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# Anthelmintic resistance in gastrointestinal nematodes in goats and evaluation of FAMACHA diagnostic marker in Uganda

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ABSTRACT

Gastrointestinal nematodes (GIN) are a challenge to goat production globally causing reduced growth, morbidity and mortality. We report here results of the first nation-wide anthelmintic resistance (AR) study and validation of assessment of clinical anaemia with FAMACHA eye scores in goats in Uganda. From August to December 2012 the efficacy of albendazole (7.5 mg/kg), levamisole (10.5 mg/kg) and ivermectin (0.3 mg/kg) against strongyle nematodes was tested on 33 goat farms in Soroti, Gulu, Mpigi, Mbarara and Ssembabule districts of Uganda. Altogether 497 goats were subjected to a total of 45 different faecal egg count reduction tests (FECRT), each involving 5–20 goats. On one farm all substances were tested. Faecal and blood samples were collected and FAMACHA eye scores evaluated on the day of treatment and 15 days later. A questionnaire survey was conducted on frequency, type and dose of anthelmintics used, farm size and grazing management system. Examination of infective third stage larvae (L3) from pooled faecal cultures demonstrated *Haemonchus* to be the predominant genus (>75%). Resistance to at least one anthelmintic group was detected on 61% of the 33 farms and in 49% of the 45 test groups. Prevalence of resistance to ivermectin, levamisole and albendazole was respectively 58%, 52% and 38%. Correlation between pre-treatment packed cell volume determinations and FAMACHA scores ($r_{498} = -0.89$) was significant. Paddock grazing system (Odds ratio 4.9, 95% CI 1.4–17.3) and large farm size of >40 goats (odds ratio 4.4, 95% CI 1.2–16.1) were significant predictors of AR. In all districts, resistance to all three anthelmintics was higher on large-scale goat farms practising mostly paddock grazing. Interestingly, resistance to albendazole, the most commonly used anthelmintic in Uganda, was lower than that to ivermectin and levamisole. We recommend adaptation of FAMACHA to goats to help restrict anthelmintic treatment to heavily infected individuals. This will limit selection pressure and hence delay development of anthelmintic resistance.



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## 1. Introduction

The majority of 13 million goats in Uganda are indigenous Mubende (60%), Small East African (35%) or Kigezi (5%) breeds (Siefert and Opuda-Asibo, 1993; van Wyk and Bath, 2002). Goats are mainly reared by small-scale farmers, comprising approximately four out of every ten households, each with an average of 2–5 goats (MAAIF, 2008). The large-scale farms, each with ≥40 goats on ≥2.5 hectares of land, have extensively cross-bred goats with imported exotic Boer goats (85%, meat) from South Africa, Savannah White (5%, meat) from Middle East and Toggenburg (10%, milk) from the Alpine region (Semakula et al., 2010). Breeding goats for small-scale farms (<40 goats) with minimum input–output productivity, are usually purchased from large-scale farmers or bucks are obtained in communal grazing systems (Semakula et al., 2010).

Gastrointestinal nematodes (GIN) remain a challenge to livestock production and productivity worldwide (Vatta and Lindberg, 2006b; Höglund et al., 2009; Hoste and Torres-Acosta, 2011), to the extent that it is regarded as the major animal health constraint on small ruminant production (Vatta et al., 2001). As in other tropical and sub-tropical regions of the world, haemonchosis is one of the most debilitating livestock diseases in small ruminants in Uganda. It causes financial losses directly through production losses or indirectly in control efforts (Katunguka-Rwakishaya and Rubaire-Akiiki, 2007; Lapenga and Rubaire-Akiiki, 2009; Ssewannyana et al., 2010). Use of chemotherapeutic drugs (i.e. anthelmintics) has for years been the most viable option when implemented with other strategies such as good farm management (Waller, 1997; Coles, 2003; Katunguka-Rwakishaya and Rubaire-Akiiki, 2007; Höglund et al., 2009; Van Wyk and Reynecke, 2011). Anthelmintic resistance (AR) is present when worms tolerate doses of an anthelmintic, which otherwise would prove lethal to the majority of individuals (http://www.scops.org.uk/what-is-resistance.html). According to World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines, anthelmintic resistance (AR) is considered to be present in small ruminant nematodes when the efficacy of an anthelmintic drug is below 95% and the lower 95% confidence interval (CI) is below 90%, whereas AR is suspected when only one criteria is met (Coles et al., 1992). Many studies have demonstrated the existence of AR in small ruminants nematodes in several countries globally (Mckenna, 1994; Waller, 1997; Coles, 1998; Chandrawathani et al., 2004; Domke et al., 2011; Maharshi et al., 2011) including several African countries, notably South Africa (Van Wyk et al., 1999; Vatta and Lindberg, 2006b; Bakunzi, 2008), Kenya (Wanyangu et al., 1996; Maingi et al., 1998; Waruiru et al., 2003), Tanzania (Keyyu et al., 2002), Ethiopia (Sissay et al., 2006), and Zambia (Gabrie et al., 2001). A recent study at a research farm in eastern Uganda indicated reduced anthelmintic efficacy to the three main anthelmintic groups in goats (Byaruhanga and Okwee-Acai, 2013).

FAMACHA was developed to estimate clinical anaemia due to *Haemonchus contortus*, based on the colour of the lower conjunctiva, graded from 1 (normal, intensively red) to 5 (almost white). While developed primarily for sheep (van Wyk and Bath, 2002; Burke and Miller, 2008) it may be similarly applicable to goats (Kaplan et al., 2004). The FAMACHA score enables identification of the most anaemic animals that require treatment instead of the whole flock (Vatta et al., 2001). This reduces the selection pressure on the worm population and thereby delays development of AR and lowers the cost of treatment (van Wyk and Bath, 2002). Therefore, the objectives of this survey were; (i) to determine the prevalence of resistance to the commonly used anthelmintics and the associated risk factors in Uganda and (ii) to investigate the use and value of FAMACHA in goats under Ugandan conditions.

## 2. Materials and methods

### 2.1. Study design and area

A cross-sectional survey on AR to albendazole (ABZ), levamisole (LEV) and ivermectin (IVM) was conducted from August to December 2012. Thirty-three farms with flock sizes ranging from 10 to 150 goats from Mbarara (00° 36′S 30° 36′E), Soroti (01°43′N 33°36′E), Gulu (02° 45′N and 32° 00′E), Mpigi (00° 14′N 32° 20′E) and Ssembabule (00° 06′S 31° 30′E) districts were studied (Fig. 1). All these districts except Gulu are part of the great cattle corridor in Uganda, where there are two rainy seasons, which determine the farming activities. The first season starts in March to mid-June, and the second is from mid-July to early November.

### 2.2. Sample size estimation and sampling

The sample size for farms was estimated based on 90% helminth prevalence (Magona and Musisi, 2002), and 2 standard errors at 95% confidence ($d = 0.1$) using a formula by Thrusfield (2007) $4P(1-P)/d^2$ to give 35 farms. These were proportionately distributed in the five districts. Multi-stage sampling was done to select five districts with high goat production from four regions of Uganda. We purposively selected two sub-counties from each district and randomly selected two villages per region. The district veterinary extension staff participated in selection of farmers to be involved in the study. Naturally infected goats of local, exotic and cross breeds both on large (≥40 goats) and small-scale (<40) farms were enrolled into the study. Currently, goat breeds in Uganda are not phenotypically distinguishable, thus breeds were classified based on farm records. During data analysis, goat breed was dichotomised to local (mainly Mubende and Small East African) and pure exotic breeds (mainly Boer) or their crosses.

### 2.3. Data collection

Farmers with a minimum of 10 goats and a history of no deworming in the past 3 months were specifically approached. After explaining the objectives of the study, the farmers who consented to participate were recruited.

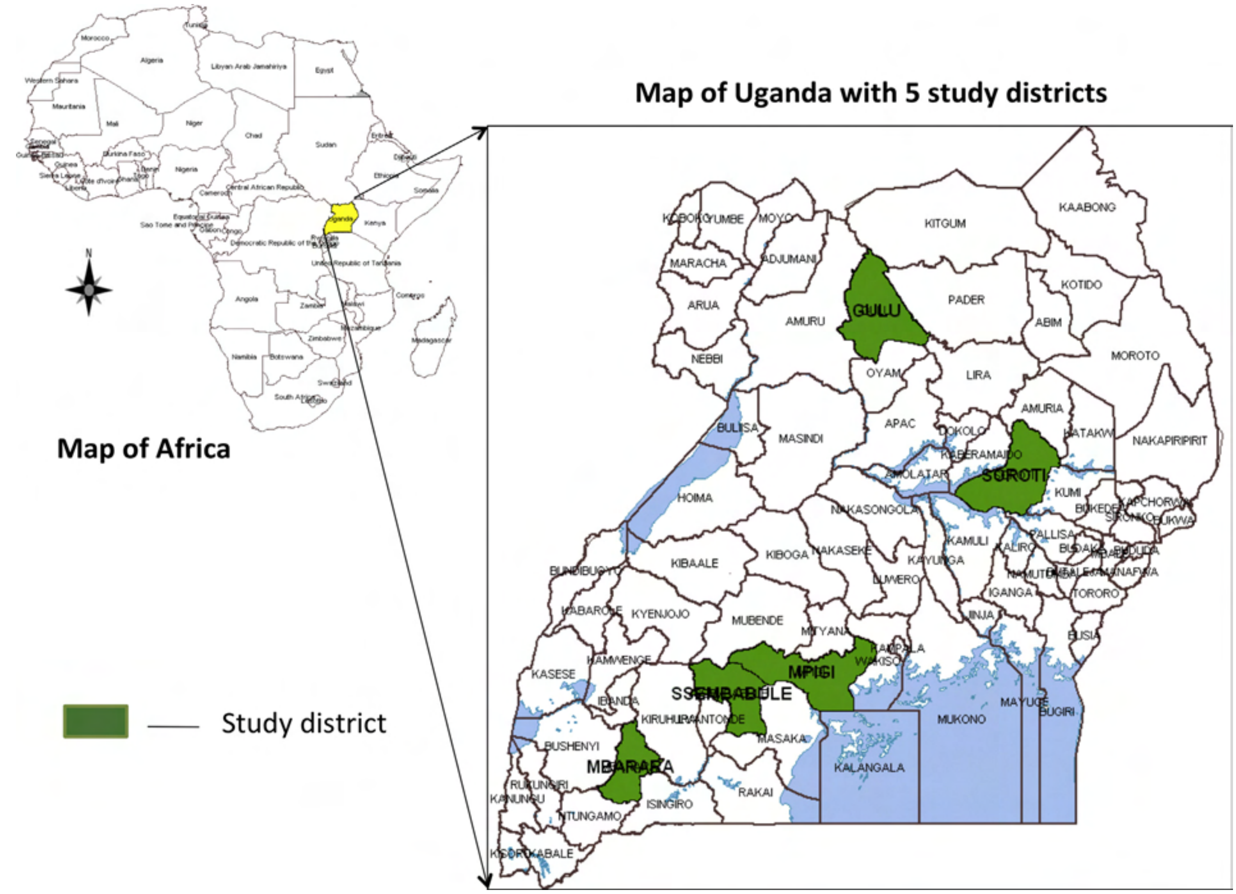


**Fig. 1.** Map of Uganda (right) extracted from Africa (left) shows the five study districts in the four regions of Uganda where the study was conducted: Mpigi and Ssembabule, central; Soroti, Eastern; Gulu, Northern and Mbarara, Western regions.

### 2.4. Questionnaire administration

A semi-structured questionnaire administered by personal interview to the household head or spouse was used to study the respective worm control practices. The frequency of anthelmintic use, rates per year (desired and practised frequencies), availability of veterinary services, type of anthelmintic used, source and route of administration, any changes in type during the year and persons responsible for administering the anthelmintic(s), were explored to evaluate potential risk factors for resistance.

### 2.5. Sample collection and processing

The goats were ear-tagged, their anaemia status categorized by FAMACHA eye score (Bath et al., 2001) and aged either by dentition (Lapenga and Rubaire-Akiiki, 2009) or from farm records. PCV was determined by the micro-haematocrit method (Hughes et al., 2005). The Red blood packed cell volume (PCV)-based classification used to develop FAMACHA in sheep as category 1 (≥28%); category 2 (23–27%); category 3 (18–22%); category 4 (13–17%) and category 5 (≤12%) (Bath et al., 2001; van Wyk and Bath, 2002), was used. We re-classified FAMACHA scores based on the corresponding PCV values. Blood samples were collected from the left jugular vein into 4 ml EDTA evacuated tubes (BDH, Plymouth, UK). Faecal samples were collected *per rectum* before treatment with either the assigned anthelmintic or distilled water as an oral placebo. Field-based FAMACHA eye scores and PCV-based FAMACHA scores, blood for PCV and faecal samples for faecal egg counts (FEC) were collected when the animals were dewormed (day 0) and again 15 days post-treatment.

### 2.6. Allocation of treatment groups

The *a priori* number of animals per treatment group was set at ≥10 goats. Where feasible, we randomly allocated the goats on farms with more than 10 goats to two or three treatment groups: (i) albendazole (7.5 mg/kg - Albafas® Norbrook, Kenya Batch 2201A 43NKL or Vermiprazol® Laboratorios Hipra, Spain Batch 42 DK-1); (ii) levamisole (10.5 and 22 mg/kg oxyclozanide - Levafas Diamond® Norbrook, Kenya Batches 2242 112NKL and 3185A 112NKL) and ivermectin (0.3 mg/kg Iverveto-1® VMD Arendonk, Belgium Batch 0113279 or Noromectin® Norbrook Newry, Northern Ireland Batch 8411-98). Where possible, at least one institutional or large-scale farm (≥40 goats) per region was included, and where possible all three anthelmintics were tested. The treatment given was 1.5 times the sheep manufacturer-recommended doses using weight of the heaviest goat for all anthelmintics (Coles et al., 1992). On 11 farms, we had two treatment groups. Buyana Stock Farm in Mpigi district had two flocks one of approximately 85 Boer or Boer cross goats separated from a larger flock of about 150 local Mubende and Small East African goats. Thus, both flocks on this farm were separately allocated and treated with ABZ or LEV. The total number of treatment groups was 50 and five untreated groups (one control per district) for comparison purposes. However, two farms with four treatment groups were excluded from FECRT analysis because of missing data on day 15. In addition, one farm had low (≤300) pre-treatment eggs per gram of faeces (EPG). Accordingly, results from 45 treatment groups (FECRT) from 33 farms are presented and discussed.

### 2.7. Faecal Egg Count Reduction Test (FECRT)

The FECRT was conducted as described in the WAAVP guidelines (Coles et al., 1992). Pre-treatment faecal samples collected on treatment day just before oral dosing or injection, were used as pre-treatment controls to assess treatment efficacy. A modified McMaster method was used to quantify strongyle nematode eggs in 3 g of faeces with a minimum diagnostic sensitivity of 50 eggs per gram (EPG) of faeces (Coles et al., 1992; Hansen and Perry, 1994). The EPG were calculated from the same animals on days 0 and 15 post-treatment. After enumeration of nematode eggs, faecal samples were pooled by goat age group, namely ≤1 year, 1–2 years and >2 years and incubated at ≈25 °C for 7 days as previously described (Rubaire-Akiiki, 1994). On day 8, nematode third larval stage (L3) was harvested using the Baermann apparatus and at least 100 L3 were differentiated by genus (Sissay et al., 2006). The response to treatment was measured by the percent faecal egg count reduction (%FECR) 15 days post-treatment.

### 2.8. Data management and analysis

Although 10 or more goats per farm were sampled, during analysis, only farms with a minimum of ≥5 goats and having a pre-treatment strongyle EPG ≥300 were included (Maingi et al., 1996). Two computer-based methods were used to calculate %FECR. Firstly, a Microsoft Excel pre-programmed method was used, which provided the percent reduction with 95% lower and upper confidence intervals (CI) and automatically classified the result (LeJambre et al., 1990). Secondly, a newer method developed by University of Zurich based on R software based on FEC hierarchical model was used (Michaela, 2012). Results from both methods and their level of agreement using the Kappa statistic were assessed. Data from the questionnaires was entered in Microsoft Excel version 3.1, exported and analysed in Statistical Package for Social Scientists (SPSS) version 17.0. Means and standard deviations for PCV, age, EPG, FAMACHA scores, and their 95% CI were calculated. To evaluate the relationship between FAMACHA and EPG, the latter was recalculated at 80% to get a proportion of *H. contortus*. Pearson and Spearman's rank correlations between EPG (*H. contortus*), PCV and FAMACHA scores were computed. Possible risk factors for AR such as flock size, frequency of deworming and grazing system were determined by detecting associations with AR status in the 45 tests using chi-square test and odds ratios. The resistance status was dichotomised to resistant (22) and susceptible (23) tests (re-coded with suspected resistant). The purely negative control groups were left out of this analysis. Where there was missing data in day 0 or day 15 assessments, the valid counts and percent were presented. The level of significance was set at $P < 0.05$.

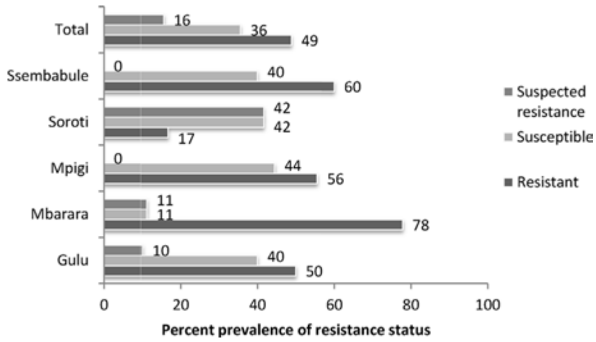


**Fig. 2.** Up to 49% ($n = 45$) of the faecal egg count reduction tests showed resistance to the tested anthelmintics, while 36% were susceptible. The five districts had varying anthelmintic resistance status with 78% in Mbarara and only 17% in Soroti district. Gulu, Mpigi and Ssembabule had similar prevalence levels.

## 3. Results

### 3.1. Comparison of AR computation methods

There was a high level of agreement between the two computer-based methods used to calculate resistance status (Kappa statistic 0.78, $P < 0.001$). Both methods classified 49% ($n = 45$) of the tested flocks with resistant or suspected resistant worm populations. Each of the two tests classified 6.7% (3) tests as susceptible or suspected resistance. The R-based method classified 36% FECRT as susceptible compared to 42% by the Excel-based method, which would help detect AR at the earliest stage. Results from the recent R software (Michaela, 2012) were then used in further analysis.

### 3.2. Prevalence of anthelmintic resistance

AR was recorded in 24 (49%) out of 45 tested flocks and to at least one anthelmintic in 20 (68%) of 33 farms throughout the country (Table 1). The proportions of flocks showing AR to IVM were 58% (tested flock, $n = 12$), 53% ($n = 17$) to LEV and 38% ($n = 16$) to ABZ. Mbarara district had 78% ($n = 9$) resistance, the highest recorded, followed by 60% ($n = 5$) in Ssembabule and 56% ($n = 7$) in Gulu (Fig. 2).

### 3.3. Nematode genera

Based on pre-treatment egg morphology, 72% of the eggs by McMaster slide count were strongyle type, 25% *Strongyloides* and 3% *Trichuris*. Fifteen days post-treatment, 90% of the eggs were strongyle type while 10% were *Strongyloides*. Pre-treatment coproculture results in ≤1

**Table 1**
Results (R-based method) of 45 faecal egg count reduction tests (FECRTs) for the 33 farms studied in the Mpigi, Ssembabule, Soroti, Gulu and Mbarara districts in Uganda. A total of 165 (ABZ), 132 (LEV) and 151 (IVM) goats treated had complete results analysed. Twelve farms had two or more FECRTs and 16 tests were performed for ABZ, 17 for LEV and 12 for IVM.

| District; region (FECRT) | Farm code | EPG Day 0[a] | Anthelmintic tested | | Ivermectin %FECR (95% CI), $n$[b] | AR Status[c] | Levamisole %FECR (95% CI), $n$[b] | AR Status[c] |
|---|---|---|---|---|---|---|---|---|
| | | | Albendazole %FECR (95% CI), $n$[b] | AR Status[c] | | | | |
| Gulu; Northern (10) | 1 | 730 | | | 87 (80–92); 10 | R | | |
| | 2 | 743; 790 | | | 90 (83–94); 10 | R | 29 (5–47); 7 | R |
| | 3 | 733 | | | 97 (92–99); 9 | S | | |
| | 4 | 1120; 1340 | | | 99 (98–100); 5 | S | 100 (98–100); 5 | S |
| | 5 | 1020 | | | 75 (70–81); 15 | R | | |
| | 6 | 833 | | | 92 (88–95); 15 | R | | |
| | 7 | 1056 | | | | | 81 (75–87); 9 | R |
| | 8 | 1131 | 98 (96–99); 13 | S | | | | |
| Mbarara; Western (9) | 9 | 1391 | | | | | 66 (58–73); 11 | R |
| | 10 | 925; 758 | 96 (91–99); 6 | S | | | 69 (58–77); 10 | R |
| | 11 | 679 | 91 (86–95); 14 | R | | | | |
| | 12 | 912; 1009 | 86 (80–91); 11 | R | 87 (84–91); 17 | R | | |
| | 13 | 1034; 1042 | 40 (28–48); 20 | R | 72 (67–78); 19 | R | | |
| | 14 | 680 | | | | | 84 (76–90); 10 | R |
| Mpigi; Central (9) | 15 | 1580, 2429 | 50 (41–57); 10 | R | | | 34 (24–45); 7 | R |
| | 16 | 2305, 3246 | 52 (46–58); 20 | R | | | 83 (46–58); 14 | R |
| | 17 | 771; 886 | 97 (93–99); 6 | S | | | 97 (93–99); 6 | S |
| | 18 | 1388; 992 | 100 (98–100); 8 | S | | | 96 (93–99); 7 | S |
| | 19 | 700 | | | 54 (40–64); 10 | R | | |
| Soroti; Eastern (12) | 20 | 1940 | | | | | 99 (98–100); 5 | S |
| | 21 | 1043 | 93 (88–97); 7 | SR | | | | |
| | 22 | 780; 729 | 94 (85–98); 7 | SR | | | 83 (72–91); 5 | R |
| | 23 | 842; 857; 1050 | 92 (86–96); 6 | SR | 95 (90–97); 7 | S | 78 (67–86); 5 | R |
| | 24 | 1480 | 97 (94–99); 5 | S | | | | |
| | 25 | 1436 | | | | | 99 (98–99); 7 | S |
| | 26 | 1488 | | | 98 (97–99); 14 | S | | |
| | 27 | 770 | | | | | 92 (85–96); 5 | SR |
| | 28 | 1783 | 100 (98–100); 6 | S | | | | |
| Ssembabule; Central (5) | 29 | 1511 | 85 (80–89); 8 | R | | | | |
| | 30 | 2319 | 99 (98–100); 13 | S | | | | |
| | 31 | 833 | | | 88 (83–93); 18 | R | | |
| | 32 | 1095 | | | | | 97 (93–99); 10 | S |
| | 33 | 950 | | | | | 83 (77–87); 8 | R |

[a] EPG for second or third treatment group on same farm.
[b] $n$ = number of goats per FECRT with complete set of data.
[c] S = Susceptible; SR = Suspected resistance; R = Resistant.

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**Table 2**
Spearman rank and Pearson correlation coefficients for red blood packed cell volume (PCV), FAMACHA eye score, *Haemonchus contortus* eggs per gram of faeces (EPG) on day 0 and 15 days post-treatment and goat age (497 goats). Negative sign = negative correlation.

| Variable | Day 0 | Correlation coefficient[a] | *P*-value | Day 15 | Correlation coefficient[a] | *P*-value |
|---|---|---|---|---|---|---|
| Field-based FAMACHA classification | | | | | | |
| PCV (%) | EPG[b] | −0.22 (4.6) | <0.001 | EPG[b] | −0.26 (6.8) | <0.001 |
| | FAMACHA | −0.19 (3.6) | <0.001 | FAMACHA | −0.26 (6.8) | <0.001 |
| | Age (years) | −0.11 (1.2) | 0.036 | Age | −0.14 (2) | 0.003 |
| EPG[b] | FAMACHA | 0.12 (1.4) | 0.008 | FAMACHA | 0.25 (6.3) | <0.001 |
| PCV-based FAMACHA classification | | | | | | |
| PCV (%) | EPG[b] | −0.23 (5.3) | <0.001 | EPG[b] | −0.26 (6.8) | <0.001 |
| | FAMACHA | −0.92 (84.3) | <0.001 | FAMACHA | −0.88 (77.6) | <0.001 |
| EPG[b] | FAMACHA | 0.23 (5.3) | <0.001 | FAMACHA | 0.33 (10.9) | <0.001 |

[a] Number in brackets is $R^2 \times 100\%$—variability explained by correlation values. Spearman rho calculated for EPG and FAMACHA; EPG and PCV; PCV and Age; Pearson for PCV and FAMACHA. Degrees of freedom = 496.
[b] EPG calculated as 80% *Haemonchus contortus*.

year age group revealed 58% *Haemonchus*, 37% *Strongyloides* and 5% others (including *Trichostrongylus* spp., *Cooperia* spp. and *Bunostomum* spp.). In the 1–2 years age group, 88% *Haemonchus*, 8% *Strongyloides* and 7% *Trichostrongylus* were found. In goats above 2 years, we found 90% *Haemonchus* and 10% *Trichostrongylus*. In coprocultures 15 days post-treatment, ≤1 year age group had 87% *Haemonchus*, 3% *Trichostrongylus* and 10% *Strongyloides*. The 1–2 years group had 95% *Haemonchus* and 5% *Strongyloides*. Above 2 years, results showed 100% *Haemonchus*. There was a significant change in proportions of nematode species post-treatment, with higher proportions of *Haemonchus*.

### 3.4. Evaluation of FAMACHA

Classification categories of FAMACHA by card in the field were 1 (3, 0.7%), 2 (50, 11.2%), 3 (158, 35.3%), 4 (161, 35.9%), 5 (76, 17%). Correspondingly, PCV-based FAMACHA categories (section 2.5) were; 1 (203, 45.4%), 2 (145, 32.4%), 3 (74, 16.5%), 4 (20, 4.5%), 5 (6, 1.3%). There was low level of agreement between the two classifications (Kappa statistic 0.015, $P = 0.22$). The latter classification therefore was used in further analysis. The Spearman rank correlation between EPG (*H. contortus*) and FAMACHA scores was strongly positive ($r_{498} = 0.93$, $P = 0.008$) as shown in Table 2. There was strong correlation between PCV and FAMACHA scores (Table 2). There was no significant relationship between age and EPG. The paired samples *t*-test showed significant differences between days 0 and 15 EPG ($t = 13.32$, $P < 0.001$), FAMACHA scores ($t = 12.03$, $P < 0.001$), and PCV ($t = -3.25$, $P = 0.001$). Therefore there was least change in PCV post-treatment.

### 3.5. Effect of treatment on PCV, EPG and FAMACHA

One-way analysis of variance (ANOVA) showed significant differences in these parameters before and after treatment across the three treatment groups (Table 3). The mean pre-treatment EPG levels of strongyle eggs were different between IVM (938 ± 830) and LEV (1422 ± 2065) but not with ABZ (1246 ± 1413) before treatment. Post-treatment (15 days) the EPGs were different between IVM (135 ± 329), ABZ (323 ± 805) and LEV (285 ± 648). PCV on day 0 was similar in all three groups but was significantly higher at day 15 in the animals treated with IVM (29.2 ± 5.2%) than in those dewormed with ABZ (27.0 ± 5.5%) or LEV (27.1 ± 5.3%). FAMACHA was lower in IVM compared to ABZ and LEV on 0 and 15 days post-treatment (Table 3). Mean PCV was in normal range (22–38%) for adult goats. Ivermectin had lower EPG, higher PCV 15 and lower FAMACHA scores at 0 and 15 days compared to ABZ and LEV treatment groups.

### 3.6. Risk factors

Up to 65% ($n = 33$) of the farmers used anthelmintics irregularly, of whom 48% had <40 goats (small-scale). Analysis of the grazing management system and flock size revealed that 63% ($n = 20$) of large-scale farms had their goats on open paddock grazing while 92% ($n = 13$) small-scale farms had tethered goats. Only 45% ($n = 33$) farmers dewormed their goats more than once per year. Farmers keeping both cattle and goats used anthelmintics more frequently than farmers with only goats. Nematode populations in goats from large-scale farms were four times more likely to develop AR compared to those on small-scale holdings (Table 4). Furthermore, open paddock grazing was associated with five times higher risk for AR compared to tethered goats. In the large-scale paddock grazing flocks, 82% ($n = 20$) were resistant compared to 24% ($n = 13$) in small-scale farms. Similarly, districts varied in resistance status with 78% ($n = 9$) prevalence recorded in Mbarara compared to 17% ($n = 12$) in Soroti (Table 4). Although 80% of the farmers attested to presence of veterinary services in their sub-counties (within 30 km distances), only 15% ($n = 33$) relied on these services for helminth control. Other (85%) farmers drenched their goats and used veterinary services for other diseases or conditions, especially when associated with high morbidity or mortality. Anthelmintics were not alternated or rotated between treatments, except on two large-scale institutional farms, with very high levels of AR.

## 4. Discussion

This study established an overall prevalence of AR in 49% of the investigated groups. This was so despite giving 1.5 times the sheep manufacturer-recommended doses

**Table 3**
Comparison of mean differences in red blood packed cell volume (PCV), FAMACHA eye score and nematode eggs per gram of faeces (EPG) by treatments on day 0 and 15 days post-treatment in 497 goats.

| | | *N* | Mean[a] | Std. error | 95% Confidence interval for mean | | ANOVA result | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower bound | Upper bound | *F*-value | *P*-value |
| EPG on day 0 (treatment day) | Albendazole | 165 | 1246.4 | 110.0 | 1029.5 | 1463.9 | 3.88 | 0.021 |
| | Levamisole[*] | 132 | 1422.4 | 179.7 | 1066.9 | 1777.8 | | |
| | Ivermectin | 151 | 937.8 | 67.5 | 804.3 | 1071.2 | | |
| EPG on day 15 | Albendazole[*] | 165 | 323.3 | 62.7 | 199.6 | 447.1 | 3.82 | 0.023 |
| | Levamisole | 132 | 284.5 | 56.5 | 172.8 | 396.2 | | |
| | Ivermectin | 151 | 134.8 | 26.8 | 81.8 | 187.7 | | |
| PCV(%) on day 0 | Albendazole | 165 | 27.0 | 0.4 | 26.2 | 27.8 | 0.71 | 0.490 |
| | Levamisole | 132 | 26.8 | 0.5 | 25.7 | 27.9 | | |
| | Ivermectin | 151 | 26.2 | 0.5 | 25.3 | 27.1 | | |
| PCV (%) on day 15 | Albendazole[*] | 165 | 27.0 | 0.4 | 26.1 | 27.8 | 8.44 | 0.000 |
| | Levamisole[*] | 132 | 27.1 | 0.5 | 26.2 | 28.0 | | |
| | Ivermectin | 151 | 29.2 | 0.4 | 28.4 | 30.1 | | |
| FAMACHA on day 0 | Albendazole[*] | 165 | 3.7 | 0.1 | 3.5 | 3.8 | 3.67 | 0.026 |
| | Levamisole[*] | 132 | 3.6 | 0.1 | 3.5 | 3.8 | | |
| | Ivermectin | 151 | 3.4 | 0.1 | 3.3 | 3.6 | | |
| FAMACHA on day 15 | Albendazole[*] | 165 | 3.3 | 0.1 | 3.1 | 3.4 | 8.24 | 0.000 |
| | Levamisole[*] | 132 | 3.0 | 0.1 | 2.9 | 3.1 | | |
| | Ivermectin | 151 | 2.9 | 0.1 | 2.8 | 3.0 | | |

[*] Tukey High Significant Difference test significant with ivermectin at $P < 0.05$.
[a] Underlined values are significantly different from ABZ or LEV treatment group means.

(Eguale et al., 2009) and by calculating the doses for each treatment group based on weight of the heaviest goat (Coles et al., 1992). These two adjustments have been previously recommended to overcome the shortfall goats have compared to sheep in digestion and subsequent bioavailability of anthelmintic drugs (Coles et al., 1992; Canga et al., 2009). In this study, the cut-off of ≥300 strongyle EPG to demonstrate the changes in FEC was used, avoiding the pitfall at lower pre-treatment levels (Githiori et al., 2005). Additionally, given the low numbers of goats at small-scale farm level in African conditions, adaptations to the recommended standard procedure were made (Section 2.8). In this study, the requisite number per FECR test was 10 goats allowing adjustments and statistical analysis for the goats not meeting the pre-treatment ≥300 EPG cut-off criterion.

The two computer-based methods used to calculate AR status yielded comparable results. In the R software method, FECRT are based on Bayesian inference via Markov chain Monte Carlo techniques, which takes into account sampling variability and between-animal variation (Michaela, 2012), whereas the Excel model is based on a beta distribution. We found that the R-based results were strict in assigning suspected resistance or susceptibility AR status and were thus preferred. The strengths of the R-based method include analysis of both paired (before and after) and unpaired datasets and it also takes into account the sampling variability in each sample. This removes the requirement of control animals on the same farm, which is a limitation especially in the study of goats on small-scale farms (Coles et al., 1992). However, the number of

**Table 4**
Risk factors identified in bivariable analysis by combining FECRT results of suspected resistance and susceptible for anthelmintic resistance in 45 FECRTs in five districts of Uganda.

| Variable | | Resistant *n*, % | Susceptible *n*, % | Chi-square, *P*-value | Odds ratio, 95% CI |
|---|---|---|---|---|---|
| Farm size | Large-scale (>40 goats) | 17 (63) | 10 (37) | 5.35, 0.02 | 4.42 1.21–16.12 |
| | Small scale <40 goats | 5 (27.8) | 13 (72.2) | | |
| Management system | Paddock open grazing | 15 (68.2) | 7 (31.8) | 6.41, 0.01 | 4.89 1.39–17.31 |
| | Tethered grazing | 7 (31.8) | 16 (69.2) | | |
| District | Gulu | 5 (50) | 5 (50) | 11.7, 0.02 | [a] |
| | Mbarara | 7 (78) | 2 (22) | | |
| | Mpigi | 5 (56) | 4 (44) | | |
| | Soroti | 2 (17) | 10 (83) | | |
| | Ssembabule | 3 (60) | 2 (40) | | |

[a] Odds ratio not calculated due to many categories.

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farms that were diagnosed with resistant nematodes was not significantly different depending on which of the two methods was used, making our results comparable to previous studies. This is in agreement with a Danish study, where comparison of four different methods of calculating AR was done (Maingi et al., 1996).

A recent review (Canga et al., 2009) showed that bioavailability of subcutaneous ivermectin (as used in this study) was 91.8% and it was more persistent in plasma (Alvinerie et al., 1993) than when administered via other routes. Additionally, Chartier et al. (1996) showed that initial biotransformation of benzimidazoles does not affect individual response of the goat. Although the intramuscular route yielded the highest bioavailability of levamisole in goats and sheep (Galtier et al., 1981), the oral route is most commonly used. Interestingly, the highest prevalence of AR was against IVM followed by LEV and ABZ. In Uganda, these anthelmintics are used in the reversed order of frequency, thus one would expect the highest levels of AR to ABZ. This was also demonstrated in the questionnaire survey indicating that 85% ($n = 33$) use ABZ, and 12% use LEV. On the other hand, IVM was mainly used at large-scale institutional farms and as part of the annual anthelmintic changes where mites are also targeted.

A recent study in Uganda (Byaruhanga and Okwee-Acai, 2013), reported reduced efficacy to ABZ, LEV and IVM, albeit with inconclusive results since all three anthelmintics were tested on one goat farm only. Nevertheless, the FECRT result was not different across these three treatment groups, contrary to our study where we detected AR to one anthelmintic and efficacy to another on the same farm. In Kenya, Wanyangu et al. (1996) reported 50% prevalence of AR to LEV in goats compared to 30% in ABZ, in agreement with our findings. Similarly, in Kenya Waruiru et al. (2003) reported 54% efficacy of LEV on a goat farm. In South Africa, efficacy of LEV was 58–75% on different farms, whereas ABZ reduced EPG by 68% (Vatta and Lindberg, 2006b). Although AR was first detected to benzimidazoles, these studies indicate higher efficacy of ABZ compared to LEV.

The significant change in the composition of genera identified in the coprocultures suggested that *H. contortus* was the main species contributing to AR. This is similar to findings in Ethiopia (Sissay et al., 2006; Eguale et al., 2009), South Africa (Van Wyk et al., 1999; Vatta et al., 2001), and Kenya (Wanyangu et al., 1996; Maingi et al., 1998). Despite using NaCl as the flotation solution, 3% *Trichuris* eggs, which are heavier than typical strongylid eggs were detected pre-treatment. For this reason, some may have been missed and none were detected post-treatment. The difference in prevalence of AR levels across the five districts can be explained by scale of production and associated differences in grazing management system. For example, all goat farms in Mbarara and Ssembabule districts were large-scale compared to one in Soroti. Similarly, the highest prevalence of AR was found on large-scale farms where goats were grazed in open paddocks, which is more prone to development of AR due to intense deworming schedules. On these farms for instance, 89% of the farmers dewormed twice or more times a year compared to 17% on the small-scale farms. The majority of the small-holder farms in this study had tethered grazing system where goats were moved to different stationary posts daily. Anthelmintic use on 83% of these farms however, was also less than twice a year. There is no doubt that a high deworming frequency creates selection pressure in the nematode populations, which increases the risk for in AR (Sissay et al., 2006).

For the first time, we have evaluated the FAMACHA diagnostic marker in goats in Uganda. The low level of agreement between field FAMACHA card scores and PCV-based classifications indicated the need for further development. Previously, van Wyk and Bath (2002) noted that there were conjunctiva colour differences between sheep and goats. The chart used was based on sheep conjunctiva colour and the PCV ranges in Section 2.5. In the current study however, when these PCV categories were applied, most of the goats with pale conjunctivae would be ineligible for anthelmintic treatment. FAMACHA scores reduced 15 days post-treatment and a matching change in PCV was achieved. The high correlations between PCV-based FAMACHA scores and PCV levels confirmed its field utility in clinical diagnosis of anaemia prior to anthelmintic treatment. Similar to high correlations used to develop the FAMACHA system (Bath et al., 2001; Sotomaiora et al., 2012), the results in this study were in agreement with previously documented relationship between PCV and FAMACHA eye scores (VanWyk and Bath, 2002; Kaplan et al., 2004). Adopting FAMACHA will reduce frequency of anthelmintics usage as only the most severely affected goats (Kaplan et al., 2004) are dewormed (Sotomaiora et al., 2012). This will reduce selection pressure, and therefore slow down development of AR (VanWyk and Bath, 2002). The system will also save the farmers more time and financial resources (Vatta and Lindberg, 2006a; Scheuerle et al., 2010).

In conclusion, this study has demonstrated that there is AR in strongyle nematodes to all major groups of anthelmintics in goats in Uganda. *Haemonchus* was the most commonly identified resistant nematode. Higher frequency of anthelmintic usage was found on large-scale farms, thus the reported high prevalence of AR on these farms. Use of the FAMACHA eye scores needs further development to support decision making for regulated anthelmintic use in goats.

## Authors' contributions

All authors contributed equally to this work. IN, JH, CRA and DM took lead in design and field implementation of the study whereas JH, CRA, and DO supervised the work and supported data analysis. WJA contributed to the study design, provided the FAMACHA kits and supported analysis and interpretation of the data. CRA, JH and DM supported the editing process. The authors read and approved the manuscript.

## Conflict of interests

The authors declare that they have no conflict of interest. Sida, which funded the research, however, had no direct role in design and conduct of the study.

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