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The importance of differential diagnosis of resistence and resilience in ruminants

Review scientific paper

THE IMPORTANCE OF DIFFERNTIAL DIAGNOSIS OF RESISTANCE AND RESILIENCE IN RUMINANTS[**]

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Abstract: Control of the gastrointestinal parasites in different systems of ruminant breeding is based on vaccination, chemotherapy, improved herd management and use of genetic potentials of host animals. Strategy of the helminths control based on frequent anthelmintics usage is dominant among the world, although it is considered unsustainable due to the appearance of increased number and species of parasites that are resistance to drugs. Development of resistance on all three groups of broad-spectrum antihelmintics (nicotinic anthelmintics, benzimidazoles and macrolides lactones) as well as increased care for consumers heath caused by appearance of used drugs residues in food, additionally complicate overall nematode control. With the aim to decrease appearance of anthelmintics resistance of gastrointestinal nematodes of small ruminants and in order to organize process of sustainable integrated parasite management, principle of targeted selective treatment is globally accepted. Implementing of this strategy has only recently become feasible, with development and practical use of systems that serve for clinical assessment of anemia in small ruminants which suffer from hemonchosis. Besides that, short term changes in body weight and body condition scoring may be indicators of diseases caused by endoparasites, as it can provide rapid identification of animals that will probably have benefits from therapy. Obtained results of quantitative coprological diagnostic tests and results for anemia assessment are criteria that provide differential diagnosis between healthy and resilient animals and easier diseases diagnostic. Since resilient animals play important role in pasture contamination, the significance of their detection is understandable.

Key words: ruminants, resistance, anthelmintics, resilience, diagnostic parameters

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INTRODUCTION

Parasitic gastroenteritis is economically the most important disease of grazing animals, which is mainly controlled, during last five decades, by adequate organization of grazing and anthelmintics usage. Grazing management systems are mainly impractical and expensive, whilst the frequent use of anthelmintics has led to problems related to increased resistance of parasites to antiparasitic drugs, especially in young ruminants (Várady et al., 2011). Appearance of resistance was observed among the world in all three broad-spectrum anthelmintics – nicotinic anthelmintics (imidazothiazole and tetrahydropyrimidine), benzimidazoles and macrolides lactones, that are used in sheep breeding for suppression of infections cause by strongyloidiasis (Coles et al., 2006, Vidyashankar et al., 2012, *Salgado and Santos*, 2016). Decreased efficiency of anthelmintics combined with attempt to reduce chemical usage in production systems have stimulated search for alternative and sustainable options for parasite control and have resulted in appearance of new classes of anthelmintics on market (amino-acetonitrile derivate and spiroindoles) (McManus at al., 2014).

Development of resistant helminth strains is an evolutionary characteristic based on intra-population selection of those parasites that carry alleles responsible for resistance on chemical components from drug. Long-lasting usage of the same antiparasitic drug, or agents that have similar mechanism of drug action, lead to the appearance of parasite resistance on drug. Once established, resistance may last several years (*Jackson and Coop*, 2000), or disappear as a result of selection and genetic drift, which act in a way to bring back sensibility in population (Petričević et al., 2007).

Since the problem of resistance is very actual, much effort have been made to control its development and to slow down the process by using different approaches. Research during last 20 years indicates that the best way to prolong the process of selection of resistant genes in parasites is the usage of combined different preparations – mixtures with two or more different chemical active substances. The other recommendation is to rotate drugs from different chemical groups that have been used for avoiding a tolerance (Lalošević et al., 2009).

One of the possibilities to increase drug efficiency is food deprivation before peroral application of anthelmintics. Thus, the amount of food is reduced in digestive tract allowing more time for absorption and distribution of medicinal substances (*Jackson and Coop*, 2000). It is important to form sheep groups with similar body weight and to give each sheep dosage that is sufficient for largest sheep in the group. This approach ensures that each animal in the group receive sufficient drug dosage, since subdosing of animals is the most harmful (Lalošević et al., 2009).

The term resilience appears in many different research studies, although its definition differs. According to Doeschl-Wilson et al. (2012), resilience is capability of an animal to maintain a good condition and usual level of activity, while being infected by parasites, no matter the level of load created by the present pathogens. According to some authors, resilience is connected to capability of host/animal to survive and stay productive no matter the parasite challenge it's exposed to (Bishop, 2012). There is one more definition in the literature for this term, according to which resilience represents ability of the host to tolerate existing parasites without showing any clinical signs of diseases (Gunia et al., 2013). *Bishop and Morris* (2007) define resilience as ability of the animals to adapt to infection with different causes of parasitical ethiology from environment. According to Storey (2015), resiliance represents adaptability to changes and owning capacity for successful adaptation, when facing parasitic

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infection, as gaining more and wider competences for stress reaction.

Since resistence and resilience are hereditary characteristics, accurate and timely definition of each parameter that helps process of animal breeding selection is important, shown by these traits. For confirmation of animals that show resiliance when facing with helminth challenge, or in which parasites are anthelmintics resistant, counting of helminth eggs number present in feces of examined animals is necessarry (FEC - Fecal Egg Count), as well as estimation of clinical anemia based on erythrocyte volumen value (packed cell volume - PCV) using FAMACHA (FAffa MAlan CHArt) test (*Malan and Van Wyk,* 1992) and parameters related to body condition (Storey, 2015).

DIAGNOSTIC VALUE OF FECAL EGG COUNTING
(FEC - FECAL EGG COUNT)

For assessment of anthelmintics efficiency in ruminants, detection of anthelmintics resistance and resilience proving, World Association for the Advancement of Veterinary Parasitology (WAAVP) recommends McMaster method (Coles et al., 1992, Storey, 2015). That is standard and most often used conventional method of quantitative coprological diagnostic in veterinary parasitology. It serves for determination of the endoparasitic infection degree and is based on counting of number of parasitic elements in fecal weigh unit (EPG - Eggs Per Gram, OPG - Oocysts Per Gram, CPG - Cysts Per Gram and LPG - Larvae Per Gram). Sensitivity of method is from 10 to 100 parasitic elements in 1 g of feces (Pereckiene et al., 2010).

Application of this procedure, until now, have been described in large and small ruminants, horses, pigs, canines, birds, rabbits, mice, turtles, lemurs and human (Bondarenko et al., 2009). Vadlejch et al. (2011) compared sensitivity and reliability of three modified McMaster techniques with the aim to estimate which modification is most suitable for routine parasitological examinations and diagnostic assessments in veterinary clinical practice (Table 1).

It was shown that the concentration McMaster method (*Roepstorff and Nansen*, 1998) is most sensitive and most reliable for helmnith egg detection. This method is fast, use largest quantity of feces (4g), have low limit of detection (20 EPG), and, thanks to centrifugation, fecal suspension is clear enough for microscopy.

Modification made by Zajček (1978) should provide better results due to lower correction factor, two procedures of centrifugation and lowest dilution ratio. Nevertheless, low dilution ratio consequently lead to presence of large amount of impurity in examined substance. This makes the process of preparation examination significantlly difficult, in which parasitic elements may be camouflaged or wrongly detect as fecal pseudoparasitical particles, thus increasing a unreliableness of this procedure. This method has middle limit detection value and is applicable with result accuracy of 100 EPG (Vadlejch et al., 2011).

Third comparable method, according to Wetzel (1951), is the simplest but with worst result. Low sensitivity and reliability of this method is most probably caused by high correction factor and absence of centrifugion. This method has high limit of detection value and gives precise results at 200 EPG (Vadlejch et al., 2011).

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Table 1. Comparative parameter values of modified McMaster techniques (Vadlejch et al., 2011)

Parameters	Modified McMaster techniques		
	Wetzel (1951)	Zajiček (1978)	*Roepstorff and Nansen* (1998)
The amount of feces (g)	2	1	4
Type of flotation solution	NaCl	$MgSO_4 + Na_2S_2O_3$	NaCl + glucose
Specific weight of solution	1.200	1.280	1.300
Centrifugation (RPM[1])	-	2000	1200
Centrifugation (RCF[2])	-	479	172
Time of centrifugation (min)	-	2	5
Time of flotation in chamber (min)	2-3	5	3-5
Number of chambers in McMaster object	3	2	2
Multiplicative (corrective) factor	67	33	20

[1]RPM - Revolutions Per Minute

[2]RCF - Relative Centrifugal Force

In different ruminant species there are certain limits of FEC procedure that may significantly impact the explication, interpretation and reliability of obtained results. In adult cattle those are: 1) limited diagnostic value, linked to infection degree that is usually not correlated with helminthes load; 2) low FEC values, that requires more sensitive flotation techniques in cattle than in sheep; 3) limited clinical value for *Nematodirus* spp., since the most damage is caused by immature stadiums of this nematode before starting laying eggs and 4) clinical form of paramphimatosis, that is usually caused by numerous immature parasites in migration, leading to absence or low number of eggs in feces (Rollinson, 2013).

In hemonchosis and trichostrongilidosis of small ruminants, FEC is in high correlation with helminth load of animals. In the case of polyparasitisms, when relatively high production of *H. Contortus* eggs may camouflage lower production of eggs by some other species (*T. colubriformis* and *T. circumcincta*), FEC has limited diagnostic values (Roeber et al., 2013). Because of that, only approximate assessment of infection intensity and decision when animals should be treated can be obtained based on numbers of eggs laid by different gastrointestinal nematode (GIN) species (Table 2).

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Table 2. Determination of rate of infection with GIN for young animals (Kahn, 2005)

Parasite	Infection rate (EPGF - Eggs Per Gram Feces)		
	Low	**Moderate**	**High**
CATTLE			
Mixed infection	50-200	200-800	800+
Haemonchus spp.	200	200-600	600+
Trichostrongylus spp.	50-100	100-400	400+
Cooperia spp.	200-300	300-2500	2500+
SHEEP			
Mixed infection	50-800	800-1200	1200+
Haemonchus spp.	100-2000	2000-7000	7000+
Trichostrongylus spp.	100-500	500-2000	2000+
Nematodirus spp.	50-100	100-600	600+
Oesophagostomum spp.	100-800	800-1600	1600+

It is known that high efficiency is expected from antiparasitics on field. Antiectoparasitics should have absolute efficiency, while it is expected to be around 95% in anthelmintics, since it is favorable to maintain small number of parasits in the body as a stimulus of immunological response of the host (Dimitrijević, 1999). In grazing animals there is always mixed infection with higher number of different gastrointestinal nematoda species. Some of them provide development of natural immunity, so that it can be decided if treatment of animal is necessary on the basis of FEC results.

Table 3. **Interpretation of clinical form apperance in cattle based on EPG**

(***Love and Hutchinson***, **2007)**

Parasite species	Egg number/1g feces (EPG)
Haemonchus	200
Trichostrongylus	50
Ostertagia	150
Oesophagostomum	100
Cooperia	500
Fasciola	50

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This is very important, since it is necessary to maintain balance between induced immunity (its development is stimulated by small number of healminth in animal) and productive capability (its decrease is affected by the same number of helmiths, that cause subclinical form of disease). Obtained EPG results might be very useful for interpretation of clinical form of some helminthosis in large and small ruminants (Table 3).

DIAGNOSTIC VALUE OF FECAL EGG COUNT REDUCTION TEST (FECRT)

Fecal Egg Count Reduction Test (FECRT) is established in early 90`s (*Coles* et al., 1992) and represents a method of choice for monitoring anthelmintics efficiency in ruminants (Dobson et al., 2011). Currently, it is the unique test that may detect resistance of all types of nematoda species in all host species (McKenna, 2013) and serve for calculation of egg count reduction in feces, by comparing mean values of FEC before treatment and after obtained treatment (Wang et al., 2017).

According to World Association for the Advancement in Veterinary Parasitology (WAAVP), there are guidelines for performing and calculating standard FECRT (Coles et al., 1992, 2006), which are improved by recommendations made by Levecke et al. (2017). In accordance with those guidelines, recommendations are: size of sample (≥10 or ≥15 animals per group for treatment, and each excretion at least 150 EPG), FEC method (McMaster), statistical analysis (FECRT based n arithmetical mean of grouped FEC after drug application) and criteria which define decreased drug efficiency (FECRT <90% or FECRT <95%, resistance is declared if the fecal egg count reduction is lower than 95%, and the lowest limit of drug efficiency is lower than 90%) (Dobson et al., 2011; Vidyashankar et al., 2012).

For performing FECRT, sample of faces is collected before dehelmintisation and EPG value is determined in it, followed by treatment. Sampling of feces is repeated 14 days after obtaining treatment and value of EPG is determined again. By using special equation, percentage decrease of eggs count in feces is calculated individually. Thereafter, average decrease for all test animals is calculated, in order to calculate overall decrease for farm or herd. This value is subsequently used for obtaining calculation related to existence or absence of drug resistance (*Kaplan and Nielsen*, 2010). If the drug is effective, no parasite should survive treatment longer than the time needed for gut emptying (usually up to 48 hours). This period of time may be prolonged for as many days as temporary suppression of eggs laying lasts (3 days – for imidazole, 8 days – for benzimidazoles, 14 to 17 days – for macrolides lactones), so that efficiency of certain drug groups is estimated only after expiration of this period (Coles et al., 2006).

If examined animals have large egg number in feces, after which dehemintization is obtained, and 10 days after FEC shows zero or very low value (lower than 5% of value before treatment), for that group can be claimed certainly that dehilmisation is successfully obtained (Coles et al., 1992).

DIAGNOSTIC VALUE „FIVE POINT CHECK" CLINICAL APPROACH

Obtained diagnostic FEC based results have to be complement with assessment related to presence or absence of clinical signs of diseases („Five Point Check"). This clinical approach means monitoring of five most common nonspecific symptoms in animals infected by parasites – anemia of mucus membrane of eye, body

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weight lost or growth and development retardation, fecal dirtiness of tail and posterior body region, submandibular edema and runny nose (Bath et al., 2010). Based on clinical symptoms severity, selection of animals that should undergo process of dehelmintisation is done. Herd health status is classified as: „good" (no need for dehelmintisation), „bad" (necessarily of dehemintisation, with control during several fallowing months) and mixed results („some animals are good, some are bad"- according to estimated symptoms severity, it is decided which animals will undergo dehelmintisation process) (Storey, 2015).

With the aim to slow down the onset of the anthelmintics resistance and organization of Integrated Parasite Management (IPM), the principle of Targeted Selective Treatment (TST) is globally accepted. Implementation of this strategy has become workable on farms only with development and practical application of FAMACHA© system for clinical estimation of anemia caused by hematophagous nematode *Haemonchus contortus* in small ruminants. Principle of TST can be expanded on other important ectoparasites, under condition that developed system is practical, economical and realistically capable to identify animals that are under risk of overloading by expected endoparasites (*Bath and Van Wyk*, 2009).

Candidates for expanded TST system manifest one of five listed clinical symptoms that served as bases for designing of practical guide for breeders. For international, multilingual usage, this system is called Five Point Check©, and represent practical expansion of TST and may be effective contribution in monitoring of endoparasite presence in small ruminants. It let users to: (a) make rapid estimation of parasitosis signs in small ruminants, (b) make effective estimation of health status of own animals, (c) identify expected parasites, (d) select anthelmintic groups for treatment (e) use practical systems for temporary identification of treated animals

and (f) familiarize with limits of the system (*Bath and Van Wyk*, 2009).

FAMACHA© system presents figure of the estimation of severity of parasite infection in ruminants and making decisions about healing, based on anemia degree of mucous membrane. Clinical anemia is represented through erythrocyte volume, and is ranged from 1 to 5 on the scale of FAMACHA card and it indicates the infection by haematophage nematode *H. contortus,* trematodes and cestodes (Ferreira et al., 2019).

Besides increased FAMACHA results, the cause for delemintisation might also be other clinical manifestations in infected animas. Based on body condition index, which is determined by BCS (Body Condition Score) by card on scale from 1 to 5, there is possibility of infection by *Telodorsagia* spp., *Trichostrongylus* spp. and nodular helminths (Mahieu et al., 2007, Arece-Garcia et al., 2016). Dirtiness of tail and posterior body region with feces is determined by DS (Dag Score) card with 1 to 5 scale and is indicator of possible presence of infection by nematodes *Telodorsagia* spp., *Trichostrongylus* spp., *Oesophagostomum* spp., *Strongyloides* spp. snd coccidia (*Eimeria* spp). Existance of nasal discharge indicates the presence of nasal miasa, pulmonal parasites and pneumonia, and is scaled by ND (Nasal Discharge) card on scale 1-5. Cold submandibular edema is, according to severity, catogorised on 1 to 5 scale, and indicates the low blood protein level in examined animals and on possibility of parasite infection caused by *H. Contortus* species*,* trematodes, cestodes and coccidia (*Eimeria* spp) (Walker et al., 2015).

Besides these five standard clinical symptoms, sometimes observation of condition of the fur is performed (low hair quality or abnormal fleece), which changes are classified on scale from 1 to 5, and may indicate the presence of nematode infection (*H.* c*ontortus*, *Telodorsagia* spp., *Trichostrongylus* spp.), coccidia (*Eimeria* spp) and ectoparasites (Vanimisetti et al., 2004; Mahieu et al., 2007)

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From the aspect of differential diagnosis of animals that show signs of resistance, i.e. resilience, same of the most important parameters are obtained FAMACHA values (*Burke and Miller*, 2008). Usage of FAMACHA© system provides small ruminants breeders to make decisions related to delelmintisation based on assessment of anemia rate caused by *H. contortus* in sheep and goats (Arece-Garcia et al., 2016). This causative agent is economically most important GIN in sheep and goats, is most comon cause of anemia during pasture season in USA, and in the cases of infections of high intensity causes death.

FAMACHA© card is developed in South Africa, and is imported in USA by American Consortium for Small Ruminant Parasite Control (*ACSRPC*). That is one of the most successful diagnostic indicators, according to which the color of eye mucous membrane is compared with 5 categories of color on control panel with colors that match the different anemia rates. Category 1 is "non anemic" state, while category 5 represent the state of "severe anemia"

(Martinez-Valladares et al., 2013). Based on results determined by card, sheep and goats with anemia are identified and exposed to selective dehelinitisation.

Selective dehelmintisation reduces the drug usage and slows down the development of anthelminths resistance in GIN. It can also help in making selective decisions related to breeding, in a way that it will identify those animals that are most sensitive to gut parasite infection, i.e. resilient animals (Rizzon Cintra et al., 2018). FAMACHA© is applied only in a cases where main causative agent of clinical disease is *H. contortus*. Before performing the test, it should be taken in consideration that some stages (eye diseases environmental stimulus and systemic diseases) cause reddish of eye mucous membrane and thus may camouflage anemia. Questionable may also be the other causes of anemia, but they are rare comparing to GIN infection during pasture season (Ferreira et al., 2019).

CONCLUSION

Since they are adapted to present parasites, resilient animals present non-identified sources of parasite infections, which can be maintained for a long period and obtained continual recontamination of pasture. They can be identified only just with implementation of quantitative coprological diagnostic and performing FEC values for each individual respectively. Resilient animal has consistently low FEC levels and low FAMACHA results, mainly are in good body condition and do not show variations in body weight. Obtained FEC values usually indicate that suspicious (resilient) animals are carriers of much higher number of parasites than it can be expected by analysis and estimation obtained on other clinical parameters. These animals should

not be dehelminted, or should be rarely dehelminted, compared to the other herd animals which show clinical signs of diseases also after dehelmintisation, due to parasite resistance. Animals infected by large number of resistant endoparasites show high FEC values, high FAMACHA results and have poorer body condition with significant body weight variations also after treatment. Short term changes in body weights, may be indicators of parasitosis, and this may provide rapid identification of animals that will probably have benefits from treatment. Without FEC information, it cannot be certainly defined if observed noted characteristic is resistance or resilience.

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