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Prevalent Bovine Internal Parasites Using Different Techniques in Communal Farms around Mafikeng

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ABSTRACT Knowledge of the nematodes species present in a particular geographical location and their biology and epidemiology has important implications for the control of gastrointestinal parasites. The main objective of the study is to compare Zinc Sulphate, Sheather's solution, and sodium nitrate and concentrated salt solution flotation techniques in the identification of bovine internal parasites around the Mafikeng area. Simple random study of analysis of bovine fecal samples collected from Mafikeng area during the dry season was used, using egg per gram centrifugal flotation techniques of feces collected the same day. Direct smears using both iodine and saline were also performed to detect motile parasites stages. Of the two direct methods of detecting motile parasitic stages, iodine was more sensitive with only twenty percent negative results compared to forty percent using saline. Also, highest egg burden of 11-15 was found in ten percent of iodine samples compared to seven percent of saline samples. Comparison of flotation techniques showed that sodium nitrite had a negative score of twenty-two percent followed by zinc sulphate, Sheather's, and concentrated salt at fifteen percent, twelve percent and six percent, respectively. Highest egg burden of 1100-1599 was found in ten percent of samples using sodium nitrate compared to eight percent, five percent and three percent for Sheather's, zinc sulphate and saturated salt solution, respectively. From the results, the researchers can conclude that saturated salt solution and Sheather's solution are methods of choice for identifying bovine intestinal parasites, and they should be used in conjunction with direct methods using iodine especially for semi-solid to loose feces. For routine diagnostic work, the researchers recommend sodium nitrite although it had a higher negative value because it is cheap, easier to use and less messy than the other techniques. Also, it is the second best in detecting moderate to heavy infection with parasites only superseded by concentrated salt solution.

INTRODUCTION

Mafikeng is a semi-arid area where bovine do very well due to the open grasslands with lush grasses and conducive for parasite invasion. Internal parasites can cause poor performance of cattle and can lead to economic loss for the owner. Prevention and correction of parasitism is based on knowledge of factors that affect both the survival of parasites in the environment and their transmission to the host (Demander et al. 2003). Farmers must understand the types of parasites they may encounter and methods for controlling them in order to minimize losses in the Mafikeng area. The negative impact of infection includes subclinical effects as well as clinical disease. Appetite suppression, reduced production, lowered weight gain, and immunosuppression have been documented in infected animals (Bandyopadhyay et al. 2010). Parasites have adapted to conditions that best enhance forage growth. Parasites have an extraordinary ability to remain on pasture. The researchers have been looking at some alternative ways to control internal parasites in cattle, one is to modify how many eggs are passed in the feces and the other is to alter how many survive the time between when they hit the pasture and when they are ingested. Prior to about 30 years ago, most of the effort was in modification of pastures and parasite survival (Gasbarre et al. 2001).

Worms are primarily a disease of pastured cattle. However, almost all cattle carry a few worms that can serve as a source of pasture contamination and infection of herd mates, especially animals younger than two years of age (Hoglund et al. 2013). Adult cattle are much more resistant to heavy infection compared to young animals. Adult worms in the stomach or intestinal tract lay millions of microscopic-sized eggs that are passed in the feces (Hawkins 1993; Gadberry and Pennington 2011; Tsotetsi et al. 2013).

Although there are many species of worm parasites harbored in the gastrointestinal and respiratory tracts of cattle, only a few target species are clinically and economically important. These include the brown stomach worm *Oster-*

tagia, the coccidia *Eimeria bovis*, and the lungworm *Dictyocaulus*. Clinically, the parasites of the stomach and intestine cause anemia, scouring, depression and even death, but clinical parasitism occurs infrequently. The effects of parasitism usually are insidious and subclinical, such as indigestion and poor feed conversion, less than expected weight gain and (for brood cows) decreased milk production. Lungworms cause verminous pneumonia and provide an environment conducive for viral and bacterial pneumonia, with labored breathing and anxiety evident. Depressed performance then may be a consequence of internal parasitism (Walker et al. 2013; Huang et al. 2014). Internal parasites are known to reduce production by fifty percent, the problem with internal parasites is that they do not only cost the farmer by the death of the bovine but the loss made through the reduced production of animal products like milk in dairy cattle and the cost of the feed, which will be devoured by the parasites residing within the bovine (Gasbarre et al. 2001; Bandyopadhyay et al. 2010).

The problem of infestation by parasites is made worse by the fact that some parasites are resistant to some de-wormers and most farmers are not aware at which egg per gram count should they start deworming. Flotation technique is regarded as a gold standard for quantifying parasites by egg per gram of feces but there are many flotation fluids in the market with different sensitivities and specificities the objectives of the study are therefore to compare these flotation fluids and recommend the ones that can be used in the laboratory.

The main objectives of the study are therefore identifying the parasites, which are zoonotic from the identified ones, categorization of infestation into subclinical and clinical stages to avoid unnecessary deworming and acquisition of drug resistance as well as cutting down costs.

Objectives

The objectives included to compare zinc sulfate, concentrated salt solution, sodium nitrite, and Sheather's flotation solutions in the identification of internal bovine parasites, to quantify internal parasites infestation into egg per gram of feces so as to know when to initiate deworming and when to stop, to evaluate the efficacy of using direct smear techniques as an adjunct to flotation technique for eggs that do not float or liquid stools where protozoan trophozoites may be present, and to identify common species of internal parasites infecting cattle around Mafikeng area.

One may never completely eliminate internal parasites from the farm. However, by understanding the lifecycle of the parasite and adopting some of the techniques described in this technical paper one can keep them at a level where they are not adversely affecting the stock.

MATERIAL AND METHODS

Material

In this study, the following equipment were used for sample analysis: Microscope (Nikon YS100), Microscope slides, Cover slips, Gloves, Tongue suppressor, Tea strainer, Pipette, Stopwatch, Hydrometer, Centrifuge machine, Centrifuge tubes (15ml), Scale (digital), Funnel, Flotation kit (Kyron laboratories)

Reagents and solvents used, such as sodium nitrate flotation fluid, zinc sulfate, sodium nitrate, sodium chloride, granulated sugar, iodine, potassium iodide, and formaldehyde were laboratory graded obtained from Merk SA or Sigma SA.

Sample Collection

Rectal samples as well as on the ground fresh defecation samples were collected from cattle in Molelloane, Mokhosane and Lokaleng areas around Mafikeng area. Since the area was not clean enough for ground samples, only the top layer was taken to avoid contamination. Samples were collected in airtight leak-proof containers to prevent desiccation and create anaerobic conditions to prevent eggs from developing and possibly hatching. Samples were then placed in a refrigerator and analyzed within two days using protocol described below.

Reagents Preparation

Physiological Saline

8.5 grams of sodium chloride was mixed in a liter of distilled water. This solution was used for direct smear technique.

Lugols Iodine

1 gram of iodine and 2 grams of potassium iodide were mixed in 100 ml of distilled water. The solution was used for direct smear technique.

Flotation Fluids

Zinc Sulfate

386 grams of zinc sulfate was dissolved in 1000 ml of distilled water and specific gravity was adjusted to 1.18.

Saturated Salt Solution

350 grams of sodium chloride was dissolved in 1000 ml of distilled and specific gravity adjusted to 1.18.

Sheather's Solution*

454 grams granulated sugar was dissolved in 355mls of tap water, gently heated to aid dissolving and allowed to cool. 6ml of formaldehyde was then added to preserve solution and inhibit mold formation. Specific gravity of solution was adjusted to 1.27.

Sodium Nitrate

Ready to use and containing 338 grams in 1000 ml distilled water and specific gravity at 1.18.

Sample Analysis

Direct Smear Techniques

A direct smear technique using both saline and iodine were used for the study. These techniques were used as adjunct to flotation techniques when looking for eggs that are too heavy to float. A matchstick-sized stool sample was placed on the microscope slide and two drops of saline in case of saline technique and two drops of iodine in the iodine technique were added to the feces and mixed thoroughly. A cover slip was then placed on top, and moved around until it lays flat. The slide was then placed in the microscope and examined under low power using subdued light and results recorded accordingly.

Concentration Techniques

Modification of the Stoll and modified Wisconsin sugar flotation method was used for the study. The same method was used using zinc sulfate, concentrated salt solution, sodium nitrate, and Sheather's* solutions. Briefly 10 ml of flotation fluid was poured into a cup containing 1 gram of feces, mixed well and passed through double layered gauze into 15 ml centrifuge tube and all liquids squeezed out of the feces. 4 ml of flotation fluid was then added and the filtrate centrifuged at 2000 rpm for 3 minutes and allowed to stand for 5 minutes. 0.075 ml of supernatant was transferred into two clean microscope slides covered with a 22x40 mm coverslip and examined under a microscope and number of eggs was counted. 0.15 is 1/100 of 15 ml (14 ml flotation fluid and one gram feces) so number of eggs in 0.15 ml x 100 is equal to total number of eggs in the gram of feces. Results were then recorded accordingly

RESULTS

The results of the project have been grouped according to the number of eggs counted per gram of feces. For the direct smear technique, eggs were recorded per egg found or seen in the slide. This was categorized from negative (where no eggs were found), low infection (eggs less than 5 found), moderate infection (eggs above 5 but less than 10 found), and high infection (eggs more than 10 found). Majority of eggs found with concentration method were *haemonchus* and the following criterion was used to differentiate the level of infection in the cattle (negative, no infection, low infection less than 200, moderate infection 200-600 and heavy infection more than 600) using the egg per gram (EPG) technique. Table 1 shows summarized results of the iodine smear technique. Sixty percent of the results showed low to negative infestation with parasites, while ten percent showed high infestation with parasites. Table 2 shows summarized

Table 1: Eggs count using the iodine technique

Number of stool samples	*Eggs found*	*Percentages*
40	0 (negative)	40
20	1-5	20
30	6-10	30
10	11-15	10

Table 2: Eggs count using the saline smear technique

Number of stool samples	*Eggs found*	*Percentages*
30	0 (negative)	30
45	1-5	45
18	6-10	18
7	11-15	7

results of the saline smear technique. From the results it could be seen that seventy-five percent of cases showed low to negative infestation with parasites, while eighteen percent showed moderate infestation with parasites. Table 3 shows summarized results of Sheather's sucrose solution. Fifty-five percent of the cases showed an egg count of less than 599, and only eight percent showed an egg count of greater than 1099. Table 4 shows summarized results of concentrated salt solution. Results show that only twenty-four percent had an egg count of less than 599. Majority of cases (73%) had an egg count of between 600-1099, and only three percent had egg per gram count of greater than 1100. Table 5 shows summary of results of zinc sulfate solution. Seventy-three percent of cases had an egg per gram count of less than 600, and only five percent had egg count of over 1099.

Table 6 shows results of sodium nitrate flotation fluid. Forty-five percent of results showed an egg count of less than 600 eggs per gram of feces, and only ten percent showed results of greater than 1099 eggs per gram of feces.

Table 3: Eggs count using the Sheather's* technique

Number of stool samples	*Eggs found*	*Percentages*
12	0 (negative)	12
0	1-99	0
43	100-599	43
37	600-1099	37
8	1100-1955	8

Table 4: Eggs count using the concentrated salt solution

Number of stool samples	*Eggs found*	*Percentages*
6	0 (negative)	6
0	1-99	0
18	100-599	18
73	600-1099	73
3	1100-1599	3

Table 5: Egg count using zinc sulfate

Number of stool samples	*Eggs found*	*Percentages*
15	0 (Negative)	15
0	1-99	0
58	100-599	58
22	600-1099	22
5	1100-1599	5

Table 6: Eggs count using the sodium nitrite techniques

Number of stool samples	*Eggs found*	*Percentages*
22	0 (negative)	22
0	1-99	0
13	100-599	13
55	600-1099	55
10	1100-1599	10

DISCUSSION

The Wet Mount

Infections caused by gastrointestinal parasites are prevalent in the Mafikeng area because of the convenient climate for the transmission of infection. Counting of eggs from feces is the commonest method for the diagnosis of gastrointestinal parasite infections, and the method is inexpensive, easy to perform and does not require specialized equipment. Results of the direct smear technique, also called the wet mount, in Tables 1 and 2 show that it is the least sensitive. It only examines a small amount of feces and it takes a very long time to examine the sample properly, however it is a useful technique in cases where faces obtained is too small to handle with any technique and when examining feces from small fish, birds and reptiles. It has been proved to be effective in liquid feces where giardiasis is suspected (Sun 1980; Janoff et al. 1989). In which use of centrifugal flotation methods will crenate protozoan trophozoites beyond recognition. The iodine method was more sensitive than saline in the moderate to high infection category (40% as opposed to 25%), which is of clinical significance in deciding when to initiate deworming. Forty percent of cases were negative for parasites using iodine as opposed to thirty percent using saline. It could therefore be argued that saline is more sensitive than iodine in quantitating parasites. Study by Huang et al.

(2014) showed that of 1259 samples from dairy cows analyzed, 81.3 percent of parasites were protozoan and 5.8 percent nematodes as opposed to the present study, where thirty percent and sixty-five percent were protozoan and nematodes respectively.

Centrifugal Flotation

The centrifugal flotation method using different standard flotation media reduces the time for eggs and oocytes to float and is therefore recommended for use by routine diagnostic laboratories and research labs, which is also recommended by the Companion Animal Parasite Council. However, the choice of flotation media is still a challenge, as sensitivity and efficacy of these flotation fluids have not been well documented (Michel 1969; Grisi and Todd 1978). Results are usually based on field samples where actual number of eggs in feces is unknown. In this study, a comparison of different flotation media was made in order to come up with the one that can best suit the laboratory. Concentrated salt solution was found to be more sensitive with only six percent of cases being negative with this technique as opposed to twelve, fifteen and twenty-two percent using Sheather's*, zinc sulfate and sodium nitrate, respectively. Concentrated salt solution also proved to be more sensitive in the moderate to high infection category with eighty percent as opposed to forty-five, twenty-seven and sixty-five percent for Sheather's*, zinc sulfate and sodium nitrate, respectively. This is a significant finding, as decision of initiation of therapy, drug resistance and level of pasture contamination is made based on this category. The high sensitivity of the saturated salt solution should be interpreted with caution since only *haemonchu*s and *ostertagia* were found, which have lower specific gravity than salt solution. If ova with higher specific gravity than concentrated salt solution like Taenia (SG1.2251) they could not have been identified. Further advantage of saturated salt solution is that it is cheaper and easily available, however it is difficult to dissolve and forms minute dark deposits, which can be misinterpreted as parasites. The main disadvantage of concentrated salt solution is corrosion of laboratory equipment and severe distortion of the ova making interpretation very difficult.

The Sheather's* is usually regarded as the gold standard for identifying most parasites since it has a specific gravity of 1.27, greater than those of most ova, which can range from 1.055 for *Ancyclostoma* to 1.225 for *Taeni*a. Results of the study show that it is the second best, as only twelve percent of the results were negative with this technique superseded only by concentrated salt solution at six percent. In some studies it has been found to be up to five times more reliable than zinc sulfate in detecting *Trichurius* species (Dryden et al. 2005). It does not crystalize and therefore can be assessed over longer periods of time. It also causes minimal distortion of ova compared to concentrated salt and sodium nitrate, however *Gardia* cysts will crenate and thus require special attention. Sheather's* solution's main disadvantage is it leaves a sticky mess, it is difficult to clean and it attracts insects.

Sodium nitrate is the most widely used flotation method in veterinary practice because of its simplicity, minimal distortion of eggs, fine mixing and slight particle flotation of feces. Results of the study show that it had the highest percentage of negatives at twenty-two percent, meaning that it is least sensitive than other techniques. However, caution should be made when interpreting these results, as field samples are being used and therefore absolute numbers of eggs are unknown and therefore true or false positives and negatives cannot be calculated. Sensitivity of sodium nitrate was notable in the moderate to high infection rate category where it was only superseded by concentrated salt solution. Sodium nitrate has been found to recover more eggs than Sheather's* when simple flotation was used (Dryden et al. 2005). Simple flotation methods are used in most veterinary clinics and diagnostic labs. A study by Christie et al. (2011) found sodium nitrate to be most sensitive and superior to zinc sulphate and sugar solutions.

Zinc sulfate centrifugal flotation is regarded as a good choice for standard fecal examinations (Zajeck et al. 2004). Results of the study show that it was least sensitive with fifteen percent negative results only beating sodium nitrite at twenty-two percent. It was worse off in the moderate to high infection category at twenty-seven followed by Sheather's* at forty-five percent. In some studies it has been found to be superior in identifying *Giardia lamblia* and recovering delicate larval stages of lung worm

parasites like oslerius and filaroides (Dryden et al. 2005, Katagari and Oliveira 2010; Christie et al. 2011). It causes minimal distortion to eggs, crystalizes but is less messy than concentrated salt solution and Sheather's*.

Factors influencing accuracy and significance of fecal egg counts should be taken into consideration when interpreting the above results. They include quantity of feces passed, the number of eggs per unit weight, eggs may not be detected due to low number or low test sensitivity, in adult cattle where they have limited diagnostic value as they do not relate to worm burden. Should be used to guide treatment decisions but should be interpreted in conjunction with nutritional status, age and management of stock (Roeber et al. 2013).

CONCLUSION

Results obtained from this study show that saturated salt solution and Sheather's solution are methods of choice for identifying bovine intestinal parasites, and they should be used in conjunction with direct methods using iodine especially for semisolid to loose feces. For routine diagnostic work, the researchers recommend sodium nitrite although it had a higher negative value because it is cheap, easier to use and less messy than the other techniques. Also, it is the second best in detecting moderate to heavy infection with parasites only superseded by concentrated salt.

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