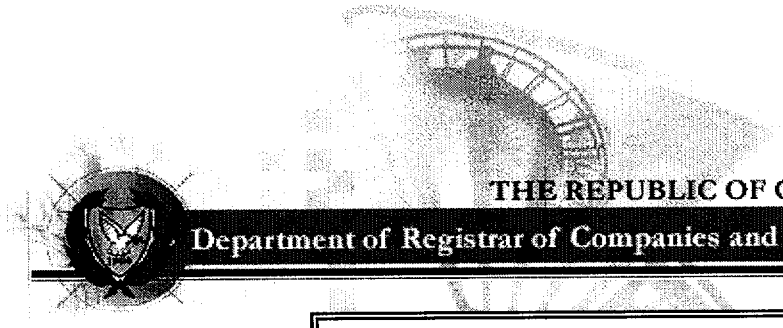

ΚΥΠΡΙΑΚΟ ΓΡΑΦΕΙΟ ΔΙΠΛΩΜΑΤΩΝ ΕΥΡΕΣΙΤΕΧΝΙΑΣ

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(54) VETERINARY COMPOSITIONS

(71) We, THE WELLCOME FOUNDATION LIMITED, of 183-193 Euston Road, London N.W.1 a company incorporated in England do hereby declare the invention for which we pray that a Patent may be granted to us and the method by which it is to be performed, to be particularly described in and by the following statement:

The present invention relates to compositions useful in veterinary medicine and more particularly in the treatment of bacterial and protozoal diseases in poultry.

Coccidiosis is a common disease in poultry caused by species of protozoal parasites of the genus *Eimeria*. The infection commonly occurs in poultry between the ages of two and fourteen weeks and can cause widespread losses if left unattended. The prevention and treatment of coccidiosis in poultry husbandry is therefore of extreme economic importance.

One medicament often used in therapeutic preparations for treating coccidiosis is sulphaquinoxaline [N-(2-quinoxalyl)sulphanilamide], which is described in the textbook of Organic Medicine and Pharmaceutical Chemistry, 4th Edition, J.B. Lippincott Co., Philadelphia, but this is unfortunately toxic in large doses. It has been commonly found that by combining sulphonamides with a particular group of compounds the efficacy of the sulphonamide as an antibacterial agent is maintained whilst the dose required is reduced. In recent years several preparations for treating coccidiosis have contained sulphaquinoxaline in combination with diaveridine [2,4-diamino-5-(3,4-dimethoxybenzyl)pyrimidine], a sulphonamide potentiator. U.K. Patent Specifications Nos. 1 028 204 and 1 108 112 describe such preparations.

Trimethoprim [2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine] is on the other hand an antibacterial agent which has been commonly used in admixture with certain sulphonamides such as sulphathiazole, sulphamethoxazole, sulphadiazine, sulphadimidine and sulphadoxin in man and mammals. However, it has not previously been suggested that trimethoprim may be combined with sulphaquinoxaline for the treatment of coccidiosis in poultry.

It has now been found that preparations containing trimethoprim and sulphaquinoxaline provide an effective and improved treatment of coccidiosis including coccidiosis caused by sulphaquinoxaline resistant strains of protozoa of the genus *Eimeria*. This admixture is also unexpectedly efficaceous against bacterial infections including those caused by certain sulphonamide resistant strains of *Escherichia coli* and in the treatment of malaria in poultry. This is particularly surprising as it has been suggested (Lewis, Anderson and Lacey, J. Clin. Path., 1974, *27*, 87-91) that potentiation between sulphonamides and trimethoprim does not occur *in vitro* when the infection is sulphonamide resistant.

Accordingly the present invention provides a veterinary composition which comprises trimethoprim or a salt thereof in admixture with sulphaquinoxaline or a salt thereof, the weight ratio of trimethoprim present to sulphaquinoxaline present being from 1:1 to 1:4.

Conveniently trimethoprim may be present as a free base and sulphaquinoxaline as a salt with a veterinarily acceptable base.

It is also possible to use trimethoprim as an acid addition salt, e.g. as a salt formed with a veterinarily acceptable acid, together with the free sulphaquinoxaline, or have both active ingredients present in their free unsalted forms.

Preferably the weight ratio of trimethoprim to sulphaquinoxaline is about 1:3.

In a preferred aspect the present invention provides a veterinary composition which comprises trimethoprim in admixture with a veterinarily acceptable salt of sulphaquinox-

aline, the weight ratio of trimethoprim present to sulphaquinoxaline present as hereinbefore defined.

The veterinary compositions of the present invention are normally in either powder or liquid concentrate form. In the powders of the present invention it is preferred to use an alkali metal salt of sulphaquinoxaline, such as its sodium salt. In accordance with standard veterinary formulation practice, conventional water soluble excipients, such as lactose or sucrose, may be incorporated in the powders to improve their physical properties. Thus particularly suitable powders of this invention comprise 50 to 100% w/w, and preferably 60-80% w/w, of a mixture of trimethoprim with sulphaquinoxaline, preferably as an alkali metal salt, and 0-50% w/w, and preferably 20-40% w/w, of conventional veterinary excipients, the ratio of trimethoprim present to sulphaquinoxaline present being as hereinbefore defined.

These powders may either be added to animal feedstuffs, for example by way of an intermediate premix, or diluted in animal drinking water. It has been found that dilution of a powder of this invention in water to give about 133 ppm of trimethoprim and sulphaquinoxaline as active ingredients is particularly efficacious for poultry. Thus 20g of powder containing 70% w/w of a mixture of trimethoprim with sodium sulphaquinoxaline may be added to 100 litres of drinking water to provide an effective treatment for poultry.

Liquid concentrates of this invention suitably contain trimethoprim and a water soluble veterinarily acceptable salt of sulphaquinoxaline formed from mixing a solution of sulphaquinoxaline with a water soluble base and in particular an organic base such as ethanolamine or diethanolamine in a veterinarily acceptable water miscible solvent. Suitable solvents include polyethylene glycol, propylene glycol, glycerol, glycerol formal or such a solvent mixed with up to 30% v/v of ethanol. It has been found that polyethylene glycol is a particularly convenient solvent for use in the liquid concentrates of this invention. Suitably a salt of sulphaquinoxaline is formed *in situ* by having an organic base present with sulphaquinoxaline in the liquid concentrate.

Thus in a further preferred aspect the present invention provides a liquid concentrate which comprises 10-50% w/v and preferably 24% w/v, of a mixture of trimethoprim with a salt of sulphaquinoxaline formed from a veterinarily acceptable water soluble organic base, in a solvent selected from glycol, propylene glycol, glycerol and glycerol formal, the weight ratio of trimethoprim to sulphaquinoxaline being as hereinbefore defined.

The liquid concentrates of this invention will be administered to the drinking water of animals, particularly poultry. They are particularly suitable for automatic addition to the drinking water of poultry by using water proportioners. The veterinary compositions of the present invention are particularly suitable for the treatment of coccidiosis in chickens.

In another aspect the present invention provides a method of treatment of bacterial diseases in poultry which comprises the oral administration of 1 to 100 mg/kg of a veterinary composition of the present invention as hereinbefore described.

Suitably 5 to 70 mg/kg, and preferably 20 to 40 mg/kg of a composition of the present invention is orally administered daily by providing the composition in the feedstuff or drinking water of poultry in the treatment of bacterial diseases.

In a further aspect the present invention provides a method of treatment of protozoal diseases, in particular coccidiosis and malaria, and bacterial infections in birds such as poultry which comprises the oral daily administration of the above mentioned dosages of a veterinary composition as hereinbefore described.

The present invention also provides a method of treatment of malaria in poultry which comprises the oral daily administration of the abovementioned dosages of a veterinary composition as hereinbefore described.

The following examples demonstrate the veterinary efficacy of the formulations and their preparation. The percentage, weight to volumn concentrations referred to herein are in g/100 ml.

Antibacterial and antiprotozoal activity of trimethoprim/sulphaquinoxaline formulations

Chicks which had been infected by sulphonamide resistant *E. coli* strain 18EC were treated with a trimethoprim/sulphaquinoxaline formulation at different dose levels, (*E. coli strain* 18EC induces a syndrome clearly resembling "Coli-septicaemia" which is a serious commonly occuring disease of broilers; see Piercy and West, J. Comp. Path., (1976) *86* 203). Similarly infected chicks were also treated with a trimethoprim formulation and the two commercially used formulations, sulphachloropyridazine and oxytetracycline, for comparison purposes.

The chicks were infected and weighed when 18 days old. Seven days after infection the birds were killed, weighed again and a post-mortem examination carried out. All the chicks, apart from the uninfected and infected untreated controls were offered medicated drinking water *ad lib* from 24 hours before infection with no further access to unmedicated

water until the treatment ended. The drum levels and treatment periods are shown in Table 1 which gives the observations recorded at post-mortem examination. The extent of lesions observed at post-mortem examination was recorded on a scale of 0 to 4 on the following basis:

	Score
No. lesions	0
Inflammatory reaction in injected air sac	1
Inflammation of both air sacs	2
Severe bilaterial airsacculitis plus pericarditis	3
All above symptoms plus fibrinous peritonitis, or death	4

TABLE 1

Group	Drinking Water Concentration (ug/ml)	Lesion Score 1	2	Distribution 3&4	7 day wt gain(g)	% of uninfected control wt gain	Mortality
1.	-	6	2	12	50.5	22.2	2/20
2.	TMP 33 SQ 100 (three days)	14	0	6	193.0	84.8	1/20
3.	TMP 33 SQ100 (five days)	16	1	3	187.5	82.4	1/20
4.	TMP 25 SQ75 (five days)	15	1	4	180.0	79.1	1/20
5.	TMP 33 (five days)	4	0	16	74.5	32.7	5/20
6.	1300 (five days)	12	0	8	176.0	77.3	0/20
7.	265 (five days)	7	1	12	91.0	40.0	3/20
8.	-	9	1	12	111.0	48.8	2/22
9.	-	Uninfected			227.5	100	0/22

Groups 1 and 8 were infected untreated controls.
Group 9 was an uninfected control.
Groups 2,3 and 4 were treated with a 24% solution of a 1:3 trimethoprim/sulphaquinoxaline mixture in polyethylene glycol suitably diluted in water.
Group 5 was treated with an 8% trimethoprim suspension suitably diluted in water.
Group 6 was treated with the sodium salt of sulphachloropyrdazine suitably diluted in water.
Group 7 was treated with a 50 mg/ml oxytetracycline solution (Terramycin injectable solution Pfizer) suitably diluted in water.

Groups of five, one week old Ranger cockerels were each infected orally with a mixture of 50,000 sporulated oocysts of the Ongar strain of *E. acervulina* and 50,000 oocysts of the Weybridge strain of *E. maxima* and 50,000 oocysts of the Weybridge strain of *E. brunetti*. This produced 72% mortality in unmedicated chicks. The chicks were treated with a 3:1 Sulphaquinoxaline/trimethoprim formulation in drinking water (final concentration 132 ppm). Five day treatments were begun on the day of infection and one and two days afterwards. Infected untreated and uninfected untreated groups were included as controls the latter receiving a water innoculation. Table 2 shows the weight gains of the chicks after the periods from 0-4, 4-7, 7-14 and 0-14 days (day 0 = day of infection). The treatment controlled both mortality and haemorrhage/diarrhoea completely.

TABLE 2

Percentage weight gain of chicks during and after treatment with a 3:1 Sulphaquinoxaline/trimethoprim formulation

Treatment	Wt. gains/chick(g)* days 0-4	4-7	7-14	0-14	Wt. gain 0-14 as % control
Unmedicated uninfected	35.5[a]	33.1[a]	97.3[a]	165.9[a]	100.0
Unmedicated infected	17.6[b]	5.2[b]	75.7[b]	98.5[b]	59.4
TMP/SQ days 0 to 5	32.1[a]	31.0[a]	85.6[b]	148.7[c]	89.6
TMP/SQ days 1 to 6	33.2[a]	29.9[a]	84.0[b]	147.1[c]	88.7
TMP/SQ days 2 to 7	32.4[a]	28.5[a]	87.6[a]	148.5[c]	89.5

*In each column, figures sharing the same superscripts are not statistically different at the 5% level.

Groups of 5, one week old Ranger cockerels were infected orally with 50,000 sporulated oocysts of the Weybridge strain of *Ei. tenella*. This produced 73.5 percent mortality in unmedicated chicks. The chicks were treated with 4:1 Sulphaquinoxaline diaveridine solution (final concentration 96 ppm) and with a 3:1 Sulphaquinoxaline/trimethoprim solution (final concentration 133 ppm). Treatments were begun on the day of infection and lasted for 3 and 4 days. The details of mortality control are shown in Table 3. The Sulphaquinoxaline/diaveridine solution gave 95% protection against mortality after a three day treatment and 100% protection for four day treatment whilst Sulphaquinoxaline/trimethoprim formulations gave 100% protection for both the three and four day treatments.

TABLE 3

Percentage protection against mortality due to Ei tenella by treatment with a sulphaquinoxaline/diaveridine formulation and Sulphaquinoxaline/trimethoprim formulation (the percentages are based on the results obtained from 50 chicks).

Treatment	Regime (days)* 0-3	0-4
Sulphaquinoxaline/diaveridine : 4:1	94.9	100.0
Sulphaquinoxaline/trimethoprim ; 3:1	100.0	100.0

Groups of five, one-week old Ranger cockerels were each infected orally with 250,000 sporulated oocysts of the sulphaquinoxaline resistant Dessord Mill Strain of *E. acervulina*. The chicks were treated with a 3:1 sulphaquinoxaline/trimethoprim formulation in drinking water (final concentration 132 ppm). Five day treatments were begun on the day of infection and one and two days afterwards. Infected untreated and uninfected untreated groups were included as controls, the latter receiving doses water by gavage as sham-infections. Table 3 shows the weight gains of the chicks after periods of 0-4, 4-7, 7-14 and 0-14 days (day 0 is the day of infection). Over the whole 14 days, all treated groups achieved weight gains significantly better than those of infected controls and about 97% over-all of the uninfected controls. The results are shown in Table 4.

TABLE 4

Weight gains per chick during and after treatment with TMP/SQ (33/99 p.p.m.) in the drinking water following infection with sulphaquinoxaline-resistant E. acervulina.

	Wt. gains/chick (g)* during days:- 0-4	4-7	7-14	0-14	Wt. gain 0-14 as % control
Unmedicated uninfected	24.8[a]	27.6[ac]	83.6[a]	136.1[a]	100.0
Unmedicated infected	20.3[b]	13.7[b]	75.2[b]	109.2[b]	80.2
TMP/SQ Days 0-5	28.4[c]	28.5[c]	82.3[ab]	139.2[a]	102.3
TMP/SQ Days 1-6	28.7[c]	22.7[d]	87.0[a]	138.4[a]	101.7
TMP/SQ Days 2-7	23.2[a]	25.0[ad]	78.1[b]	126.3[c]	92.8

*In each column, figures showing the same superscripts are not statistically significantly different at the 5% level.

EXAMPLES

Example 1

A 24% solution of a 3:1 sulphaquinoxaline/trimethoprim formulation was prepared having the following composition:

Trimethoprim	6g
Sulphaquinoxaline	18g
Monoethanolamine	4g
Polyethylene Glycol 200 to	100ml

The trimethoprim was dissolved in 70ml of the Polyethylene Glycol 200 heated to 70 - 80°C. The Monoethanolamine and sulphaquinoxaline were added and the mixture stirred until the contents had dissolved. The solution was cooled to room temperature and made up to volume with Polyethylene Glycol 200. The resulting solution was throughly mixed to ensure its homogeneity.

Example 2

A soluble powder containing a 3:1 formulation of sulphaquinoxaline/trimethoprim was prepared having the following composition:

Sulphaquinoxaline Sodium	10.73g
Trimethoprim	3.3 g
Sodium Lauryl Sulphate	0.1 g
Lactose	5.87g

The Lactose, trimethoprim and sulphaquinoxaline sodium were throughly mixed together in a mixer. The sodium lauryl sulphate was dissolved in a small quantity of water and this solution was used to granulate the powders. The resulting granules were sieved and dried and the dried granules again sieved. The sieved dried granules were thoroughly mixed so that they were in a form suitable for administration to animal feedstuffs or animal drinking water.

WHAT WE CLAIM IS:-

1. A veterinary composition which comprises trimethoprim or an acid addition salt thereof in admixture with sulphaquinoxaline or a salt thereof, the weight ratio of trimethoprim present to sulphaquinoxaline present being from 1:1 to 1:4.
2. A veterinary composition as claimed in claim 1 wherein the weight ratio of trimethoprim present to sulphaquinoxaline present is about 1:3.
3. A veterinary composition as claimed in either claim 1 or claim 2 wherein the trimethoprim is present as the free base.
4. A veterinary composition as claimed in any one of claims 1 to 3 wherein the sulphaquinoxaline is present as a salt with a veterinarily acceptable base.
5. A veterinary composition as claimed in any one of claims 1 to 4 in the form of a powder.
6. A veterinary composition as claimed in claim 5 wherein the sulphaquinoxaline is present in the form of an alkali metal salt.
7. A veterinary composition as claimed in claim 6 wherein the alkali metal salt of sulphaquinoxaline is the sodium salt.
8. A veterinary composition as claimed in any one of claims 5 to 7 wherein the powder comprises 50 to 100% w/w of a mixture of trimethoprim and sulphaquinoxaline.
9. A veterinary composition as claimed in any one of claims 5 to 8 wherein the powder comprises 60 to 80% w/w of a mixture of trimethoprim and sulphaquinoxaline.
10. A veterinary composition as claimed in any one of claims 1 to 4 in the form of a liquid concentrate.
11. A veterinary composition as claimed in claim 10 comprising trimethoprim and a water soluble veterinarily acceptable salt of sulphaquinoxaline.
12. A veterinary composition as claimed in claim 11 wherein the salt of sulphaquinoxaline is obtained by mixing a solution of sulphaquinoxaline with a water-soluble base in a veterinarily acceptable water-miscible solvent.
13. A veterinary composition as claimed in claim 12 wherein the water soluble base is an organic base.
14. A veterinary composition as claimed in either claim 12 or claim 13 wherein the base is ethanolamine or diethanolamine.
15. A veterinary composition as claimed in any one of claims 12 to 14 wherein the water-miscible solvent is polyethylene glycol, propylene glycol, glycerol, glycerol formal or such a solvent mixed with up to 30% v/v of ethanol.
16. A veterinary composition as claimed in any one of claims 10 to 15 comprising from 10 to 50% w/v of a mixture of trimethoprim and sulphaquinoxaline.
17. A veterinary composition as claimed in any one of claims 10 to 15 comprising about 24% w/v of a mixture of trimethoprim and sulphaquinoxaline.
18. A method for the treatment of protozoal diseases in birds comprising the administration of a veterinary formulation as defined in any one of claims 1 to 17.
19. A method as claimed in claim 18 wherein the protozoal disease is coccidiosis.
20. A method for the treatment of bacterial diseases in birds comprising the administration of a veterinary composition as defined in any one of claims 1 to 17.

21. A veterinary composition as claimed in claim 1 and substantially as hereinbefore described with reference to the Examples.

22. A method for the treatment of birds as claimed in any one of claims 19 to 21 and substantially as hereinbefore described.

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