1	1	Spring		0,92351	0,39342	0,88850	0,87291	0,67824	0,66222	0,14064	0,00764
2	1	Summer	0,92351		0,39628	0,95406	0,94295	0,72104	0,70163	0,12389	0,00454
3	1	Autumn	0,39342	0,39628		0,48110	0,43592	0,61788	0,70163	0,46087	0,02653
4	2	Spring	0,88850	0,95406	0,48110		0,99489	0,79320	0,76603	0,18202	0,01050
5	2	Summer	0,87291	0,94295	0,43592	0,99489		0,77497	0,74934	0,14024	0,00527
6	2	Autumn	0,67824	0,72104	0,61788	0,79320	0,77497		0,94896	0,22474	0,00955
7	3	Spring	0,66222	0,70163	0,70163	0,76603	0,74934	0,94896		0,30264	0,02048
8	3	Summer	0,14064	0,12389	0,46087	0,18202	0,14024	0,22474	0,30264		0,10922
9	3	Autumn	0,00764	0,00454	0,02653	0,01050	0,00527	0,00955	0,02048	0,10922	

Descriptive statistics for Figure 9

	Descriptive Statistics				
Effect	Level of Factor	Level of Factor	N	Total Mean	Total Std.Dev.
Total			24	51,4167	68,1392
Farm*Season	1	Spring	2	9,0000	12,7279
Farm*Season	1	Summer	3	14,0000	5,2915
Farm*Season	1	Autumn	3	54,0000	17,0880
Farm*Season	2	Spring	2	17,0000	7,0711
Farm*Season	2	Summer	3	17,3333	10,0664
Farm*Season	2	Autumn	3	30,6667	44,4672
Farm*Season	3	Spring	2	34,0000	0,0000
Farm*Season	3	Summer	3	88,6667	62,7482
Farm*Season	3	Autumn	3	166,6667	131,0013