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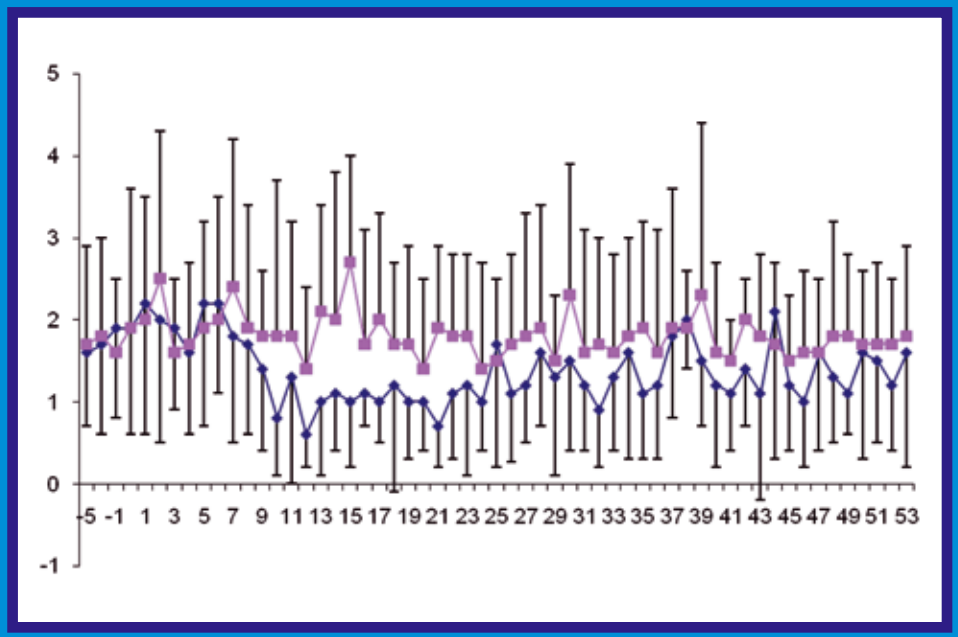

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Original Research Paper

THE EFFICACY OF ANTIHISTAMINE FEXOFENADINE VERSUS METHYLPREDNISOLONE IN THE TREATMENT OF ATOPIC DERMATITIS IN DOGS

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Summary: The objective of the study was to establish efficacy of antihistamine fexofenadine in atopic dogs.
Thirty atopic dogs over the age of 6 months, of different breeds and sex were randomized into two groups with 15 dogs each. The group F received fexofenadine orally; the group M received methylprednisolone for the period of 6 weeks. CADESI and pruritus score were measured three times during the study (at the baseline, after 3 weeks and after 6 weeks of treatment).
Statistically significant discrepancies in CADESI score in comparison with baseline were observed in both groups, namely for 3rd (P=0,007 for group M and P=0,019 for group F) and 6th treatment week (P<0,001 for group M and P<0,001 for group F). Between the groups statistically significant difference in CADESI score for the 6th week of treatment was observed in favour of group F (P=0,012). Statistically significant lowering of strengths of itching score at week 3 (P=0,002) and week 6 (P=0,004) were observed in group M compared to baseline. The difference in strength of itching score in group F was not statistically significant at week 3 of treatment (P=0,215) but was at week 6 of treatment (P=0,002). Between the groups the difference was statistically significant at baseline (P=0,048), although not at week 3 and 6 of treatment. The results of the study are showing comparable improvement of clinical signs for fexofenadine treatment versus methylprednisolone treatment, nevertheless fexofenadine needed longer period of time to reach efficacy endpoint.

Keywords: fexofenadine hydrochloride; methylprednisolone; atopic dermatitis; atopy; glucocorticoids; antihistamines

Introduction

Atopy is the genetic predisposition of the body to develop immunoglobulin E (IgE) antibodies in response to environmental allergens, which is clinically manifested by a combination of immediate and delayed signs of allergy (1,2,3,4). Symptomatic treatment of atopic dermatitis in dogs often includes glucocorticoids, antihistamines, or a combination of both. While glucocorticoids are highly effective in this indication, their use in long-term treatment causes serious adverse reactions in most patients. Therefore glucocorticoids are mainly recommended in acute allergic episodes and for initial treatment of atopy (5,6). The evidence for antihistamine therapy in dogs is still limited but there is a consensus that they offer some benefit, which is worth trying (7). There are some data regarding antihistamines which block H1 receptors could contribute to pruritus controll associated with atopic disease in dogs (8,9,10). The realistic success ranges from 5 - 30% for any given antihistamine (11). The sedative effect of many antihistamines may also contribute to the control of pruritus. Some other antihistamines such as tricyclic piperidine antihistamine (azatadin) and hydroxyzine hydrochloride (HCl) may stabilize mast cells and decrease mediator release following antigen challenge in allergic patients. Antiserotonine-, an-

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algesic- and antianxiety activity are also expressed by some antihistamines and these may contribute to their effectiveness. In a retrospective study of 55 cases of pruritus in atopic dogs, 30% of the cases responded to antihistamines well enough that the clients were satisfied with the results and the dogs did not require systemic glucocorticoids (12). There are some products that combine antihistamines and glucocorticoids. When prednisone and trimeprazine were given in combination at the same doses 76.7% responded satisfactorily (13). By controlling whichever of the pruritus that is mediated by histamine the patient moves closer to going below its purity threshold.

Fexofenadine is a second-generation antihistamine and it is indicated in humans for the relief of symptoms associated with seasonal allergic rhinitis and chronic urticaria. It is a pharmacologically active metabolite of terfenadine, an antihistamine of the first generation. The second-generation antihistamines are more lipophobic than those of the first generation; therefore they do not cross the blood–brain barrier to produce CNS effects and are devoid of anticholinergic activity when used in therapeutic doses (14). In humans it has been shown as highly effective (15,16,17,18,19,20). Fexofenadine has been shown to inhibit the expression of the cell surface adhesion molecule ICAM-1 on human conjunctival epithelial cells (21). It also inhibits eosinophil-induced release of soluble ICAM-1 and induction of inflammatory mediators (e.g. GMCSF, IL-8) in nasal epithelial cells cultured from biopsies taken from patients with SAR and calcium ionophore-induced release of eosinophil cationic protein from eosinophils (22). In addition, fexofenadine decreases adhesion and chemotaxis of eosinophils to human endothelial cells (23). Consequently, these data suggest fexofenadine has anti-allergic properties in addition to its antihistamine activity in humans.

Fexofenadine does not interact with muscarinic receptors in people and does not provoke drowsiness, urinary retention, dry mouth, and constipation (24), which might offer a potential advantage compared with desloratadine, the recently approved active metabolite of loratadine. Fexofenadine is devoid of adverse cardiac effects, and changes in human electrocardiogram parameters are not significantly different from those observed with placebo (25). It is also well tolerated in people with renal or hepatic impairment, in children and the elderly (26). Fexofenadine has also been shown to have a favorable effect on nasal congestion. This therapeutic advantage might be related to its significant antiallergic properties (25).

Fexofenadine (as cetirizine and loratadine) may have additional activity at cells involved in the inflammatory response. These actions include possible inhibition of mediator release from mast cells, action on leukotrienes and prostaglandins involved in the late phase allergic response (27,28,29,30).

Cumulatively, these benefits distinguish fexofenadine from the other antihistamines and make it an optimum therapeutic option for treating allergy-mediated respiratory and dermatologic diseases in humans (25).

Any data on the efficacy, safety and recommended doses of fexofenadine hydrochloride in dogs with atopic dermatitis were found in reviewing the literature. Our decision to conduct a study to investigate the efficacy of fexofenadine in the treatment of atopic dogs was therefore made.

Material and methods

Thirty dogs of different breeds, age and sex with signs of atopic dermatitis and meeting the criteria proposed by Willemse (31) were included in the study. Presentation of the breeds was as follows: 4 german shepherds, 3 chow chows, 3 golden retrievers, 3 mixed breeds, 2 shar-peis, 2 west highland white terriers, 2 labradors, 2 tibetan terriers and one of dalmatian, french bulldog, boxer, cairn terrier, russian terrier, schipperke, basset hound, great dane and cocker spaniel. The age of dogs included ranged from 9 to 125 months (on average 39.5 months). Female gender dominated (18 dogs of 30) and weights ranged from 7.0 kg to 69 kg (on average 27.35 kg).

Other differential diagnoses were excluded before the inclusion into the study. Parasitic diseases were excluded by negative skin scrabs and use of Stronghold spot-on® (Pfizer Ltd., Sandwich, United Kingdom) as a diagnostic therapy trial to exclude scabies. Parasitic diseases were prevented during the study using Frontline spot-on® (Merial, Lyon, France). Prior inclusion into the study individually adjusted elimination diet was fed to the dogs. The dogs have been given elimination diet already 3 months before inclusion into the study and all the time of the study (6 additional weeks).

The study protocol included consent of the dog's owner to participate in the study, data on the animal and the history of the disease and on previous treatments. The use of the following medications was prohibited: glucocorticoids (3 weeks or less before inclusion), antihistamines (14 days or less before inclusion), cyclosporines (1 month or less before inclusion), EFA (14 days or less before inclusion), vitamin E supplements (14 days or less before inclusion), antipruritic substances as SRI (Clamipramin) and SSRI (Fluoxetin) (14 days or less before inclusion), antiseborrheic, keratolitic and antiseptic shampoos (14 days or less before inclusion), immunotherapy (never).

Next conditions were incorporated among the exclusion criteria: the cases with their history, previous therapies and outcomes of the treatments which were not well documented, presence of concurrent illnesses, that could influence the results of the study (like heart disease), serious impairment of kidney or liver function, planned or coincidental pregnancy, FAD symptomatic dogs, concurrent food allergy or intolerance cases not controlled with the diet, presence of the ectoparasites, symptoms of bacterial or fungal concurrent infection.

A random sample was obtained by alternate allocation of dogs to one or the other investigational group. Fifteen dogs were treated for 6 weeks with methylprednisolone (Medrol®, Pharmacia Enterprises S.A.) 0.5 mg/kg/24 hours for the first 5 days, then with 0.5 mg/kg/48 hours (group M), and 15 dogs with fexofenadine (Telfast®, Aventis pharma) 18 mg/kg/24 hours (group F). CADESI-02 (Canine Atopic dermatitis Extent Severity Index) values and pruritus values were taken three times during the study (baseline, after 3 weeks of treatment and after 6 weeks of treatment). Presence and intensity of erythema with lichenification and excoriation of the skin was estimated on altogether 40 different parts of the body. In accordance to photo scale, which was produced prior the study on basis of photographs of clinical cases, every each parameter was scored from 0 to 3 (0 = no changes). The measurements were performed single blindly, thus leaving assessors unaware of previous measurements. Acquired CADESI score was statistically compared between the groups and periods of the treatment.

The pruritus of the skin was estimated on basis of owner observations in each dog. To estimate the pruritus, visual analogue scale was used, for owners to give their observations by scoring from 0 to 100 (0= no pruritus). The estimate of the owner based on observations regarding intensity, frequency and duration of the pruritus. The owners were instructed to observe licking of the paws and inguinal area, biting of the paws and body, scratching the head and the body as well as rubbing of the head and the body to objects.

The results acquired at the first visit (inclusion day) were compared to the results of the second visit (3 weeks of treatment) and the third visit (6 weeks of the treatment). Results of the second visit were compared to results of the third visit as well. After this, results were compared also between the groups. For the statistical analysis of the obtained data SPSS statistical package version 14 was used. Statistical evaluation of the data between the groups was performed by one-way T test, while statistical evaluation of the data between the visits in each of the groups was performed by ANOVA. Differences were considered significant when $P<0.05$.

The study was approved by the Ministry of Agriculture, Forestry and Food, by its Decision No 4.4.-43/05 issued on 26 October 2005.

Results

The results of the study are presented in Table 1 and Figures 1-2.

Side effects during the therapy trial:

Owners of dogs with numbers 27 and 33 (group M) reported increased drinking of the animals. Three owners of dogs from group M (dogs with numbers 14, 25 and 29) reported on severe fatigue with one of them also with increased eyes discharge. Eyes discharge was reported also by three owners of dogs from group F (dogs with numbers 11, 16 and 20) at the second visit, one of them also with nasal discharge. Dog number 1 (group F) had for a short while no appetite, although appetite returned when diet flavour changed. Dog from group M with number 15 had transient diarrhoea, with rattle and bacterial inflammation of the abdominal skin occurring at the visit 3. The occurrence of rash was noted also in dog number 13 (group F) at visit 2. By the visit three the rash didn't resolve, on the other hand the dog appeared to be in heat. Due to sporadic and mild nature of difficulties, none of the dogs was excluded from the study.

Table 1: CADESI (Canine Atopic dermatitis Extent Severity Index) evaluation at visits 1, 2 and 3 (baseline, after 3 weeks of treatment and after 6 weeks of treatment) for group M (methyilprednisolone treated) and for group F (fexofenadine treated)

Group M	1.VISIT	2.VISIT	3.VISIT	Group F	1.VISIT	2.VISIT	3.VISIT
3	28	29	25	**1**	66	42	16
4	13	23	9	**2**	134	40	13
8	40	29	15	**5**	19	2	2
10	37	23	11	**6**	46	14	13
12	62	17	17	**7**	49	27	18
14	46	18	7	**9**	24	14	5
15	50	14	17	**11**	27	10	5
17	11	4	5	**13**	26	26	22
19	27	12	12	**16**	43	30	7
23	83	43	32	**18**	22	9	3
25	60	20	15	**20**	36	10	6
27	38	11	15	**22**	34	10	2
29	28	8	8	**24**	37	5	5
32	45	44	27	**26**	18	4	2
33	73	44	11	**30**	20	2	2

Majority of dogs from both study groups experienced lowering of CADESI score parameters from the start to conclusion of the treatment, which was later on statistically evaluated. The dogs with numbers 3 in group M and number 13 in group F stepped out, since they haven't experienced lowering of the scores.

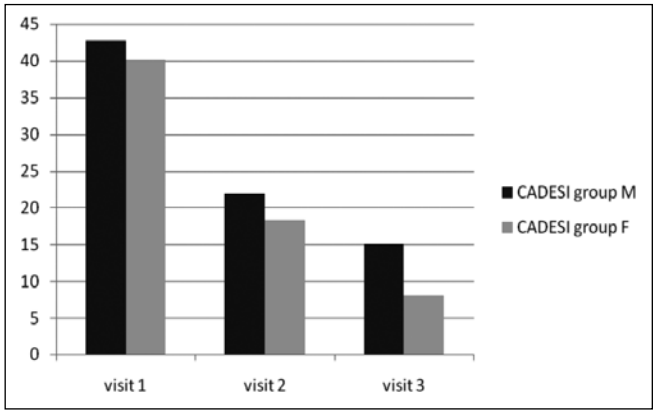

Figure 1: Mean values of CADESI (Canine Atopic dermatitis Extent Severity Index) at visits 1, 2 and 3 (baseline, after 3 weeks of treatment and after 6 weeks of treatment) for group M (methylprednisolone treated) and for group F (fexofenadine treated)

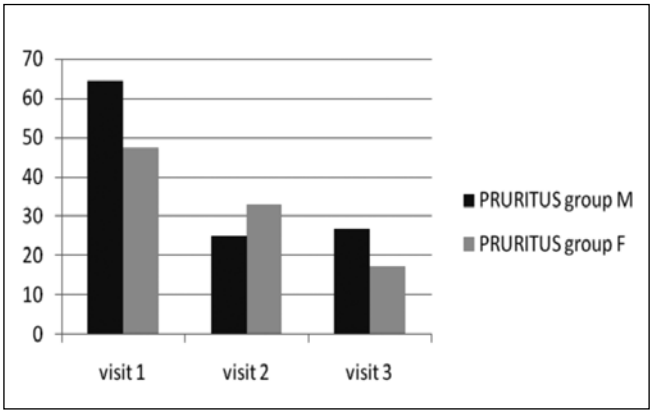

Figure 2: Mean values of pruritus in percent at visits 1, 2 and 3 (baseline, after 3 weeks of treatment and after 6 weeks of treatment) for group M (methyilprednisolone treated) and for group F (fexofenadine treated)

Discussion

Antihistamines are commonly used for treatment of atopic dermatitis in dogs, both as single agents, and as synergistic medications used to reduce required dosages of glucocorticoids. While the role of histamine-regulated responses in atopic dermatitis is well-established, antihistamines are not as effective as glucocorticoids in the management of the pruritus in atopic dogs (5). The efficacy of an-

tihistamines is notoriously unpredictable and individualized in a given patient. Part of this variation may be dose-related because antiallergic effects are concentration dependent and some dose ranges are antiallergic whereas others may enhance mediator release (32). We also know that not all the effects of histamine in dogs are antagonized by H1 blockers therefore antihistamines of the second generation, that additionally may block mediator release (33) should be more effective. However the data demonstrate that these drugs are much more effective when given before the allergy symptoms take hold and that they will be most effective if used regularly as directed, starting just before the allergy season which is hard to obtain in treatment of atopic dogs.

In our study Fexofenadine was used that was not used in clinical trials in dogs before. A comparable efficacy of fexofenadine versus methylprednisolone was found, as shown above and discussed below. Two non-responders were observed concerning CADESI score (table 4) but even in these two dogs the pruritus was reduced for more than 50% at the end of the study (data not shown).

Some non-desired effects were observed during the treatment: polydipsia, fatigue, eye discharge, diarrhoea and pyoderma were observed in group M. In group F eye and nasal discharge, loss of appetite and skin rash were reported in few cases. Similar effects (fatigue, diarrhoea) were described in use of fexofenadine in people, but the incidence was similar to placebo (34). According to these data and since observed phenomena in our study were sporadic they can hardly be contributed to the drugs used in short –term therapy. Many of them may also have been symptoms of AD. While the side effects of long-term use of methylprednisolone were already well recognised, fexofenadine long-term use should be additionally investigated.

Developing *fexofenadine* usage in human medicine produced pharmacological studies in dogs. Different ECG parameters regarding possible drug effects were followed and established, that *fexofenadine* perorally in dosage of 30 mg/kg/ body mass twice daly is free of lenghtening of QT interval in dogs. Plasma concentrations of *fexofenadine* in dogs were thus reaching 9-time therapeutic plasma concentrations of adult human, who received maximal recommended daily dose of fexofenadine (16). Therefore when formulating the study design we didn't want to exceed the maximal dose that was reported to be safe in dogs. On the other hand our decision about the dose to be used (18 mg/kg b.w.) was based on the ratio calculated for those antihistamines for which the doses for humans and for dogs were known. The mean doses used in veterinary medicine in dogs are 7.5 times higher than the human recommended dose. The mean human recommended dose of fexofenadine hydrochloride is 180 mg/day. If this dose is adjusted to the average human body mass approximately 75 kg, we get a dose of 2.4 mg/kg b.w. The dose used in the study in dogs was 18 mg/kg b.w. or 7.5 times the human recommended dose. This was the highest acceptable number of tablets administered in a single dose. Optimisation of dosage regimen is left to be determined with further investigation.

In the study CADESI-02 scale was used and was the most up-to-date rating system at the time of the study. CADESI evaluation system is the only system allowing statistical evaluation and monitoring of the effects of the treatment in atopic dogs. A reduction in the mean CADESI scores was observed in both groups of dogs and continued throughout the study period (Figure 1). The difference in the scores at the second visit (3 weeks of treatment) was of statistical significance compared to baseline in both groups ($P = 0.002$ for group M; $P = 0.019$ for group F). A statistically significant difference in the mean scores compared to baseline was also found in both groups at the end of the study ($P < 0.001$ for group M; $P < 0.001$ for group F). No statistically significant differences between the groups were found at the first visit ($P = 0.775$) and at the second visit ($P = 0.535$), while at the third visit a statistically significant difference ($P = 0.012$) was recorded in favour of group F. It has thus been demonstrated that the clinical picture improved significantly after both investigational therapies. In the last three weeks of the treatment, an even greater improvement was observed in dogs treated with fexofenadine compared to methylprednisolone. Improvement was manifested as reduction or disappearance of skin lesions, i.e. erythema, lichenification and excoriation, as evaluated by the CADESI scoring system. The authors of a similar study (11) set the level of significance for the change of the CADESI score at 50% ($CADESI_{50}$) reduction from baseline. Considering the recommendations of the above authors, we can see that both active substances used in our study reduced the CADESI score by more than 50%, which demonstrates that both medicinal products are sufficiently effective in the treatment of dogs with atopic dermatitis.

The severity of pruritus was markedly reduced compared to baseline in group M at the second visit

(3 weeks of the treatment) and stayed at a similar level until the end of the study (Figure 2). The difference in the severity of pruritus in group M was statistically significant compared to baseline at the second visit (P = 0.002) and at the third visit (P = 0.004). In group F, the severity of pruritus was gradually decreasing throughout the study period, but the change from baseline at the second visit was of no statistical significance (P = 0.215). However, a statistically significant difference was demonstrated at the third visit (P = 0.002). This finding demonstrates that both fexofenadine and methylprednisolone therapy resulted in a significant reduction in pruritus in atopic dogs. With methylprednisolone, a significant reduction in pruritus was found as soon as after three weeks, while with fexofenadine a longer period was needed for a similar effect. The between-group comparison by visit revealed a statistically significant difference at the first visit (P = 0.048), when the mean severity of pruritus was greater in group M, while no statistically significant differences were found at the second (P = 0.47) and third visit (P = 0,263). Preliminary study, that was done on 8 dogs remitted encouraging results (35), so the study presented in this article was designed. A comparison of the results of our study with those of a similar study (11), where a reduction in the severity of pruritus by 50% was considered satisfactory, reveals that the mean pruritus severity was reduced in our population of dogs by more than a half (pruritus$_{50}$) over a treatment period of 6 weeks. Therefore both investigational active substances resulted in a satisfactory reduction of the severity of pruritus in dogs with atopic dermatitis.

Conclusion

Based on the results of this study, we can conclude that both of the active substances, fexofenadine hydrochloride in oral doses of 18 mg/kg b.w. once daily and methylprednisolone in doses of 0.5 mg/kg b.w. administered for 5 days, followed by doses od 0.5 mg/kg b.w. every other day, were effective in reducing the severity of pruritus and the presence of skin lesions (CADESI score) in dogs with atopic dermatitis.

The results of this study suggest that *fexofenadine hydrochloride* could be accepted in the doctrine of treatment of atopic dermatitis in dogs.

However, additional studies will be needed to further substantiate our conclusions and confirm the findings of this study.

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UČINKOVITOST ANTIHISTAMINIKA FEKSOFENADINA V PRIMERJAVI Z METILPREDNIZOLONOM PRI ZDRAVLJENJU ATOPIČNEGA DERMATITISA PSOV

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Povzetek: Namen našega dela je bil ugotoviti učinkovitost antihistaminika feksofenadina pri psih z atopičnim dermatitisom. Trideset psov z diagnozo atopični dermatitis, starih nad 6 mesecev, različnih pasem in obeh spolov smo naključno razvrstili v dve skupini po 15 psov. Skupina F je 6 tednov peroralno prejemala feksofenadin, skupina M pa metilprednizolon. Meritve CADESI in oceno srbeža smo opravili 3-krat v času raziskave (pred pričetkom zdravljenja, 3. teden in 6. teden zdravljenja). V primerjavi z izhodiščno vrednostjo smo ugotovili statistično značilno odstopanje vrednosti CADESI pri obeh skupinah, in sicer 3. ($P = 0,007$ za skupino M in $P = 0,019$ za skupino F) in 6. teden zdravljenja ($P < 0,001$ za skupino M in $P < 0,001$ za skupino F). Med skupinama smo 6. teden zdravljenja ugotovili statistično značilno razliko v vrednostih CADESI v prid skupine F ($P = 0,012$).

Glede na izhodiščno vrednost je bilo znižanje jakosti srbeža v skupini M tretji ($P = 0,002$) in 6. teden zdravljenja ($P = 0,004$) statistično značilno. Za skupino F razlika v jakosti srbeža 3. teden zdravljenja ni bila statistično značilna ($P = 0,215$), je pa bila statistično značilna 6. teden zdravljenja ($P = 0,002$). Med skupinama je bila razlika statistično značilna pred pričetkom zdravljenja ($P = 0,048$), medtem ko 3. in 6. teden zdravljenja razlik ni bilo več.

Rezultati raziskave kažejo, da sta obe preizkušani zdravili učinkovito znižali parametre, ocenjevane po sistemu točkovanja CADESI, in zadovoljivo zmanjšali jakost srbeža, vendar je feksofenadin v primerjavi z metilprednizolonom za navedeni učinek potreboval daljši čas. Feksofenadin bi bilo torej primerno vključiti v doktrino zdravljenja atopičnega dermatitisa psov.

Ključne besede: feksofenadin hidroklorid; metilprednizolon; atopični dermatitis; atopija; glukokortikoidi; antihistaminiki

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Original Research Paper

IMPACT OF FEEDING OF FLAXSEED AND PROBIOTICS ON MEAT QUALITY AND LIPID OXIDATION PROCESS IN PORK DURING STORAGE

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Summary: In our experiment effect of flaxseed, mixed in standard diet (alone and in combination with probiotic bacteria) during feeding period, on lipid oxidation and sensory properties of pork (thigh muscles) stored at freezing (-21 °C) and chilling (4 °C) conditions was studied. Oxidation processes expressed as changes in malondialdehyde (MDA) content in thigh muscles were monitored. Addition of flaxseed had impact on increasing of fat content in muscles. Results confirmed that feeding the flaxseed significantly ($P < 0.05$) increased oxidation processes during storage in comparison to control group. Sensory examination of pork from pigs fed with flaxseed showed significantly different properties (taste and odour) compared to control group. Therefore, if feedstuff contains plant oils with high proportion of polyunsaturated fatty acids (PUFAs), addition of adequate amount of antioxidants is recomended.

Key words: flaxseed; lipid oxidation; pork

Introduction

Lipids are inseparable part of human nutrition and they have no replacement in various chemical and biochemical processes. Mainly, they are important as an intake of energy into the organism, and partially, lipids are utilised as a source of substances necessary for synthesis of components important for homeostasis. Besides nutrition and dietetic properties, lipids have also impact on sensory perceptions, and they have important influence on food odour and taste (1).

The main contribution of fats in nutrition is presence of essential polyunsaturated fatty acids (PUFAs). It is confirmed, that consumption of n-3 PUFAs has positive impact on human organism. In general, it is recommended to decrease the consumption of saturated and *trans* fatty acids and to increase the intake of PUFAs. Various scientific studies were focused on increasing the PUFAs content in animal products by adding plant oils into the animal feedstuff. The amount and composition of dietary fatty acids influence the quality of fat tissue in pigs (1, 2). Flaxseed presents a rich source of linoleic acid (C18:3, n-3) and in poultry (3) and rabbits (4) addition of flaxseed into the diet significantly changed proportion between PUFA/SFA and concentration of n-3 acids in animal muscles was increased.

Probiotic bacteria are known for their action on the host body including its immune system. Despite the considerable body of evidence about probiotics, the mechanism of their effect has not been explained completely. One of the presumed mechanisms of the inhibitory effect of probiotics on pathogens of digestive tract is the competition for the intestinal mucosa receptors (5, 6, 7). The results of different studies suggest that the action of probiotics may be modulated by dietary PUFAs as polyunsaturated fatty acids increase the colonisation of small intestine with lactobacilli.

It is well known that lipid oxidation is one of the major causes of lipid-rich foods deterioration. Higher content of PUFAs in meat have negative influence on stability of lipids during storage. The

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oxidative deterioration of the polyunsaturated lipids of foods leads through formation of hydroperoxides to short-chain aldehydes, ketones and other oxygenated compounds, which are considered to be responsible for development of rancidity in stored foods (8, 9).

The aim of our experiment was observation of lipid oxidation changes in pork thigh muscles during storage at chilling (4 °C, 11 days) and freezing conditions (-21 °C, 9 months) after feeding flaxseed alone or in combination with probiotic bacteria. Oxidative changes of lipids were compared with changes in sensory properties of stored meat.

Material and methods

Thirty six piglets at the age of 14 days were involved in our experiment. Piglets were divided into three groups. Groups were fed from 10 days before weaning until 35 days after weaning according to following schemes: first group (K) was fed with standard diet OŠ-02 NORM TYP (Spišské Vlachy, SR), second group (MK) was fed with standard diet enriched with flaxseed (10 % in mixture; composition of fatty acids in flaxseed is presented in Table 1). Third group (LMK) was fed with standard diet in combination with flaxseed (10 % in mixture) and probiotic bacteria strains (L81 – *Lactobacillus plantarum* and 2I3 – *Lactobacillus fermentum*) in form of probiotic cheese (4g per pig per day).

On the 35th day of age pigs were slaughtered. Bleeding of pigs followed the stunning. Procedure was performed according to the rules established for slaughtering of animals and was performed by trained veterinary surgeon. Afterwards, all pig carcasses were weighed, deboned and had skin removed. Thigh muscles were packed under vacuum conditions. One part of samples was stored at chilling conditions (4 °C) for 11 days, and second part at freezing conditions (-21 °C) for 9 months.

Table 1: Fatty acid composition of flaxseed used in diet (percentage)

Fatty acid	Proportion (%)
16:0 Palmitic	5.1
18:0 Stearic	3.7
18:1 *n*-9 Oleic	18.4
18:2 *n*-6 Linoleic	16.1
18:3 *n*-3 α-Linolenic	56.8

Chemical composition of meat samples

Determination of water content, dry matter content and fat content in percentage was performed according to Veterinary laboratory methods (10).

Evaluation of Thiobarbituric acid reactive substances (TBARS)

Decomposition of fats was assessed by TBARS assay. Evaluation of TBARS was performed according to Marcincak et al. (11) and measured spectrophotometrically at 532 nm (Helios γ, v. 4.6, Thermo spectronic, Great Britain). Results were calculated on amount of malondialdehyde in 1 kg of sample. Individual analysis were carried out on 1, 3, and 11 day of storage at chilling conditions and 0, 3, 6, and 9 month of storage at freezing conditions.

Sensory evaluation

Thigh muscles were used for sensory evaluation. Samples were evaluated 24 hours after slaughter processing of pigs (fresh meat) and after 9 months of storage at freezing conditions (- 21 °C). Professional evaluation committee presented panel of 7 assessors and they worked according to Methods intended for sensory evaluation of meat (12). The samples were boiled and 5 point schema and triangle test were applied for evaluation (13).

Statistical analysis

Statistical processing of results was performed by Graph Pad Prism 3.0 (1999). Results are expressed as arithmetic mean (x) and standard deviation (± s.d.). Increase of malondialdehyde between different groups during storage was compared by one-way ANOVA test. Tukey comparison test was used to compare statistical differences between values and $P < 0.05$ was considered as statistically significant difference. Values of observed parameters, which are presented in tables, are mean values obtained by calculation from six samples of meat.

Results

Results of determination of chemical composition of thigh muscles are shown in Table 2. Feeding of flaxseed, as a source of PUFAs caused increase in fat content in samples from experimental groups (MK and LMK). Statistically significant differences

were recorded between control and experimental groups ($P < 0.05$). Comparison of protein content revealed higher values in samples from group LMK than in control samples ($P < 0.05$); however protein content in samples from group MK and in control group was similar.

Table 2: Chemical composition of thigh muscles (percentage)

	Water (%)	Fat (%)	Proteins (%)
K	76.75 ± 1.29^{a}	3.54 ± 1.08^{a}	16.83 ± 0.23^{a}
LMK	76.73 ± 0.69^{a}	6.29 ± 1.19^{b}	17.28 ± 0.14^{b}
MK	76.43 ± 0.32^{a}	5.26 ± 0.63^{b}	16.98 ± 0.15^{a}
^{a,b} – values with different labelling in column are statistically different			

In diagram 1 results of TBARS determination in samples of muscles stored at chilling conditions (4 °C, 11 days) are shown. Immediately after the slaughtering, amount of malondialdehyde (main product of lipid oxidation) in thigh muscles (MK and LMK) increased rapidly. Statistically significant difference was present between experimental and control groups ($P < 0.05$) in the amount of MDA. Storage of samples (3 and 11 days) caused increase in MDA in all groups, although the increase was higher in samples from experimental groups. Increase of MDA in control group was significantly lower ($P < 0.05$) in comparison to experimental groups, what reflects significantly lower oxidation stability of meat samples obtained from animals fed with flaxseed alone or in combination with probiotic bacteria (*Lactobacillus*).

The oxidative changes were increased also during the storage of meat samples at freezing conditions (Graph 2). In experimental groups MK and LMK level of lipid decomposition, expressed as amount of MDA present in muscles, was significantly higher in comparison to control group ($P < 0.05$) already 24 hours after slaughter, and thereafter during the whole storage period. However, there was no significant difference between groups MK and LMK. At the end of nine month of storage, amounts of MDA in groups MK and LMK were twice as high (2.28 and 2.99 $mg.kg^{-1}$) as in control group (1.08 $mg.kg^{-1}$).

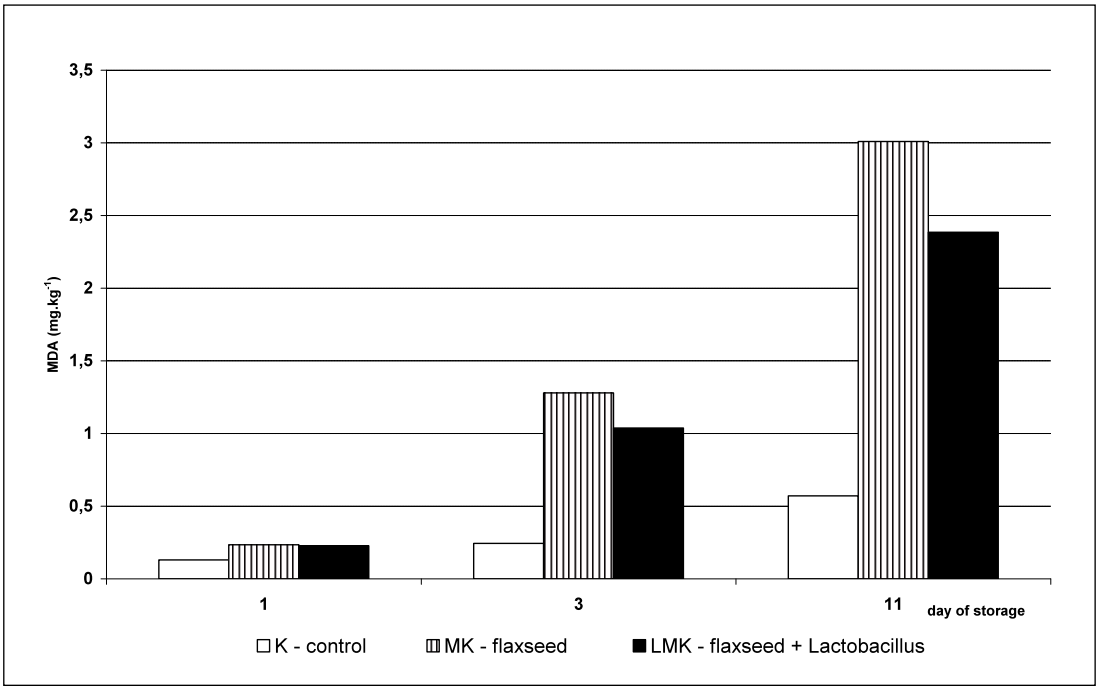

Diagram 1: Thiobarbituric acid reactive substances calculated as an amount of malondialdehyde in thigh muscles stored at chilling conditions (4 °C)

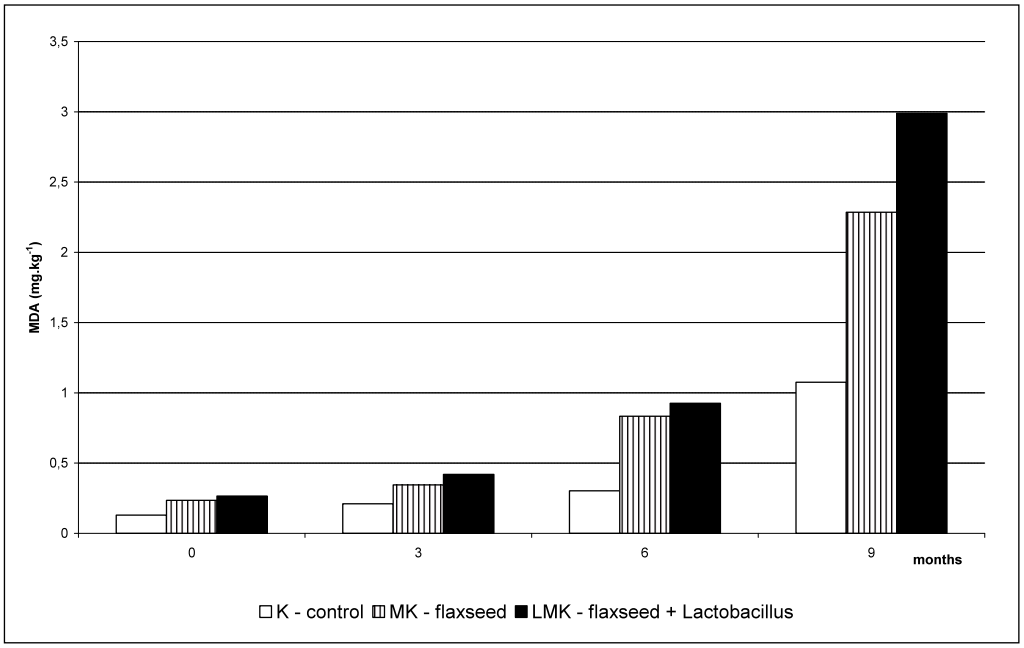

Diagram 2: Thiobarbituric acid reactive substances calculated as an amount of malondialdehyde in thigh muscles stored at freezing conditions (-21 °C)

Sensory evaluation of samples of thigh muscles was performed by professional evaluation committee. Taste, odour, succulence and texture of samples were evaluated. Immediately after slaughter, no statistically significant differences were recorded. However, control samples were evaluated as better in their sensory properties (table 3). Minimal differences were recorded between groups MK and LMK. In samples stored for 9 months at freezing conditions, higher final score, using 5 point schema, was given to samples from control group ($P < 0.05$). The most significant differences were in odour and taste of meat samples, which were considered negative properties in experimental groups.

Using triangle method, meat samples from experimental groups stored at freezing conditions were evaluated as significantly different in comparison control samples, and 28 samples from total 30 samples were correctly distinguished by a panel of 7 assessors (99.9 %). Once more, taste was the most significant resolution property in meat samples.

Table 3: Results of sensory evaluation using 5 point schema

	After slaughter	9 months freezing
K	18.4 ± 1.2^{a}	17.4 ± 2.1^{a}
MK	17.6 ± 0.9^{a}	15.1 ± 1.9^{b}
LMK	17.4 ± 1.4^{a}	14.3 ± 3.2^{b}
^{a,b} – values with different labelling in column are statistically different		

Discussion

Results of the present study showed that it is possible to raise a content of fat in pig muscle fairly quickly by adding flax into the diet of animals. It is generally known and confirmed by many studies, that feeding of flaxseed increased proportion of 18:3 n-3 and 20:5 n-3 PUFAs in fatty tissues and meat of animals (14, 15, 16). The fatty acid pattern in the diet substantially influenced the n-6/n-3 PUFAs ratio in meat (6). Kastel et al. (17) showed that elevated intake of n-3 PUFAs induced significantly higher levels of γ-linolenic, eicosapentaenoic and docosahexaenoic acids and reduction in arachidonic acid in blood serum of germ-free piglets.

Increased content of PUFAs in meat of pigs fed with flaxseed resulted in excessive production of lipid oxidation metabolites during storage of samples. Significant differences were recorded already 24 hours following slaughter processing. Storage of samples at chilling conditions (4 °C) for 11 days caused rapid growth of lipid decomposition processes. Rey et al. (16) showed that addition of 5 g of flaxseed oil per kg of feedstuff in pigs significantly increased lipid decomposition rate in muscles (*m. longissimus dorsi*) after 3, 6 and 9 days of storage when compared to control group without addition of flaxseed oil in the diet.

Time of storage in freezer influenced decomposition changes of fats in thigh muscles in all three

described positive effect of PUFAs on adhesion of probiotic strains in pig intestines. However, effect of probiotic bacteria on oxidative stability of PUFAs was not confirmed in our experiment. These results are in accordance with previously published data (2, 14, 16, 18), who stated that meat obtained from pigs fed diet fortified with flaxseed, had lower oxidative stability and higher concentration of TBARS during storage.

Sensory evaluation of pork, stored in a freezer, confirmed markedly worse final score of samples with higher content of PUFAs in the diet of pigs. These results are in agreement with Bryhni et al. (2) who stated that after 1 month of freezer storage meat and fat from pigs on the high PUFA diets showed more fishy and rancid odours and less meat odour than samples from pigs on the low PUFA diets.

In the present study, both sensory traits and TBA were affected by diets with PUFAs content. This is in agreement with Bryhni et al. (2) and Cameron and Enser (19), who showed that saturated and monounsaturated fatty acids were generally positively associated with the meat quality and stability, whereas PUFAs were correlated negatively. Although high PUFAs level might contribute to a healthier meat, it is important to be aware of reduced storage stability and problems connected with fat oxidation. Therefore, when plant oils with higher content of PUFAs are fed, it is advisable to use them together with appropriate amount of antioxidants.

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VPLIV HRANJENJA PRAŠIČEV Z LANENIMI SEMENI IN PROBIOTIKI NA KAKOVOST MESA IN OKSIDACIJO MAŠČOB MED SKLADIŠČENJEM MESA

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Povzetek: V poskusu smo proučevali oksidacijo maščob in senzorične lastnosti prašičjega mesa stegenskih mišic, ki je bilo skladiščeno bodisi v zamrznjenem stanju (-21 °C) ali ohlajeno (4 °C). Meso smo pridobili od prašičev, ki so bili hranjeni z lanenim semenom, mešano krmo, standardno krmo in standardno krmo z dodatkom probiotičnih bakterij. Oksidacijski proces smo spremljali s spremembami v vsebnosti malondialdehida (MDA) v stegenskih mišicah. Dodatek lanenega semena krmi je povečal vsebnost maščob v stegenskih mišicah in s tem tudi statistično značilno ($P < 0,05$) pospešil oksidacijske procese med hrambo mesa. Tudi senzorično ocenjevanje svinjine prašičev, ki so bili krmljeni z lanenim semenom, je pokazalo očitno razliko (v okusu in vonju) v primerjavi s kontrolno skupino. Ugotavljamo, da je pri krmljenju živali z rastlinsko hrano, ki vsebuje veliko večkrat nenasičenih maščobnih kislin (PUFA), smiselno dodajanje zadostne količine antioksidantov.

Ključne besede: laneno seme; oksidacija maščob; svinjina

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Original Research Paper

ERYTHROCYTE SURFACE SIALIC ACID DEPLETION AS PREDISPOSING FACTOR TO ERYTHROCYTE DESTRUCTION IN SHEEP EXPERIMENTAL MODEL OF AFRICAN TRYPANOSOMOSIS: A PRELIMINARY REPORT

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Summary: Changes in erythrocyte surface (ESSA) and free serum sialic (FSSA) profiles that could occur sequel to trypanosome infection with consequent destruction of red blood cells by mononuclear phagocytic system were investigated in an experiment in which 8 sheep were infected with *Trypanosoma congolense* (*T. congolense*), while six other sheep served as uninfected controls. The infection with *T. congolense* caused rapid decline in ESSA concentration and packed cell volume (PCV) in sheep. Concomitantly, rise in mean FSSA concentration was observed in the *T. congolense* infected sheep. Major periods of decline in ESSA concentration and greatest increase in FSSA concentration coincided with period of highest parasitaemia levels. The ESSA concentration in the infected sheep stabilized as the infection aged but the concentration of this parameter remained at comparatively lower levels than that in the control sheep and relative to the pre-infection value on day 0 of infection. This ESSA level in *T. congolense*-infected sheep was maintained with only minor fluctuations up to the termination of the experiment. PCV, ESSA and FSSA concentrations remained relatively unchanged in the control group throughout the course of the experiment that lasted for 53 days. The post-infection mean values of FSSA and ESSA in the *T. congolense*-infected and control sheep were 2.3±0.5mg/ml and1.7±0.9mg/ml, and 1.9±0.2mg/ml and 2.1±1.2mg/ml, respectively. The respective values of PCV, ESSA and FSSA concentrations in the infected sheep differed significantly ($P<0.05$) from those in the control sheep. Further investigations to elucidate the possible roles of sialyltransferase in the recovery of ESSA and, consequently, erythrocyte mass in trypanosome-infected animals are undoubtedly needed.

Key words: sialic acid; sialidase; trypanosomosis; *Trypanosoma congolense*; anaemia

Introduction

Anaemia is one of the principal features and, perhaps, a major cause of tissue pathology and death in the acute phase of trypanosomosis (trypanosomiasis) in livestock (1-8). Although varying reports on the mechanisms of its development in trypanosome-infected animals abound (9), the general consensus is that the anaemia in both human and animal trypanosomoses is predominantly the result of haemolytic crisis, in which the erythrocytes are being destroyed by cells of mononuclear phagocytic system (10,11). The role of trypanosomal sialidases is one of the most documented of such mechanisms (12-17). Sialidases hydrolyse the glycosidic linkage between sialic acids and the underlying sugars thereby cleaving off the sialic acids (neuraminic acids), which are found ubiquitously distributed on terminal positions of macromolecules and cell membranes in the body (18-20).

Occurrence of sialidases has been reported in *Trypanosoma vivax* (*T. vivax*), *T. congolense*, *T.*

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brucei, *T. rhodesiense, T. evansi, T. rangeli* and *T. cruzi* (12-17). These enzymes, when released by infecting trypanosomes, contribute to the development of anaemia by liberating the sialic acid on erythrocyte surface, an action that demasks galactose residues via which such desialylated erythrocytes bind to cells of the mononuclear phagocyte system through a specific receptor and are ultimately taken up and degraded (11,20-26).

A good understanding of pathophysiological mechanisms involved in the development of the anaemia in trypanosome-infected animals is pivotal to the identification of molecular targets that could be exploited biotechnologically to evolve the necessary panacea to the menacing effects of the infection in both humans and animals.

Sheep were reported to be the most suitable models in the study of erythropoiesis (27,28). We report in this paper some changes in the packed cell volume (PCV), erythrocyte surface (ESSA) and free serum sialic acid (FSSA) concentrations induced by experimental *T. congolense* infection of sheep.

Materials and methods

Experimental animals

Fifteen normal healthy sheep of the Yankasa breed with ages that ranged between 18 and 24 months were purchased from a livestock market within an area apparently free of tsetse, in Katsina State of northern Nigeria. The ages of these animals were confirmed using the dental eruption pattern described (29). Fourteen of these sheep were used in the experiment, while the remaining one served as donor animal. The animals were on arrival accommodated in a fly-proof experimental animal house and adequately fed on high plane of diet.

The sheep were also provided with water *ad-libitum*. Series of treatments were administered to the animals that include deworming using albendazole (Albendazole®, Pantex, Holland B.V.) at a dose rate of 5mg per kilogram body weight, antibiotic therapy with Oxytetracycline (Trodax®) at a dose rate of 20mg per kilogram body weight. The animals were also sprayed against external parasites using diazinon (Diazintol®, Animal Care, Nig. Ltd.) at a concentration of 2ml per litre of water (162mg/ml concentration). The individual animals were ear-tagged for proper identification. The sheep were allowed to acclimatize for a period of three months during which they were subjected to such routine handlings as collection of blood samples, which were used for parasite screening and establishment of baseline haematological values and determination of rectal temperature, each, twice a week. Prior to the commencement of the experiment, the sheep were certified to be free of trypanosomes based on weekly haematocrit (30).

Trypanosome Stock

The parasite *T. congolense* (NITRE-53624609) used in this experiment was donated by the Nigerian Institute for Trypanosomiasis Research, at Vom, Plateau, Nigeria. The trypanosome was isolated from a pregnant cow in Karu of Nasarawa State of Nigeria. The stabilate of this parasite was inoculated into two Sprague Dawley rats; one intraperitoneally and the other intradermally. The infected rats were immediately transported to Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. These rats were kept in separate cages and fed adequately with pelleted chicken feeds. Blood sample was collected from each of these rats everyday for determination of parasitaemia. When parasitaemia was at swarming degree in the rat that was inoculated intraperitoneally on day 6 post-inoculation, it was anaesthetised with chloroform and its jugular veins were then severed to collect sufficient blood. This blood, which was contained in a vacutainer and anticoagulated with heparin, was used to inoculate the donor sheep.

Detection of parasitaemia in the donor sheep

Beginning from day 1 post-infection, blood sample was collected from the donor sheep every day into vacutainer containing an anticoagulant, ethylenediaminetetraacetic acid (EDTA). This blood was used to detect the appearance of the parasites in the peripheral circulation in order to determine the prepatent period and parasitaemia level that would be sufficient to infect the experimental sheep using the modified method of Paris et al (31).

Animal allocation and infection with T. congolense

The parasites, *T. congolense*, were first detected in the blood of the donor sheep on day 11 post-infection. By day 15 post-infection, parasitaemia was

at its peak with a parasite count of $1x10^5$ trypanosomes per millilitre ($1x10^5$ tryps/ml) of blood. On this day of infection (tagged day 0 of infection), the experimental sheep were allocated to two groups. The first group comprised of 8 sheep and was infected with trypanosomes (and was therefore termed the infected group), while the second group, which consisted of 6

sheep served as the un-infected control group. These groups were closely matched on the basis of haematocrit index (infected group, 32.8±2.6%; control, 32.5±3.3%). On this day 0, each of the animals in the infected group was inoculated via the jugular vein with 2 ml of blood containing $1x10^6$ *T. congolense* organisms. Estimation of the number of trypanosomes was done using the modified method of Paris et al (31).

Haematological and parasitological analyses

Beginning from day 0 of infection and throughout the experimental period that lasted for 53 days, 0.5 ml of blood sample was collected every day from each of the animals in both the infected and control groups into a vacutainer containing EDTA as an anticoagulant. This blood was used for detection and estimation of parasitaemia level in the infected group using the modified method of Paris et al. (31) and estimation of packed cell volume using the standard microcapillary method.

Preparation of erythrocyte membranes (Ghosts) and determination of erythrocyte surface sialic acid (ESSA) concentration

Beginning from day 0 of infection and up to the termination of the experiment, 2 ml of blood was collected everyday from each of the animals in the two groups into a screw- capped test tube containing 0.3 ml of reconstituted acid citrate dextrose (an anticoagulant). This blood was used for the preparation of erythrocyte ghosts according to the method of Dodge *et al.* (32). ESSA concentration was determined using 50µl of the suspension of washed erythrocyte ghosts incubated with 100µl H_2SO_4 for 1hour at 80°C in order to

liberate bound sialic acid. Sialic acid concentration was subsequently measured in the mixture using thiobarbituric acid assay (TBA assay) (33,34).

Determination of free serum sialic acid (FSSA) concentration

Beginning from day 0 of infection and up to the end of the experiment, 1 ml of blood sample was collected everyday from each of the animals in the two groups into vacutainer without anticoagulant. The blood was allowed to clot at refrigeration temperature until serum became expressed. The serum was aspirated and dispensed into serum vials and stored at -20 °C until needed for the assay of sialic acid. FSSA concentration was determined by TBA assay (33,34).

Statistical analysis

The mean values of both ESSA and FSSA concentrations in the infected group were compared statistically with those in the control group using student's t-test (35).

Results

Parasitaemia

T. congolense organisms were first detected in the blood of some of the sheep in the infected group on day 8 post-infection. By day 11 post-infection, all sheep in the infected group were showing parasitaemia. Hence, mean prepatent period in this experimental infection was 9.5 ± 1.3 days. Parasitaemia was intermittent in individual animals of the infected group. Mean parasitaemia level rose on day 8 from $1.0x10^3$ trypanosomes per millilitre (tryps/ml) of blood (i.e. a 1+ parasitaemia score) to a peak value of $5x10^5$ tryps/ml (i.e. a 5+ parasitaemia score) on day 23 post-infection (Fig. 1). This was immediately followed by a slight drop in the mean parasitaemia level to values that ranged between 1.0×10^5 and 5.0×10^5 tryps /ml. This fluctuating parasitaemia level was maintained up to day 36 post-infection after which a sudden drop to a basal level of 1.0×10^3 tryps/ml was observed. This basal mean parasitaemia level was maintained, with only occasional spikes, until the termination of the experiment (Fig. 1). All the sheep in the control group remained aparasitaemic throughout the course of the experiment. Thus, the time interval days of 9 and 36 represents the major period of high parasitaemia levels.

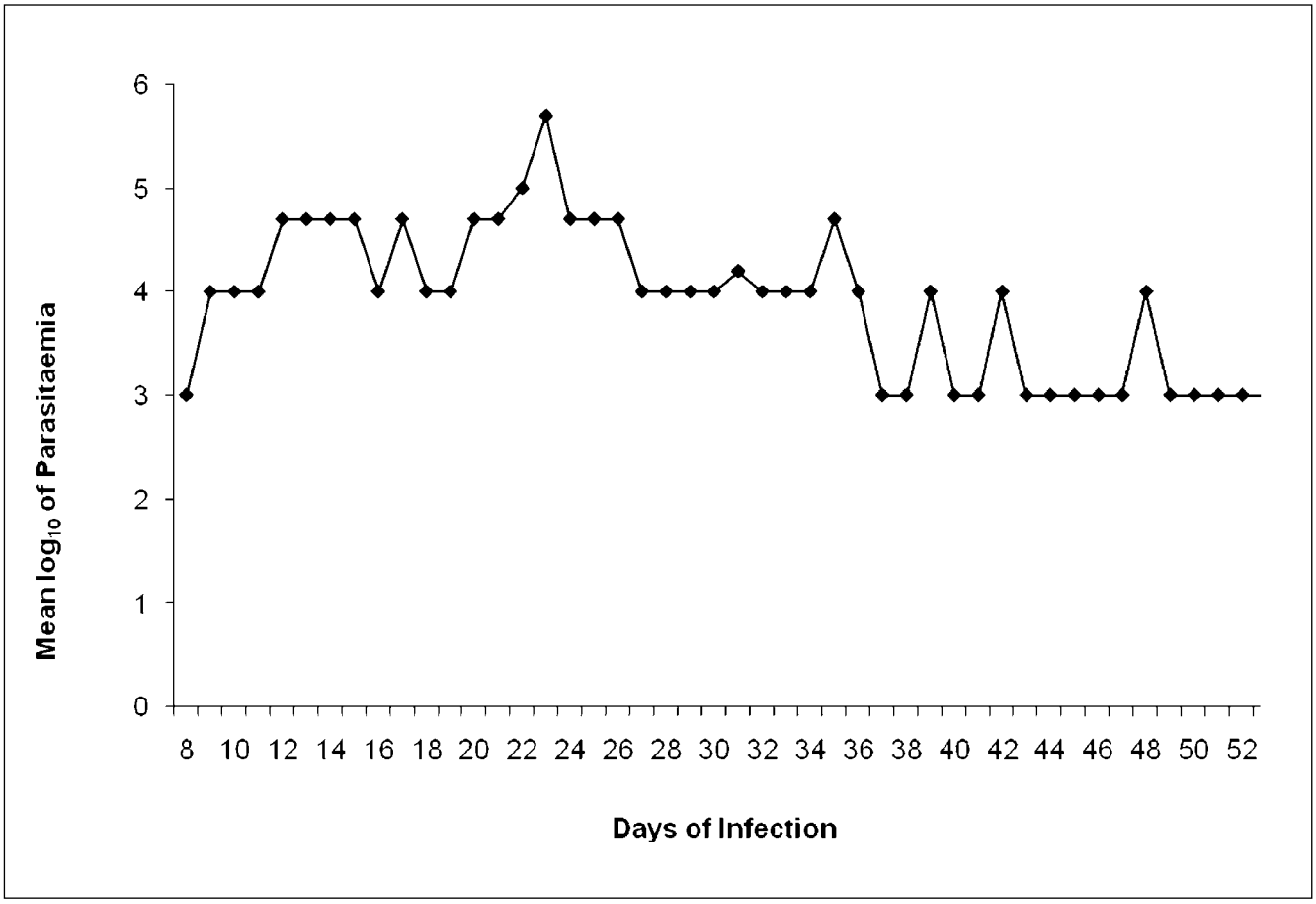

Figure 1: Mean $\log_{10}$ of Parasitaemia in *T. congolense*-infected sheep

Packed cell volume (PCV)

The mean pre-infection PCV in the *T. congolense*-infected and control sheep were 33.4 ± 3.3 and 33.5 ± 2.4 %, respectively. Following infection with the trypanosome, there was a gradual and progressive drop in the mean PCV value in the infected group until a minimum value of 16.7 ± 2.3 % was recorded on day 32 post-infection (Fig. 2). Thereafter, a staggering increase to a highest value of 21.3 % was observed on day 41 post-infection. The mean PCV then stabilized at values that fluctuated between 17 and 20 % up to the end of the experiment (Fig. 2). The post-infection mean PCV in the control group remained normal relative to the pre-infection one (Fig. 2). The difference between the post-infection mean PCV values in the *T. congolense*-infected and control groups (which were 23.9 ± 5.4 % and 31.5 ± 2.7 %, respectively) was significant ($P<0.05$).

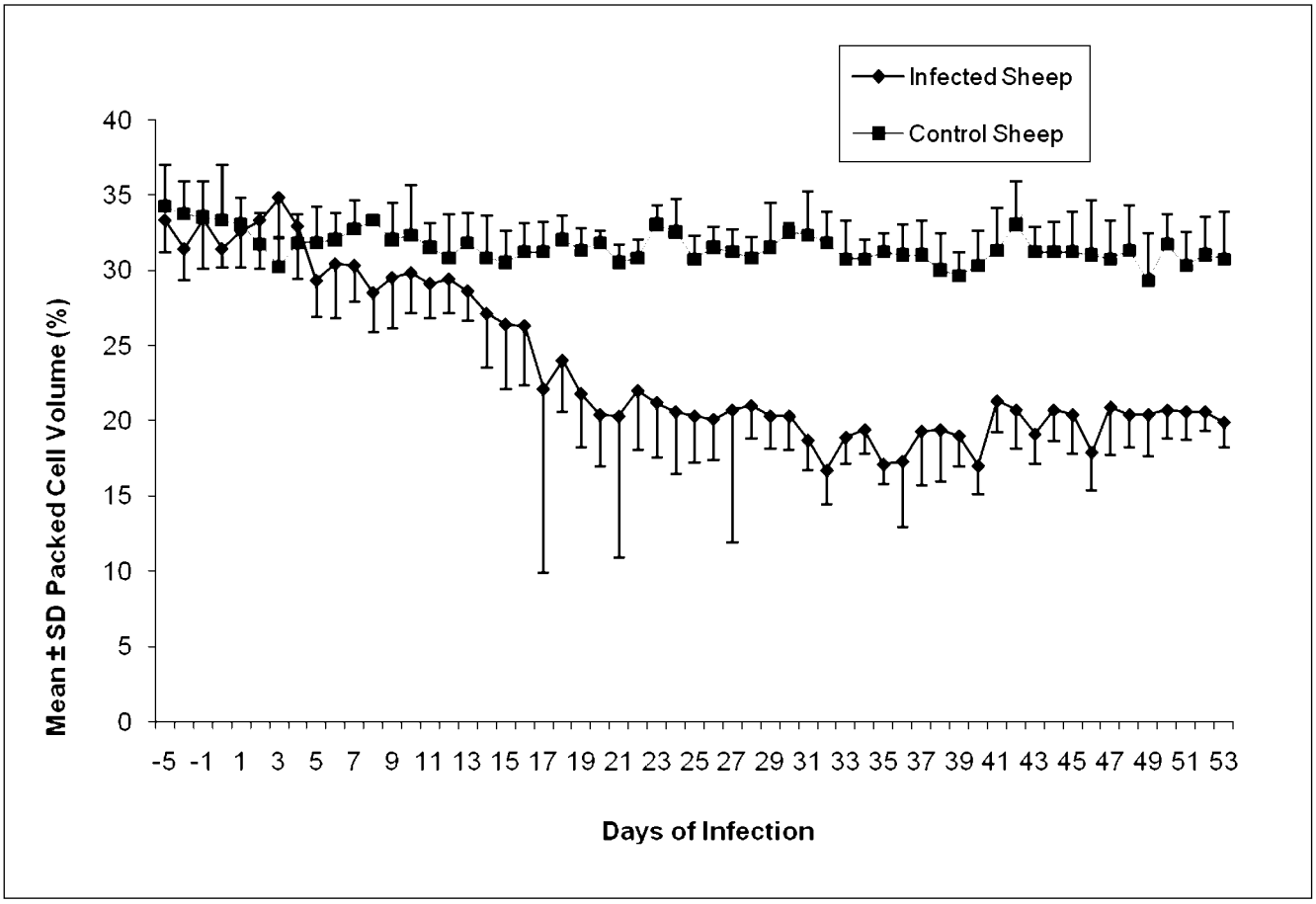

Figure 2: Mean packed cell volume in *T. congolense*-infected and control sheep

FSSA concentration

The pre-infection mean FSSA concentrations in the infected and control groups were 1.6 ± 0.7 mg/ml and 1.9 ± 1.0 mg/ml, respectively. Following infection with trypanosomes, a gradual rise in the value of this parameter was observed in the infected group beginning from day 5 of infection to reach a peak value of 4.0 ± 2.1 mg/ml on day 16 post-infection (Fig. 3). This peak value was then followed by a drop in the mean FSSA concentration to a level (1.9 ± 0.7 mg/ml) that was comparable with that in the control group on day 20 post-infection (Fig. 3). Thereafter, mean FSSA concentration stabilized with only some occasional surges up to the end of the experiment (Fig. 3). The mean FSSA concentration in the control sheep remained at the same level, with only minor fluctuations, relative to the pre-infection value on day 0 of infection. Mean FSSA level was highest between days 6 and 23 post-infection (Fig. 3). The mean post-infection FSSA concentration (2.3 ± 0.5 mg/ml) in the *T. congolense*-infected sheep was significantly higher ($P<0.05$) than that (1.9 ± 0.2 mg/ml) in the control sheep.

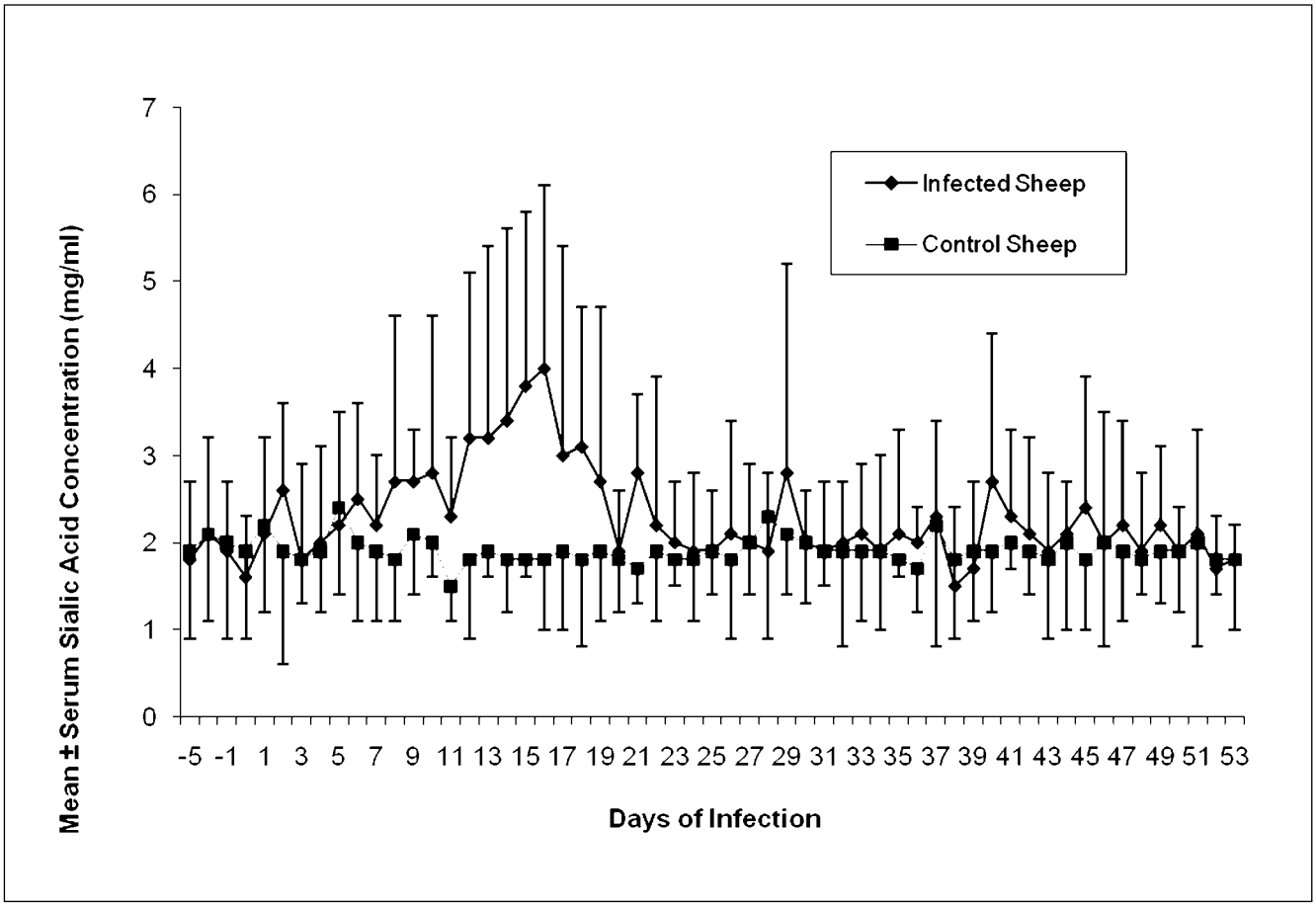

Figure 3: Mean serum sialic acid concentration in *T. congolense*-infected and control sheep

ESSA concentration

Mean ESSA concentration values in the *T. congolense*-infected and control sheep were comparable up to day 8 post infection. Following this period, a dramatic fall in ESSAconcentration was then observed in the infected group to reach the lowest level of 0.6±0.4 mg/ml on day 12 post-infection. Major decrease in ESSA concentration was observed between days 9 and 23 post-infection (Fig. 4). Thereafter, the value stabilized. Post- infection mean ESSA level was persistently lower than that in the control group up to day 53 when the experiment was terminated. The time interval between days 7 and 24 appears to be the period of greatest sialic acid loss from the erythrocyte surfaces (Fig. 4). Post-infection mean of ESSA level in the control sheep remained fairly unchanged during the course of the experiment (Fig. 4). Unlike with FSSA concentration, the post-infection mean ESSA concentration (1.7±0.9 mg/ml) in the infected group was significantly lower than that (2.1±1.2 mg/ml) in the control group.

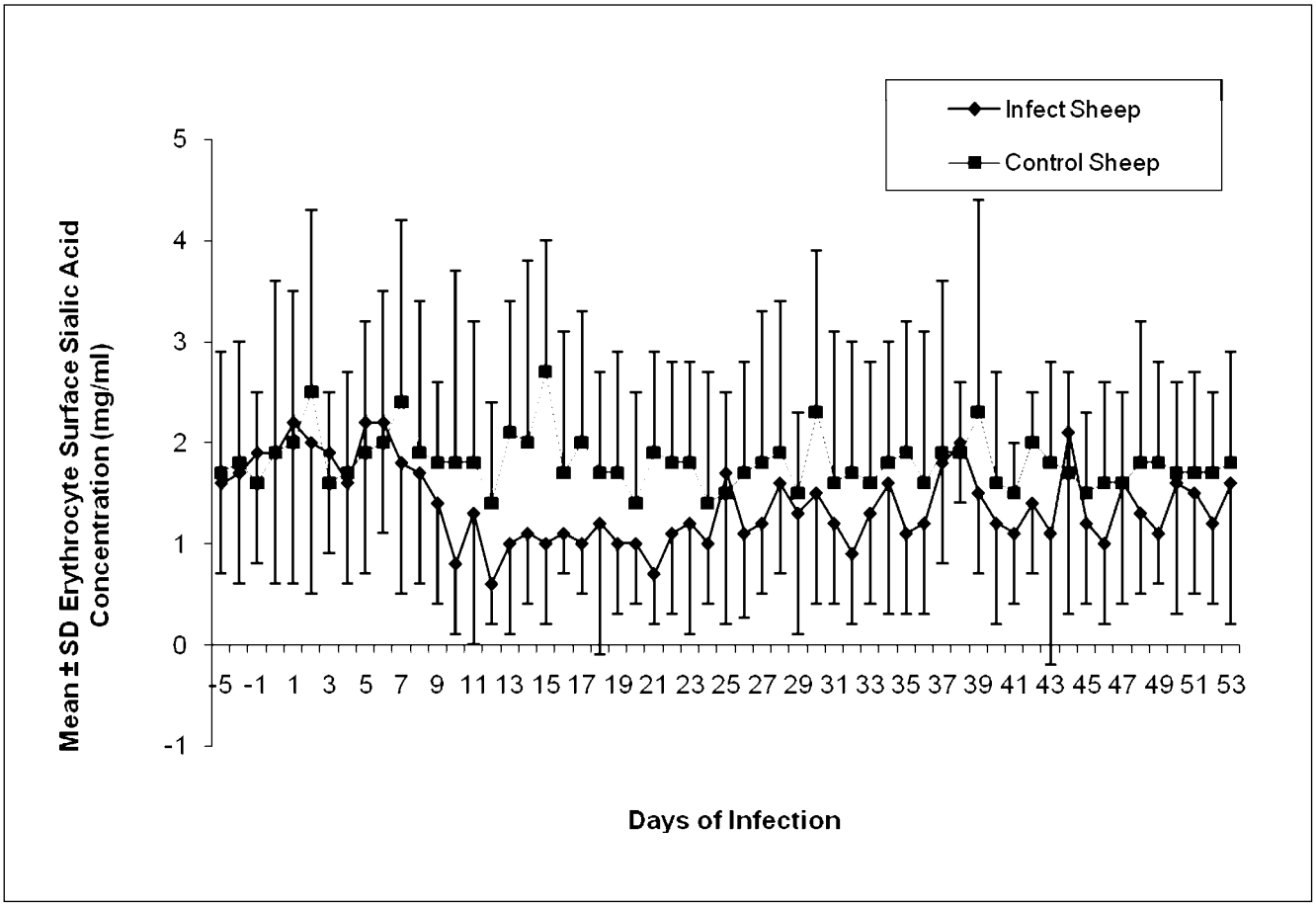

Figure 4: Mean erythrocyte surface sialic acid concentration in *T. congolense*-infected and control sheep

Discussion

The findings in this study re-affirm those in the previous reports (17,20,21). The significant reduction in ESSA concentration in the *T. congolense*-infected sheep, as observed in the present study, was most probably the result of elaboration and subsequent release of the sialic acid-hydrolyzing enzyme, sialidase, by the infecting trypanosomes (6,12-14,16,17,26). This is more so since bloodstream *T. congolense* sialidase was reported to play a contributory role in the development of anaemia in mice (6). The rise in the FSSA concentration, which accompanied reduction in ESSA concentration, suggests that erythrocytes were the major source of the FSSA in the infected sheep. The concurrence of decline in ESSA concentration with fall in packed cell volume, especially, in the major period of high parasitaemia levels as observed in the present study was earlier reported in *T. vivax*-infected cattle (Esievo et al., 1982), *T. evansi* sialidase hydrolysis of ghost red blood and brain cells (16) and *T. congolense*-infected mice (6) and is a strong basis on which trypanosomal sialidase could be implicated as being responsible for the reduction in ESSA and consequent decline in erythrocyte mass, which ultimately results in anaemia (6,20,21).

The pathophysiological mechanisms involved in the trypanosomal sialidase-induced destruction of red blood cells is that removal of sialic acid from the exposed epitopes of the erythrocytes causes some physicochemical alterations. These changes predispose them to bind to β-D-galactose-specific lectin on surfaces of macrophages and, consequently, resulting in the uptake and clearance of the desialylated erythrocytes (16,20,36). Indeed, the finding in numerous reports (24,26,37) that β-Glycosidically linked β-galactoside residues inhibited the rate-limiting binding step in the erythrophagocytic step is a strong evidence to aver that

anaemia development in trypanosome infected animals is consequential, at least in part, to loss of ESSA. This is because the terminal galactosyl residue of lactose enables it to bind to galactose-specific lectins of macrophages (6), thereby competitively inhibiting similar binding and subsequent destruction of desialylated erythrocytes. Thus, we may infer that anaemia developed in the *T. congolense*-infected sheep in the present study as a consequent effect of the parasite's sialidase-removal of sialic acid from the erythrocytes, which rendered them prone to erythrophagocytosis.

Even though ESSA concentration in the *T. congolense*-infected sheep stabilized at the later stages in the course of the infection as observed in this study, its failure to return to normality or levels that would be comparable with that in the control sheep would exacerbate the anaemic condition. This is because the red blood cells during this time would have reduced life span on account of their reduced ESSA (20,36). The perpetually reduced ESSA in the *T. congolense*-infected sheep may be attributable, among other factors, to the combined effect of trypanosomal sialidase, which continued to cleave off the sialic acid from the erythrocytes, and autoinduction of the activity of sialate-pyrvate-lyase (aldolase, EC 4.1.3.3). This latter enzyme, which is localized in the cytosol of mammalian cells, regulates the recycling of the sialic acid by hydrolyzing it to pyruvate and the corresponding acyl-mannosamine (20,38). Sialate-pyruvate-lyase occurs in bacteria but has never been reported in trypanosomes (20).

Although sialyltransferase activity was not investigated in this study, the return to normality in the FSSA concentration, following an initial rise, in the *T. congolense*-infected sheep may be the result of the activity of the enzyme, which depletes FSSA to resialylate cells, glycoconjugates and glycolipids (39-44). It may also be reasonable to attribute staggering increase and subsequent stabilization of PCV in the *T. congolense*-infected sheep to the activity of the sialyltransferase since resialylation of the erythrocytes would retard the rate of their binding to macrophages and therefore prolongs their lifespans. *T. congolense* infection of sheep resulted in depletion of ESSA with rapidly developing anaemia, thus, re-affirming the findings in previous reports (6,20,21) that the effect of trypanosome infection on ESSA may be a major mechanism of erythrocytes destruction. Further investigations to elucidate the possible roles of sialyltransferase in the recovery of ESSA and, consequently, erythrocyte mass in trypanosome-infected animals are undoubtedly needed. The revelations from previous, present and the recommended future studies could be exploited to biotechnologically develop an appropriate panacea to the detrimental effects of trypanosome infection in humans and animals.

Acknowledgement

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ZMANJŠANJE SIALIČNE KISLINE NA POVRŠINI ERITROCITOV KOT PREDISPONIRAJOČI DEJAVNIK ZA RAZPAD ERITROCITOV PRI OVCAH, POSKUSNO OKUŽENIH S TRIPANOSOMO: PRELIMINARNO POROČILO

Adamu S., Useh N. M., Ibrahim N. D. G., Nok A. J., Esievo K. A. N.

Povzetek: V poskusu, v katerega je bilo vključenih osem ovc, ki so bile okužene s *Trypanosomo congolense* (*T. congolense*), in šest ovc, ki so služile kot neokužena kontrola, smo ugotavljali spremembe površine eritrocitov (ESSA) in prostih serumskih sialnih profilov (FSSA), ki se lahko pojavijo po okužbi s tripanosomo, ter posledično uničenje rdečih krvniček, ki ga povzroči fagocitni sistem enojedrnih celic. Okužba s *T. congolense* je povzročila hitro znižanje koncentracije ESSA in hematokritske vrednosti pri ovcah. Poleg tega je bilo opaženo povečanje srednji vrednosti FSSA pri ovcah, inficiranih s *T. congolense*. Glavna obdobja, v katerih je prihajalo do znižanja vrednosti ESSA ter povišanja vrednosti FSSA, so sovpadala z obdobjem najvišjega nivoja parazitemije. Vrednost ESSA se je pri okuženih ovcah ustalila, ko je okužba zastarala, vendar pa je vrednost omenjenega parametra ostala na primerjalni ravni kot pri kontrolnih ovcah in sorazmerna z nivojem pred okužbo v začetku poskusa. Raven ESSA se je pri ovcah, okuženih s *T. congolense*, ohranila z manjšimi nestalnostmi do konca poskusa. Vrednosti hematokrita, ESSA in FSSA so ostale nespremenjene v kontrolni skupini med celotnim poskusom, ki je trajal 53 dni. Srednje vrednosti FSSA in ESSA po okužbi pri ovcah, okuženih s *T. congolense*, ter pri kontrolnih ovcah so bile 2.3 ± 0.5mg/ml in 1.7 ± 0.9mg/ml, ter 1.9 ± 0.2mg/ml in 1±1.2mg/ml. Posamezne vrednosti hematokrita, ESSA in FSSA so se značilno razlikovale med skupino okuženih ovc ter kontrolno skupino. Potrebne pa bodo še nadaljnje raziskave, ki bodo osvetlile možne vloge sialtransferaze v ponovnem normaliziranju ravni ESSA in posledično mase eritrocitov pri živalih, okuženih s tripanosomo.

Ključne besede: sialna kislina; sialidaza; tripanosomiaza; *Trypanosoma congolens*; anemija

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Original Research Paper

PORCINE RESPIRATORY DISEASE COMPLEX (PRDC) - A META-ANALYSIS AND SYSTEMATIC REVIEW OF THE EFFICACY OF ENROFLOXACIN

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Summary: Porcine respiratory disease complex (PRDC) is an economically significant disorder characterised by slow growth, decreased feed efficiency, lethargy, anorexia, fever, cough and dyspnea. A meta-analysis is a method of surveying and combining results of several independent clinical trials. Systematic reviews are concise summaries of the best available evidence that address sharply defined questions; they seek to assemble and examine all the high quality evidence on any given subject. This includes a comprehensive search of all potentially relevant articles and the use of explicit, reproducible criteria in the selection of articles for review. Enrofloxacin is a fluoroquinolone chemotherapeutic that was developed exclusively for use in veterinary medicine. The overall result of meta-analysis indicates a high efficacy of enrofloxacin ($P < 0.001$) in treating PRDC. The mortality rate was lower in the groups which used enrofloxacin ($P = 0.037$). Low resistance of all bacteria that cause respiratory tract infections was characterized from 0 to 7,6 %. The high efficacy of enrofloxacin in PRDC and its beneficial economic effect has been confirmed by meta-analysis and systematic review.

Key words: fluoroquinolones; meta-analysis; pigs; porcine respiratory disease complex

Introduction

The aim of the study is examining the effectivness of enrofloxacin in treatment of PRDC. Efficacy of treatment, influence on mortality and susceptibility of pathogens were the main criteria for assessment.

Porcine respiratory disease complex (PRDC) is a multi-factorial respiratory syndrome and is economically significant for pork producers throughout the world. PRDC is characterised clinically by slow growth, decreased feed efficiency, lethargy, anorexia, fever, cough and dyspnea and is common in pigs around 10 to 20 weeks of age. Because PRDC is not caused by a single organism the pathogens isolated from pigs vary between and within production units (1).

Most commonly porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), porcine circovirus type 2 (PCV2), pseudorabies virus (PRV), *Mycoplasma hyopneumoniae, Bordetella bronchiseptica, Actinobacillus pleuropneumoniae, Pasteurella multocida, Streptococcus suis* and *Haemophilus parasuis* are evidenced in PRDC incidences (2). Beside pathogens also environmental factors, new technologies (early weaning, multisite and age segregated systems), tendency to increase the size of finishing units and their numbers of sourcing farms, lower weaning age are contributors for PRDC (3).

A number of tests can be used to determine when these infections are occurring: post-mortem examination with organism identification, serological profiling, polymerase chain reaction (PCR) (4).

Control of PRDC is first based on the proper management of production imports such as the environment, nutrition, biosecurity and husbandry procedures (4).

In addition is recommended other management strategies that help to limit the impact of MH what include a balanced and stable sow herd with fewer than 30% replacement gilts, closing the herd or minimizing the number of sources to procure pigs, mul-

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tisite production, biosecurity to prevent the spread and introduction of disease, reduction of stress on pigs, optimal stocking density, and adequate ventilation, air quality and room temperature (5).

Antibiotic therapy is often used to control PRDC. Feed, water and injectable medications are available. Treatment programs to eliminate infection within a group of pigs or pulse medication are procedures that is reducing clinical disease (4).

The successful use of combination therapies with antibiotics has also been reported (6).

Vaccination is a common control method for PRDC control (4). The economic benefit of MH vaccination has been demonstrated (7).

Vaccination programs for PCV 2, PRRS, SIV and PRV are implemented in countries where vaccines are available.

Once we understood what is circulating, then control programs can be in place (vaccination, medication) to try to avoid clinical outbreak.

A meta-analysis is a method of surveying and combining results of several independent clinical trials. The term meta-analysis was first used by Glass in 1976, who, with his associate Mary Lee Smith, statistically compiled the results of 375 studies dealing with the efficacy of psychotherapy.

The most frequently used definition is the one by Huque: meta-analysis refers to a statistical analysis that combines or integrates the results of several independent clinical trials considered by the analyst to be combinable (8). Besides quantitative integration, a meta-analysis vastly improves the potential for uncovering and studying any differences in the available scientific material and provides a basis for plausible explanations of them. It can also lead to new discoveries.

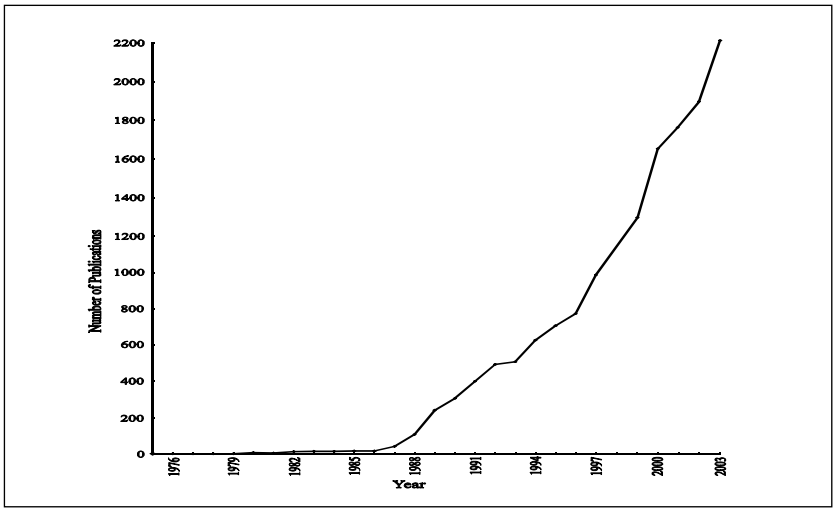
A brief summary of the procedures involved in a meta-analysis would comprise the following logical steps:

1. the definition of the problem and the inclusion criteria for the studies;
2. positioning, classifying and coding the characteristics of individual studies and the quantitative measurement of their characteristics;
3. the integration of the results and a comparison with the characteristics of the studies (analysis and explication of results); and
4. reporting of the results (9).

Systematic reviews are concise summaries of the best available evidence that address sharply defined questions; they seek to assemble and examine all the high quality evidence on any given subject. This includes a comprehensive search of all potentially relevant articles and the use of explicit, reproducible criteria in the selection of articles for review. When the results of primary studies are summarized but not statistically combined, the review may be called a qualitative systematic review. A quantitative systematic review, or meta-analysis, is a systematic review that uses statistical methods to combine the results of two or more studies (10).

This analytical method is of particular importance in the assessment of therapeutic efficacy when individual studies do not provide an overview over all studies on a topic. As their samples are too small, individual studies cannot provide a quantitative evaluation of the effect of treatment, nor can they test null hypothesis. Prior to meta-analysis, the traditional method was a narrative discourse on previous findings, which, however, could be misleading and subjective.

In the past few years, meta-analysis has been increasingly used in all fields of science. This is particularly evident for the medical science, as shown in graph 1.



Graph 1: Number of publications 1975-2003 that used meta-analysis (modificated after Petitti (11))

Investigating the efficacy of certain drugs can be done in several ways: by *in vitro* susceptibility profiling of certain bacteria, by clinical trials or by using meta-analyses or systematic reviews.

Enrofloxacin is a fluoroquinolone chemotherapeutic that was developed exclusively for use in veterinary medicine. Shortly after its administration, extremely low concentrations have a bactericidal effect against most Gram-negative and Gram-positive bacteria and will also act against mycoplasmata. It acts against both bacteria in the multiplication phase and dormant microorganisms. It is effective in the presence of oxygen and, owing to this phenomenon, it does not damage beneficial anaerobic intestinal microflora.

The efficacy of fluoroquinolones is related to both the maximum concentration and the time above their MIC value. *In vitro* pharmacokinetic models have shown that maximum concentrations of active substances, 8 times in excess of their MIC, have been able to reduce the number of bacteria by up to 99 % and inhibit their growth for up to 24 hours. The intensity of exposure may be quantified as the ratio between the area under the time-concentration curve (AUC) and the minimum inhibitory concentrations for the causative pathogens (MIC); a short term for this ratio is AIUC – area under the inhibitory plasma concentration curve. For example, if in an enrofloxacin therapy the AIUC is higher than 125, the probability of a clinical and microbiological cure is above 80 %, otherwise it is only 42 % or 26 % in respect of a microbiological cure. Resistance to fluoroquinolones is also reduced to a minimum if these parameters are taken into consideration – C_{max}/MIC ratio is at least 8-10 and AUC/MIC at least 100-125 (12, 13).

Material and methods

Table 1: Short survey of studies included in meta-analysis and systematic review

Author, year of publication, number of animals included	**Meta-analysis and systematic review parameters**
	Comparative control group
1. Altrock 1998 (14)	- lesions - incidence of bacteria - *in vitro* susceptibility testing of *P. multocida, B. bronchiseptica, A. pleuropneumoniae, H. parasuis, B-haemolytical streptococci*
	- 23 antimicrobial agents (*in vitro* susceptibility)
2. Köfer et al. 1992 (15)	- lesions - incidence of bacteria - *in vitro susceptibility* testing of *P. multocida, B. bronchiseptica, A. pleuropneumoniae, H. parasuis, P. haemolytica, Streptococcus* spp.
	- 8 antimicrobial agents (*in vitro* susceptibility)
3. Rose et al. 1996 (16); n = 551	- clinical efficacy - mortality
	- comparative control group (cefquinome, amoxicillin)
4. Kobish et al. 1990 (17); n = 69	- clinical efficacy - mortality
	- negative control group - positive control group
5. Awad-Masalmeh and Schuh 1990 (18); n = 129	- clinical efficacy - *in vitro* susceptibility testing of *P. multocida, B. bronchiseptica, A. pleuropneumoniae, M. hyopnemoniae* - economic parameters
	- autogenous vaccines - 4 antimicrobial agents (*in vitro* susceptibility)

6. Ganter et al. 1995 (19); n = 64	- *in vitro* susceptibility testing of *P. multocida, B. bronchiseptica, A. pleuropneumoniae, H. parasuis*, S. suis 2
	- 10 antimicrobial agents (*in vitro* susceptibility)
7. Pommier et al. 1998 (20); n = 326	- clinical efficacy - economic parameters
	- comparative control group (spiramycin)
8. Flaßhoff 1996 (21); n = 40	- susceptibility testing of α-hemolytic streptococci, *P. multocida, B. bronchiseptica*
	- 8 antimicrobial agents
9. Wu et al. 1997 (22)	- *in vitro* susceptibility testing of *Mycoplasma hyopneuminiae* and *M. hyosynoviae*
	- 11 antimicrobial agents
10. Hannan et al. 1997 (23)	- *in vitro* susceptibility testing of *Mycoplasma hyopneuminiae*
	- 2 antimicrobial agents
11. Kobayashi 1996 (24); n = 92	- *in vitro* susceptibility testing of *Mycoplasma hyosynoviae* and *M. hyorhinis*
	12 antimicrobial agents
12. Herrerias et al. 1995 (25); n = 150	- clinical efficacy
	- control groups (norfloxacin, trimetoprim-sulfametoksazol)
13. Asawa et al. 1995 (26)	- *in vitro* susceptibility testing of *A. pleuropneumoniae*
	19 antimicrobial agents
14. Dom et al. 1994 (27)	- *in vitro* susceptibility testing of *A. pleuropneumoniae*
	9 antimicrobial agents
15. Yeh and Park 1996 (28)	- *in vitro* susceptibility testing of *A. pleuropneumoniae*
16. Salmon et al. 1995 (29)	- *in vitro* susceptibility testing of *A. Pleuropneumoniae, P. multocida, S. typhimurium, S. cholerae-suis, E. coli*, S. suis, β- hemolytic streptococci
	- 8 antimicrobial agents
17. Hornedo et al. 1988 (30); n = 6	- clinical efficacy
	- negative control (untreated animal)
18. Gutierrez et al. 1993 (31)	- *in vitro* susceptibility testing of *A. pleuropneumoniae*
	- 41 antimicrobial agents
19. Wallgren et al. 1998 (32); n = 64 Parenteral aplication	- clinical efficacy - lesions - re-isolation of *A. pleuropneumoniae* - economic parameters
	- control (uninfected animals, infected untreated animals) - treatment (penicillin, danofloxacin, ceftiofur, tiamulin)
20. Wallgren et al. 1998 (33); n = 112 Peroral aplication	- clinical efficacy - lesions - re-isolation of *A. pleuropneumoniae* - economic parameters
	- control (uninfected animals, infected untreated animals) - treatment (, penicillin, chlortetracycline, florfenicol, tilmicosin, tiamulin)

21. Pijpers et al. 1998 (34); n = 21	- clinical efficacy - lesions - re-isolation of *A. pleuropneumoniae* - economic parameters
	- inoculation with *A. pleuropneumoniae* - negative control (untreated animal) - treatment (oxytetracycline)
22. Chung and Yeh 1993 (35); n = 25	- lesions - economic parameters
	- negative control - treatment (ceftiofur, oxytetracycline)
23. Gutierrez Martin and Rodriguez Ferri 1993 (36)	- *in vitro* susceptibility testing of *Pasteurella multocida* subspecies *multocida*
	- 41 antimicrobial agents
24. Ikoma et al. 1994 (37); n = 155	- clinical signs
	- comparative treatment (danofloxacin)
25. Heinen et al. 1998 (38); n = 8	- pharmacokinetic study and *in vitro* susceptibility (*P. multocida, A. pleuropneumoniae, M. hyopneumoniae*)
26. Stipkovits et al. 1994 (39)	- *in vitro* susceptibility testing of 7 bacteria
	- 3 combinations of antimicrobial agents
27. Kołodziejczyk et al. 1999 (40); n = 2065	- clinical efficency
	- *in vitro* susceptibility testing of 7 bacteria - negative control groups
28. Ganter and Amtsberg 1996 (41)	- *in vitro* susceptibility testing of *Pasteurella multocida* and *Streptoccocus suis*
	- 10 antimicrobial agents
29. Silva et al. 1999 (42); n = 84	- *in vitro* susceptibility testing of *P. multocida, A. pleuropneumoniae, B. bronchiseptica*
	- 7 antimicrobial agents
30. Smith et al. 1991 (43); n = 64	- clinical efficacy - lesions
	- infected control groups - uninfected control groups
31. Madsen and Larsen 1996 (44); n = 350	- treatment (attempt to eradicate pleuropneumonia and enzootic pneumonia) - treatment - control after treatment
32. Larsen et al. 1998 (45); n = 135	- treatment (attempt to eradicate pleuropneumonia and enzootic pneumonia) - lesions - clinical efficacy
	- vaccination against *A. pleuropneumoniae* - disinfection
33. Hofmo and Luim 1998 (46); n = 51	- treatment (attempt to eradicate pleuropneumonia and enzootic pneumonia) - serology
	- 2 antimicrobial agents (tiamulin, benzilpenicillin) - vaccination

34. Bada et al. 1995 (47)	- *in vitro* susceptibility testing of *A. pleuropneumoniae* - 10 antimicrobial agents
35. Habrun et al. 1997 (48)	- *in vitro* susceptibility testing of *A. pleuropneumoniae, P. multocida*
36. Friis and Szancer 1994 (49)	- *in vitro* susceptibility testing of *M. hyopneumoniae* and *M. hyosynovie* - 4 antimicrobial agents
37. Molnar 1992 (50)	- *in vitro* susceptibility testing of *A. pleuropneumoniae*
38. Scheer at al. 1996 (51)	- *in vitro* susceptibility testing of *A. pleuropneumoniae, P. multocida* - 8 antimicrobial agents
39. Flores et al. 1998 (52)	- *in vitro* susceptibility testing of *A. pleuropneumoniae* - 6 antimicrobial agents
40. Stephano et al. 1988 (53); n = 20	- treatment - clinical efficacy - positive control group - negative control group
41. Aarestrup et al. 2000 (54)	- *in vitro* susceptibility testing of *A. pleuropneumoniae*
42. Simon et al. 1990 (55); n = 60	- clinical efficacy - economic parameters - control group - tiamulin
43. Semjen et al. 1988 (56); n = 280	- clinical efficacy - economic parameters - 2 antimicrobial agents (tiamulin, tilozin)
44. Laak et al. 1991 (57)	- *in vitro* susceptibility testing of *M. hyopneumoniae, M. hyosynovie* and *M. flocculare* - 17 antimicrobial agents
45. Glaswisching et al. 1989 (58) n = 190	- clinical efficacy - untreated control group
46. Friis et al. 1994 (59)	- *in vitro* susceptibility testing of *M. hyopneumoniae* and *M. hyosynovie* - 4 antimicrobial agents
47. Chou et al. 1995 (60)	- *in vitro* susceptibility testing of *P. multocida* - 3 antimicrobial agents
48. Awad-Masalmeh et al. 1994 (61)	- *in vitro* susceptibility testing of *P. multocida* - 7 antimicrobial agents
49. Trolldenier 1996 (62)	- *in vitro* susceptibility testing of *P. multocida* and *M. haemolytica* - 14 antimicrobial agents
50. Bole-Hribovšek and Zdovc 2002 (63)	- *in vitro* susceptibility testing of *P. multocida*
51. Ikoma 1994 (64); n = 78	- clinical efficacy - lesions - control group - kanamicin

52. Udovičič et al. 1996 (65); n = 77	- treatment - clinical efficacy
	- vaccination
53. Werner-Tutschu et al. 1997 (66)	- *in vitro* susceptibility testing of *P. multocida* and *B. bronchiseptica*
	- 19 antimicrobial agents
54. Shin et al. 2004 (67)	- *in vitro* susceptibility testing of *A. pleuropneumoniae, P. multocida* and *B. bronchiseptica*
	- 8 antimicrobial agents
55. Aaerestrup and Jensen 1999 (68)	*In vitro* susceptibility testing of *A. pleuropneumoniae* (7 antimicrobial agents) – determination of MIC

It is evident from the table 1 that 55 studies were included in the survey (references from 14 – 67): 19 examined the usage of enrofloxacin in mixed respiratory infections, 14 studies of pleuropneumonia, 9 for enzootic pneumonia, 6 for pasteurellosis and 3 for atrophic rhinitis. There are 3 studies which dealt with pleuropneumonia and enzootic pneumonia.

Eleven clinical studies were included in two meta-analyses (efficacy and mortality), other studies provided a review of susceptibility status for PRDC pathogens to enrofloxacin and other comparable antibacterials. Mortality was reported in three studies only, however actual causes of deaths were not reported.

The total number of animals included in the meta-analysis (efficacy), was 3954, of which 1745 were from the enrofloxacin-treated groups of different studies and 2209 from their control or alternative treatment groups; and 668 (mortality), out of which 218 were from the enrofloxacin-treated groups and 450 from their control or alternative treatment groups.

A lot of studies examined the susceptibility profile of respiratory pathogens; all were comparative, except for two by Aaerestrup *et al.* (54). In total, there were 7866 strains of respiratory pathogens examined for their susceptibility to enrofloxacin. Some of the studies also dealt with the economic parameters and the pathoanatomical signs (e.g. lesions) in respiratory organs and their changes.

Evaluation method

The results of meta-analyses are presented graphically. The graphs show compiled data on authors of individual studies, the year of study publication or performance, a numerical comparison between the treated and the control group (shown as effect, expressed as odds ratios) and the mean values of effect size with 95% confidence intervals.

The odds ratio (OR) was used as a scale of magnitude for the effect size. Characteristic of OR is that a value of 1.0 means that a certain therapy has no effect; values below 1.0 indicate that the tested therapy (in our case the use of enrofloxacin) is better than that of the control or the comparator therapy group. Values above 1.0 indicate the advantage of the control or comparator therapy over the tested therapy. When in a graph the study presentation with its mean value and the two lines for the confidence interval does not intersect the value of 1.0, we speak about statistical significance at different levels, e.g. in 95-percent confidence intervals the P level of statistical significance equals 0.05; in 99-percent intervals it equals 0.01, etc.

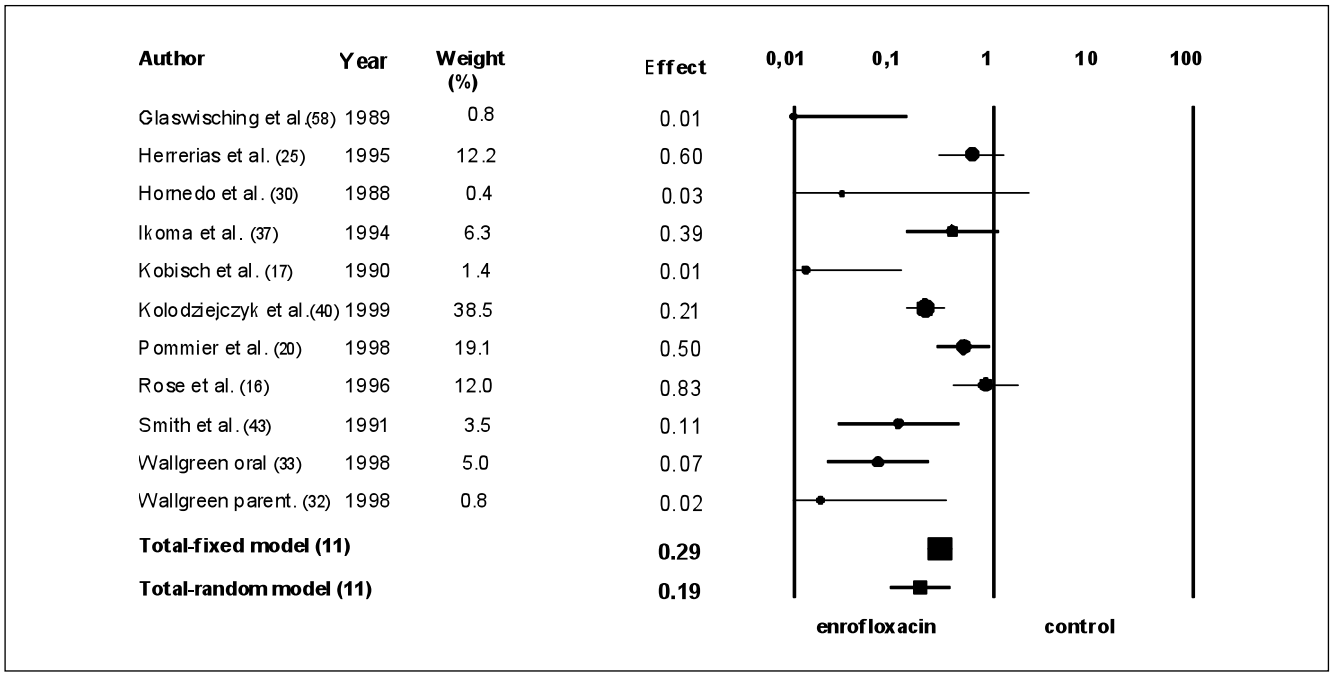
In cases, however, where mean values with confidence intervals intersect the 1.0 value line, we cannot speak about statistical significance.

The total effect size is always conditioned by weights of individual studies; therefore, in a meta-analysis we speak about a weighted total value of the effect size which, the same as for individual study is presented by mean value and confidence intervals. In the graph the weights for individual studies are shown as bigger or smaller full circles (●). This means that studies with smaller weights have smaller circles and those with larger weights have larger circles. The total effect size is shown as a full square (■). We also reviewed *in vitro* susceptibility of individual microbes to enrofloxacin as well to other antimicriobial substances and evaluated the MIC values.

The Comprehensive Meta-Analysis (69) computer programme was used for statistical analysis.

Results

Graf 2: Respiratory tract infections - efficacy of enrofloxacin treatment comparing with control group.

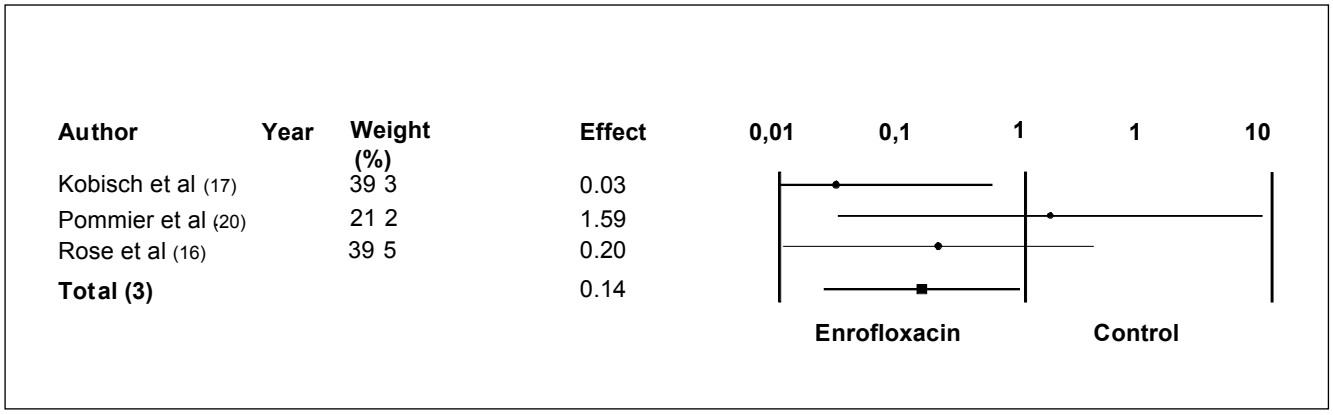


Homogeneity testing: Q = 43.1, d.f. (Q) = 10, P = 0.0003

Q.....heterogeneity value (χ^2 distribution);
d.f.....degrees of freedom ; P.... probability

It is evident from the graph that in the comparison of efficacy there is a statistically significant difference between the groups (P < 0.001).

Graph 3: Respiratory tract infections – comparison of mortality between enrofloxacin and control group. The mortality rate was lower in the groups which used enrofloxacin (P = 0.037) than in the comparative control groups



Homogeneity testing: Q = 2.62, d.f. (Q) = 2, P = 0.27

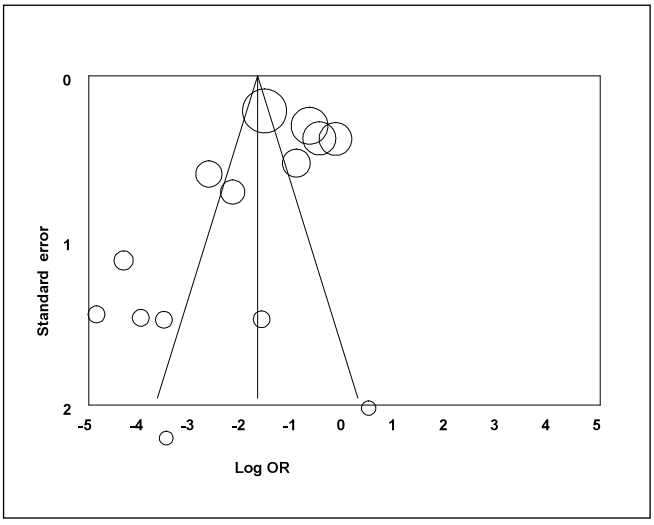
Table 2: In vitro susceptibility of respiratory pathogens to various antimicrobial substances (% of resistant strains) – summary of results from the reviewed articles

Antibacterial	*A. pleuropneumoniae*	*P. multocida*	*B. bronchiseptica*	*H. parasuis*
Gentamicin	6.4% (n = 235)	3.9% (n = 1142)	3.7% (n = 162)	9.5% (n = 42)
Trimethoprim/ sulfonamide	16.5% (n = 260)	19.9% (n =1141)	68.1% (n = 207)	30.9% (n = 42)
Enrofloxacin	2.3% (n = 2943)	2.8% (n = 2987)	7.6% (n = 904)	0.0% (n =124)
Kanamycin	3.3% (n = 87)	6.6% (n = 872)	7.0% (n = 106)	3.1% (n = 31)
Neomycin	10.3% (n = 223)	8.5% (n =1036)	3.4% (n = 148)	4.8% (n = 42)
Ampicillin	14.0% (n = 205)	15.1% (n = 1408)	57.7% (n =153)	23.3% (n = 30)

5.4% of all strains of *M. haemolytica* (n = 908) were resistant to enrofloxacin.

Table 3: Minimal inhibitory concentrations (MICs) for respiratory pathogens (enrofloxacin)

Bacteria	**MIC (µg/ml)**	**Bacteria**	**MIC (µg/ml)**
P. multocida	≤ 0.03-0.5	*M. haemolytica*	≤ 0,03-1.0
B. bronchiseptica	0.125-0.25	*A. pleuropneumoniae*	≤ 0,03-0.06



Graph 4: Funnel plot standard error by effect size (odds ratio – OR)

The funnel plot interprets dispersion of data. If heterogeneity is insignificant, data form a funnel with a wide opening of the funnel at the bottom of the graph and spire on the top. This way of presentation shows good agreement with the calculation of heterogeneity and funnel plot.

From funnel plot (graph 4.) it is evident that the studies included in the meta-analysis were heterogenous (P < 0.001).

Discussion

Graphs 2 and 3 demonstrate that enrofloxacin effectively treats respiratory tract infections in pigs (P < 0.001), and that in the enrofloxacin groups there were fewer deaths (P = 0.037).

In the study of efficacy and from graph 4 (funnel plot), we established that there was a heterogeneity of included studies, therefore, the total effect size was also calculated according to the random model.

The reasons for heterogeneity might probably be explained in terms of the larger number of included studies which dealt with different respiratory tract infections and were compiled in the joint meta-analysis, along with different sample sizes in individual studies. The susceptibility analysis, which excluded the four studies which stood out from the rest (17, 30; 32, 58,), demonstrated that these studies were not the cause of heterogeneity.

The causative pathogens are also characterized by a considerably low resistance of all bacteria that cause respiratory tract infections whereas the high efficacy of enrofloxacin is indicated by the low MICs of bacteria which are the causative pathogens of respiratory tract infections (Table 2. and 3.)

As it is so effective in treating PRDC, usage of enrofloxacin has benefits also regarding economic

parameters: eg. gain of animals, feed conversion ratio, mortality ect. in all reviewed trials where these parameters were examined.

The cause of vaccine failure is often unknown, and requires deeper understanding of the pig immune system, pathogenesis of the microbe and other potential factors (early weaning, multisite and age segregated systems, increased size of units and numbers of sourcing farms).The use of antimicrobials for control for PRDC remains still necessary in addition with other management strategies: balanced and stable sow herd, biosecurity to prevent the spread and introduction of disease, reduction of stress, optimal stocking density, ventilation, air quality etc.

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METAANALIZA IN SISTEMATIČNI PREGLED UČINKA ENROFLOKSACINA NA PRAŠIČJI RESPIRATORNI BOLEZENSKI SINDROM (PRDC)

Ščuka L., Golinar-Oven I., Valenčak Z.

Povzetek. Prašičji respiratorni bolezenski sindrom (angl.: porcine respiratory disease complex - PRDC) je ekonomsko pomembna motnja. Zanj je značilna večja smrtnost živali, manjši dnevni prirast, zmanjšan apetit, daljše pitanje živali, kašelj, težko dihanje, pljučnica. Metaanaliza je metoda pregledovanja in kombiniranja rezultatov, dobljenih v različnih neodvisnih kliničnih poskusih. Sistematični pregledi so kratki povzetki najboljših dosegljivih dognanj na natančno definirana vprašanja. Poskušajo zbrati in pregledati vse kakovostne podatke o obravnavani temi. To vključuje obširno iskanje vseh potencialno pomembnih člankov in uporabo jasno ponovljivih kriterijev pri izboru študij, uporabljenih za pregled. Enrofloksacin je fluorokinolonski kemoterapevtik, ki so ga razvili izključno za rabo v veterinarski medicini. Celotni rezultati metaanalize kažejo na visoko učinkovitost enrofloksacina ($P < 0,001$) pri zdravljenju PRDC. Smrtnost je bila manjša v skupinah, zdravljenih z enrofloksacinom ($P = 0,037$). Odpornost proti vsem bakterijam, ki povzročajo okužbe dihal, je bila nizka (0 % - 7,6 %). Z metaanalizo in sistematičnim pregledom smo dokazali visoko učinkovitost enrofloksacina pri PRDC in njegovo ekonomsko korist.

Ključne besede: fluorokinoloni; metaanaliza; prašiči; prašičji respiratorni bolezenski kompleks

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Article in a journal or newspaper: Fuji J, Otsu K, Zorzato F, et al. Identification of mutation in porcine ryanodine receptor asociated with malignant hyperthermia. Science 1991; 253: 448-51.

Article in proceedings of a meeting or symposium: Schnoebelen CS, Louveau I, Bonneau M. Developmental pattern of GH receptor in pig skeletal muscle. In: the 6th Zavrnik memorial meeting. Lipica: Veterinary Faculty 1995: 83-6.

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