The In Vitro Studies on the Effect of *Aloe vera* ((L.) Webb. and Berth.) and *Aloe spicata* (L. f.) on the Control of Coccidiosis in Chickens

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ABSTRACT

An in vitro trial was undertaken to determine the effect of *Aloe vera* and *Aloe spicata* on the inhibition of the sporulation of avian coccidia oocysts. Petri dishes containing coccidia oocysts isolated from droppings of coccidiosis-infected chickens were randomly assigned to 0%, 15%, 30%, and 45% aloe extract, and the control, sulphachlopyrazine sodium monohydrate (Esb3®, Novartis AG, Basel, Switzerland). The Petri dishes were incubated at 25°C for 48 hours. *Aloe spicata* inhibited sporulation better than *A vera* ($P < 0.05$). Increasing aloe concentration reduced the number of coccidia oocysts that sporulated ($P < 0.05$). *Aloe spicata* had the least number of sporulated oocysts at 30% concentration while *A vera* had the least number of sporulated oocysts at 45% treatment concentration. Aloes can, therefore, be used to control coccidiosis in chickens, especially among the resource-poor smallholder farmers.

INTRODUCTION

Aloe vera and *Aloe spicata* belong to the lily family. They are related to garlic, onion, and asparagus. They have been reported to have medicinal properties.[1] *Aloe vera*, for example, is thought to help in the external healing of many kinds of wounds, to be excellent in soothing minor burns, scrapes, ulcers, and to alleviate arthritis, constipation, and piles. The leaf and juice may be used in animals internally or externally. It is believed to possess pharmacological antibacterial, antivenin, and immunological properties. The potential for using *A vera* and *A spicata* in livestock health management is, however, not documented.

There is little documentation on broiler production in the smallholder sector. Most studies are on village chicken production[2] of which farmers are engaged in both village chicken and broiler chicken production. Of the several constraints to chicken production, the major chicken disease problems are coccidiosis and Newcastle disease.[3] Incidences of coccidiosis, among other diseases, were reported to be high in chickens age 3-4 weeks.[3,4]

In developing countries, poultry production is largely based on traditional extensive poultry production systems.[5] Village chickens represent an appropriate system to provide high-quality protein to the fast growing human population. They also provide income to resource-poor smallholder farmers, especially women. However, high mortality rates, mainly due to diseases, including coccidiosis, constitute one of the greatest constraints on chicken development. Resource-poor smallholder farmers usually use various aloe species to reduce chicken mortalities. Common examples of aloe used in Southern Africa include *A vera*, *A excelsa*, *A christiana*, and *A spicata*. They are easy to use and are available all year round.[6] There is, thus, a need to investigate and document the therapeutic importance of the aloes, which may in turn result in the preservation and protection of the natural plant resource-base. Smallholder farmers are able to contribute in the conservation efforts if they use the herb for ethno-veterinary and medicinal purposes.

Although ethno-veterinary medicines are widely used, the optimum dosage and the dose response characteristic are not known.[7] Dose response curves are difficult to determine when using ethno-veterinary medicines. The objective of the current study was, therefore, to determine the optimum concentration of the commonly used aloes in Zimbabwe, *A vera* and *A spicata*, to inhibit sporulation of coccidia oocysts.

MATERIALS AND METHODS

Study Site

The trial was carried out in the Parasitology Laboratory, Department of Paraclinical Veterinary Studies, University of Zimbabwe.

Experimental Design and Experimental Procedure

Extraction of aloe juice

Fresh aloe leaf extract of the broad leaf variety was used. The aloe leaves were bought at Greendale in Harare, in March 2004. The leaves were thoroughly washed using water before being minced in preparation for extracting juice. Thorns on the leaves were removed using a scalpel blade after which the leaves were cut into small cubes for easy mincing. Aloe juice was collected in a bucket from the mincer and then stored in sample bottles. Before use, the juice was thoroughly mixed to obtain a uniform solution, using a kitchen blender. The aloe extracts were stored for 6 days in a refrigerator at 4°C pending use to avoid oxidation, thereby maintaining the active ingredients in the liquid extracts.

Isolation of Coccidia From Poultry Droppings

Preparation of saturated salt solution

About 400 g of sodium chloride were mixed with 1000 mL of distilled water in a 10-L plastic container at ambient temperature.[8] The prepared saturated salt solution was used in the isolation of coccidia from coccidiosis-infected chicken feces.

Isolation of coccidia

The flotation method was used to test for the presence of coccidia oocysts in poultry droppings. Fresh chicken feces (2 g) of birds suspected of being infected with coccidia parasites were thoroughly mixed with a saturated salt solution using a pestle and mortar. The mixture was filtered through a strainer into a 250-mL plastic beaker. The saturated salt solution makes the oocysts float. The oocysts in suspension were withdrawn using

a 5-mL Pasteur pipette and mounted onto a slide for counting of oocysts under a light microscope. Oocysts were counted using the McMaster technique.[8]

Fresh chicken feces of birds suspected of coccidia oocysts infection were used. The oocysts were washed and separated from the litter using a standard 63 µm Tyler mesh sieve.[9] Initially, the droppings were mechanically mixed with water using a pestle and mortar. The oocysts were then washed with water and filtered first through the 180 µm Tyler mesh sieve. A small hand-held sprayer and flexible hose attached directly to the water faucet at a sink, ideally suited for this cleaning process, was used. The coarse residual material retained in the sieve was discarded. The filtrate containing oocysts was collected in a 5-L beaker and allowed to stand for 30 minutes in a refrigerator at 4°C until the oocysts settled to the bottom.

After the oocysts had settled, the supernatant was siphoned off and the sediment containing oocysts were transferred to a 106 µm Tyler mesh sieve through which the oocysts were flushed with water into a 5-L beaker. The filtrate was once more collected and allowed to settle in a refrigerator (4°C) for 30 minutes. Thereafter, the supernatant was discarded and the sediment flushed with water using a 63 µm Tyler mesh sieve. The process was repeated using the same sieve. The last sediment was flushed, this time into a smaller container (250-mL plastic beaker). The water was then siphoned out and a potassium dichromate solution (2.5%) was mixed with the concentrated oocysts to a depth of 40 mm above the oocysts. One part oocysts were diluted with 4 parts 2.5% potassium dichromate. Potassium dichromate was added to inhibit bacterial growth that may hinder the sporulation of oocysts.

Sample preparation for incubation and counting

The prepared oocysts-potassium dichromate-water mixture was centrifuged for 5 minutes at a gravity of 2000 rpm so as to increase oocysts counts in 1 mL oocysts-water-potassium dichromate mixture/solution. The supernatant was discarded until 15 mL of solution was left. Fifteen petri dishes, 3 for each treatment, were labeled and prepared for incubation, and 1-mL aliquots of oocyst solution were withdrawn using a 5-mL Pasteur pipette and randomly allocated to each of the prepared petri dishes into which various *A vera* and sulphachlopyrazine sodium monohydrate (Esb3®, Novartis AG, Basel, Switzerland) concentrations were added. The oocyst solution was thoroughly mixed mechanically each time 1 mL of oocysts solution was withdrawn. The *A vera* extract concentrations used were 0%, 15%, 30%, and 45%. The Esb3® (0.02 g) per petri dish was introduced into 3 petri dishes as a control. The different dilutions of *A vera* consisted of 100% *A vera* extract. To make a total volume of 20 mL for each treatment, the *A vera*, water, and oocysts volumes were prepared as shown in Table 1. All the petri dishes were incubated at 25°C for 48 hours. The same preparation procedure for incubation was done for *A spicata*.

The McMaster oocyst counting technique

One gram of feces was suspended in 30 mL of saturated salt solution. Dilutions of the saturated salt solution were calculated, as different weights were obtained for oocysts in each petri dish. The suspension was stirred well to obtain a completely homoge-

Table 1. Preparations for the Aloe Extract Concentrations.

	Volume (mL)			
Concentration (%)	**Aloe**	**Water**	**Oocysts**	**Total Volume**
0	0	19	1	20
15	3	16	1	20
30	6	13	1	20
45	9	10	1	20
Esb3®	0	19	1	20

neous distribution of the oocysts in the liquid. All 3 counting chambers of the counting cell were filled with the aid of a Pasteur pipette. After 5 minutes, the oocysts floated on the surface of the concentration solution and stuck to the cover slip. The oocysts were then counted under a microscope at a low magnification (×10 µm). The counting cells had 3 compartments, each compartment having a surface of 10 × 10 mm^2; the space between objective glass and cover slip was 1.5 mm. Each compartment contained 0.15 mL liquid, and at least 4 compartments were counted. The following formula was used to obtain the actual number of oocysts:

$$EPG = X \times 200$$

where EPG = eggs (oocysts) per gram of feces, and X = the number of eggs/oocysts counted in 1 counting cell.

If 2 or more counting cells were filled and counted, then X changed as per the formula below.

$$X = \text{Total number of eggs} \div \text{Total number of counting cells}$$

The coccidia oocysts were then counted under a light microscope using the McMaster technique (quantitative method).[8] The counts were converted to a percentage of both the sporulated and unsporulated coccidia oocysts.

Experimental design

Oocysts were randomly allocated to 4 concentrations (v/v; 0%, 15%, 30%, and 45%) of either *A vera* or *A spicata* extracts. Three replications were made for each concentration. Esb3® was applied to 6 petri dishes at a recommended application rate of 0.1% (w/v). Oocysts were incubated for 48 hours at 25°C.

Statistical Analyses

The following model was used to analyze the effect of each aloe on sporulation of coccidia oocysts:

$$Y_{ijk} = \mu + S_i + C_j + (SC)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} = the j^{th} concentration of the i^{th} species, µ = overall mean, S_i = effect due to species (i = 1 and 2), C_j = effect due to concentration (j = 1, 2, 3, 4, and 5), $(SC)_{ij}$ = species and concentration interaction, and ε_{ijk} = residual error.

The data were converted to percentages and tested for normality using a univariate procedure.[10] The data was not normally distributed, and arcsine transformations were performed. The collected data was then analyzed using the General Linear Model procedures.[10] Tukey's test was used to compare differences among means.

RESULTS

Aloe spicata and *A vera* had significant effects on the sporulation of coccidia oocysts ($P < 0.05$). Increase in aloe extract concentration had an effect on the sporulation of coccidia oocysts ($P < 0.05$): the number of sporulated oocysts decreased with increasing aloe treatment concentrations (Table 2). The 45% concentration had the least number of sporulated oocysts for both aloes. The 15% *A vera* concentration and the 30% *A vera* concentrations were not significantly different from the 0% *A vera* concentration in inhibiting coccidia oocysts sporulation, while the 15% *A spicata* concentration was not different from the 0% and 30% *A spicata* concentrations (Table 2). Conversely, the 15% and 30% *A vera* treatments were not significantly different from the Esb3® treatment, whereas for *A spicata*, only the 0% concentration was not different from the Esb3® treatment.

Table 2. Arcsine Oocyst Mean Values (±SE) Following *Aloe vera* and *Aloe spicata* Treatment on Sporulation.

	Sporulated Oocysts (counts/g feces)		Unsporulated Oocysts (counts/g feces)	
Concentration (%)	***A vera***	***A spicata***	***A vera***	***A spicata***
0	0.7^{ab}	1.2^{a}	0.6	0.07^{c}
15	0.5^{ab}	1.0^{ab}	0.5	0.14^{bc}
30	0.5^{ab}	0.7^{b}	0.6	0.37^{a}
45	0.3^{b}	0.8^{b}	0.8	0.27^{ab}
Esb3®	1.3^{a}	1.3^{a}	0.1	0.04^{c}
SEM	0.18	0.08	0.21	0.038

Lsmeans with the same superscript within a column are not different ($P > 0.05$).

The Esb3® treatment had the highest number of sporulated oocysts in comparison to petri dishes with aloe extracts, while the 45% concentration had the least number of sporulated oocysts for *A vera* and the 30% concentration had the least number of sporulated oocysts for *A spicata* (Table 2). The 45% concentration was significantly different from the Esb3® treatment ($P < 0.05$). There was no significant difference in the number of unsporulated oocysts in all the *A vera* treatments ($P > 0.05$). However, for *A spicata*, there was a significant difference ($P < 0.05$), with the 30% concentration having the highest number of unsporulated oocysts and the 0% and Esb3® having the least number of unsporulated coccidia oocysts.

The 2 aloe species *A vera* and *A spicata* were significantly different in inhibiting sporulation of coccidia parasites ($P < 0.05$). Table 3 illustrates that *A spicata* inhibited sporulation better than *A vera*. As shown in Table 4, Esb3® was not effective in inhibiting sporulation. The formulated treatment concentrations were significantly different in inhibiting sporulation ($P < 0.05$). There was no interaction between the aloe species and treatment concentrations ($P > 0.05$).

Table 3. Arcsine Oocyst Mean Values (±SE) Following *Aloe vera* and *Aloe spicata* Treatment.

Species	Sporulated Oocysts (counts/g feces)	Unsporulated Oocysts (counts/g feces)
A vera	1.0[a]	0.5[a]
A spicata	0.7[b]	0.2[b]
SEM	0.06	0.07

Lsmeans with the same superscript within a column are not different ($P > 0.05$).

Table 4. Arcsine Oocyst Mean Values (±SE) Following Different Aloe Concentrations.

Concentration (%)	Sporulated Oocysts (counts/g feces)	Unsporulated Oocysts (counts/g feces)
0	0.9[ab]	0.4[ab]
15	0.8[b]	0.3[ab]
30	0.6[b]	0.5[ab]
45	0.5[b]	0.6[a]
Esb3®	1.3[a]	0.1[b]
SEM	0.10	0.11

Lsmeans with the same superscript within a column are not different ($P > 0.05$).

DISCUSSION

The observation that aloe extract concentrations had an effect on the sporulation of coccidia oocysts indicates that aloe extracts are able to kill or inhibit growth and development of oocysts. The finding that *A spicata* had the least sporulation at 30% while *A vera* was most effective at 45% could suggest that the former is more effective in treating coccidiosis. The observation that Esb3® could not inhibit sporulation could be explained by the fact that, because it is a bactericidal drug as well, it killed the bacteria present, thereby enhancing sporulation of oocysts. The effect of bacteria on sporulation of coccidia oocysts has been documented earlier.[9] Potassium dichromate killed bacteria in a sample containing coccidia oocysts, thereby enhancing sporulation of coccidia oocysts.[9] It could be that bacteria, if present, could interfere with the sporulation of oocysts, possibly by competing for nutrients and/or feeding on the oocysts. This also explains the differences in the 0% treatment concentration values of the aloes that was expected to be equal. It is not known whether the formed sporozoites were capable of infecting chickens or not, depending on the coccidia species. It could be that although the coccidia sporulated, they were not in the infective state. Thus, more studies are required to determine the effect of the aloes on infectivity of the sporulated coccidia oocysts.

The differences between the 2 aloe species in inhibiting sporulation of the coccidia oocysts may be due to differences in chemical compositions. The inability of the aloe species to completely inhibit sporulation of the coccidia oocysts is an indication that

the herb may not be highly effective at the sporulation stage of the coccidia life cycle. This explains why it is essential to be familiar with the life cycle of the coccidia parasite, before invention of an efficient vaccine for coccidiosis disease.[11]

The findings of the *A vera* and especially *A spicata* extracts of following the pattern of control group 5 (Esb3®) of coccidiostat denotes that the herbs may act on the parasites in the same manner the coccidiostat would within the chicken. In addition, both the Esb3® and the aloes are used internally for the control of coccidiosis, while sporulation of oocysts occurs outside the host animal. It follows that the aloes may not work effectively at the sporulation stage but at other stages of the life cycle of the parasite. The optimum concentration of *A vera* that could be used to control coccidiosis could not be established. This calls for further studies to be undertaken to evaluate the optimum concentrations that should be used for which the herbs could effectively disturb the growth and development of coccidia oocysts. It is also necessary to undertake in vivo experiments to validate the effect of aloes on sporozoites and possibly the incorporation of aloes as anti-coccidials in poultry feeds, and to monitor chicken performance.

CONCLUSION

Aloe spicata inhibited sporulation of coccidia oocysts to more acceptable levels than *A vera* extract concentrations. However, the optimum concentration levels for *A vera* and *A spicata* that could be used for the control of coccidiosis could not be established.

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