

Anti-parasitic activities of four synthetic chemicals anthelmintics on *Haemonchus contortus*

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Abstract

Synthetic anthelmintic (AHs) have always been used in veterinary medicine. The increasing development of resistance to these AHs in livestock parasites threatens animal health and global production, calling into question their effectiveness. *In vitro* egg hatch inhibition assay (EHA) and larval development test (LDT) performed showed ovicidal and larvicidal activities of four AHs compared to the control (PBS). However, a suspicion of *Haemonchus contortus* resistance to Bolumisole M1 (IC₅₀ = 0.122µg/mL) and Ivomec-D (IC₅₀ = 0.130µg/mL) was recorded after the EHA. Similarly, resistance was observed to Bolumisole M1 (IC₅₀ = 0.024µg/mL), Ivomec-D (IC₅₀ = 0.07µg/mL) and Kelanthic (IC₅₀ = 0.144µg / mL) following the LDT. These results show that cases of parasite resistance to AHs exist in small ruminants in Burkina Faso. Therefore, a reasoned use of AHs could be a good approach in the control of gastrointestinal nematodes of small ruminants in Burkina Faso. Subsequent *in vivo* studies should be conducted to confirm this resistance in the real conditions of these ruminants.

Keywords: *Haemonchus contortus*; Synthetic chemicals anthelmintics; Small ruminants; Resistance

1. Introduction

Small Ruminants (SR) breeding is majoritary represented in tropical and subtropical countries with 95% of *caprines* and over 75% of *ovines* in 2010 [1]. In Burkina Faso, breeding represents one of the principal pillars of subsistence means to the population and accounts for 9 million cattles, 14 million caprines, 9 million ovines, and 44 million poultry [2]. However, its productivity is confronted by agroecological and sanitary constraints [3]. Among sanitary constraints, gastro-intestinal strongles infection represent one of the main health constraints capable to cause significant economic losses [4,5]. Thus, the use of synthetic *anthelmintics* (AHS) remains one of the solutions to fight against these infestations [6]. For more than 50 years, several AHS families have emerged [7]. Families of macrocyclic lactones, imidathiasoles and benzimidazoles are the most used in the city of Ouagadougou in Burkina Faso.

However, the intensive and sometimes inappropriate use of these AHs has led to the appearance of strains of resistant *nematodes* [6]. It is in this mind that this present work was initiated in order to compare the *In vitro* antiparasitic efficacy of four AHs regularly used in veterinary clinics in the city of Ouagadougou on eggs and L3 infective larvae of *Haemonchus contortus* (*H. contortus*), abomasal nematode in small ruminants [8]. The introduction should be typed in Cambria with

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2. Material and methods

2.1. Study sites

The study was conducted in Ouagadougou city and at Joseph KI-ZERBO University. A survey was realized in veterinary establishments to collect data on synthetic AHs used in the breeding of SR. Then, we performed *In vitro* test to compare the level of effectiveness of four AHs the most used by the veterinary establishments investigated.

2.2. Biologic material

The biological material used for the study was the caprines abomasum collected in Saaba slaughterhouse for *H. contortus* females adults collection. Rats droppings were used to prepare feeding solution [9].

2.3. Used products

Four (4) synthetic anthelmintics were used for the *In vitro* tests. There are anthelmintics with commercial names, *Benzal*300*, *Bolumisole M1*, *Kelanthic* and *Ivomec-D*.

2.4. Survey of terrain

A survey was led with twelve (12) veterinary establishments in Ouagadougou city. The informations collected were: i) the identification of diagnostic strategies, traitement strategies and the inventory of AHs used ; ii) the identification of commercial names ; the traitement period and the traitement per animal means cost. The data collected served to classify the AHs used and to choose the four first for *In vitro* tests.

2.5. *In vitro* tests

2.5.1. Collect of *H. contortus* eggs

The technic of Jabbar et al. [10] was used. The caprines abomasum was collected in Saaba slaughterhouse and conserved in a cooler. At the laboratory, the abomasum was placed into a beaker, washed with water, then incised longitudinally. The abomasum contents was empty in Petri box then, female worms of *H. contortus* were identified and placed in other Petri box containing distilled water for washing. These females adults worms were placed in a porcelain mortar. Using a pestle, they were crushed slightly to release eggs. The homogenate obtained was diluted with distilled water, filtered through sieves of different stitch (1mm to 100 µm) and recuperated in a tube of 15 mL. 10 µL of eggs solution obtained was deposited on a blade and observed to microscope (x10) to count eggs. The eggs solution were adjusted approximatively to 200 eggs per mL of solution.

2.5.2. Eggs hatch inhibition assay (EHA)

EHA was realized according to the procedure describe by [11]. For this, 100 µL of egg suspension was put into a culture plaque of 96 wells. In each well, we added 100 µL of each AHs at different concentrations (0.05 - 0.1 - 0.15 - 0.2 - 0.25 µg/mL) prepared with PBS solution (0.1 M phosphate, 0.05 M NaCl, pH 7.4) as a negative control. Then, the plaque were incubated at 27°C for 48 hours. After 48 hours of incubation, two formalin drop (10%) were deposited in each well to stop eggs hatching. Each concentration was repeated 3 times. L1 larvae and eggs were counted using microscope (x 10) and the hatching percentage were calculated according to the formula :

$$\text{Hatching rate} = \frac{\text{L1 larvae number}}{\text{eggs number} + \text{L1 larvae number}} * 100$$

2.5.3. Larval development assay (LDA)

The method used is that of Vernerova et al. [12]. 100 µL of egg solution was deposited in each well of culture plaque and incubated at 27°C for 24 hours. After 24 hours of incubation, 50 µL of feeding solution + 100 µL of each AHs at different concentrations (0.05 - 0.1 - 0.15 - 0.2 - 0.25 µg/mL) were added at larval solution then, incubated at 27°C for 6 days. After this, the test was stopped in adding two to three formalin drop (10%) in each well. A negative control (PBS)

was constituted. Each concentration was repeated 3 times like the negative control. Larval development rate was calculated according to the formula :

$$\text{larval development rate} = \frac{\text{L3 larvae number}}{\text{L1 larvae} + \text{L3 number}} * 100$$

2.6. Statistic analysis

For EHA and LDA, the means percentages obtained were calculated and the concentrations effect determinate by unparametric test of Kruskal-Wallis. Analysis was performed with software SPSS at 5% significativity. The comparison of four efficiency was achieved from the determination of Inhibitor Concentrations 50 (IC₅₀) using software Polo Plus (Version 1.0) for Windows. The four AHs IC₅₀ had served to appreciate *H contortus* resistance level in the following manner to considering the Minimal Inhibitor Concentration (MIC) beforehand defined by several authors : MIC > 0.1µg/mL indicated a resistance suspicion at Benzal*300 and at Kelanthic [12] ; MIC > 0.02µg/mL indicated a resistance suspicion for Ivomec-D [13] and Bolumisole M1 [14].

3. Results and discussion

3.1. Synthetic chemical anthelmintics used by veterinary establishments

In all veterinary establishments investigated its resort that sixteen (16) AHs are used to treat the animals affected by gastro-intestinal parasitisme (Figure 1). Almost this AHs some AHs was more used than other in particular *Kelanthic*, *Benzal*300*, *Ivomec-D* and *Bolumisole M1* which was the most prescribed (13,8 % means for each AHs).

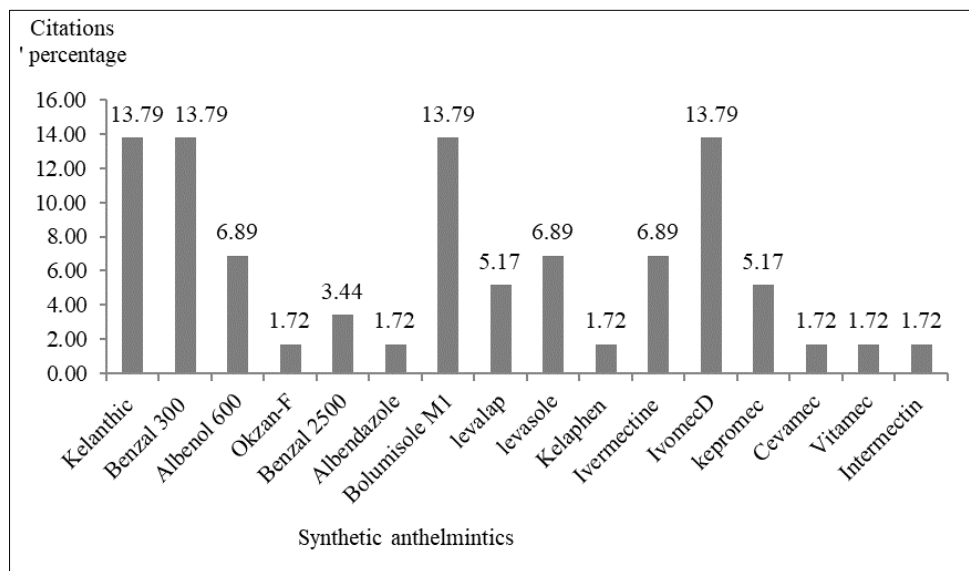


Figure 1 Synthetic chemical anthelmintics used in Ouagadougou veterinary establishments

3.2. In vitro assay

EHA and LDA were realized to evaluate In vitro anthelmintics efficacy of four AHs the most used which are: *Benzal*300*, *Bolumisole M1*, *Kelanthic*, and *Ivomec-D*.

3.2.1. Eggs hatch inhibition assay (EHA)

Figure 2 shows that the inhibition rates increased significantly ($p < 0.05$) with increasing concentrations for *Benzal*300* ($n=5$; $dl=4$; $p=0.0138$), *Bolumisole M1* ($n=5$; $dl=4$; $p=0.0157$), *Kelanthic* ($n=5$; $dl=4$; $p= 0.0212$) and *Ivomec-D* ($n=5$; $dl=4$; $p= 0.0144$). *Benzal*300* and *Kelanthic* showed higher inhibition rates ranging from 18% to 93% and from 62% to 89% respectively at doses of 0.05 to 0.25 µg/mL. These levels were found to be low for *Bolumisole M1* and *Ivomec-D* especially at low doses of 0.1 and 0.05 µg/mL.

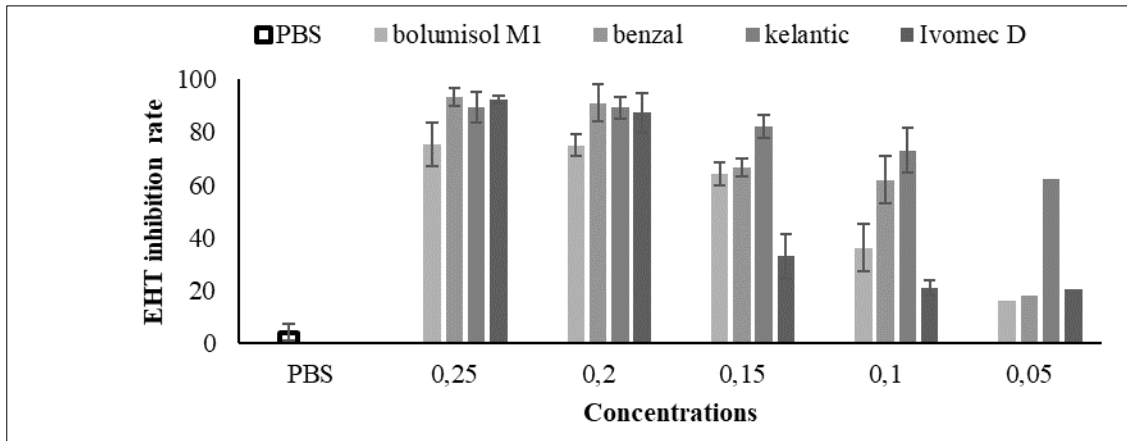


Figure 2 Profil of the concentration-response effect of *H. contortus* eggs hatch inhibition at different concentrations.

3.2.2. Larval development assay (LDA)

For LDA, the results showed a significant increase ($P < 0.05$) of synthetic anthelmintics compared to the control ($15.76 \pm 6.94\%$). *Bolusisole* M1, *Ivomec-D* and *Benzal**300 showed higher inhibition rates ranging from 56% to 68%; from 48% to 76% and from 42 to 64% respectively at doses of 0.05 to 0.25 $\mu\text{g/mL}$. These levels were found to be low for *Kelanthic* especially at low concentrations (0.1 and 0.05 $\mu\text{g/mL}$).

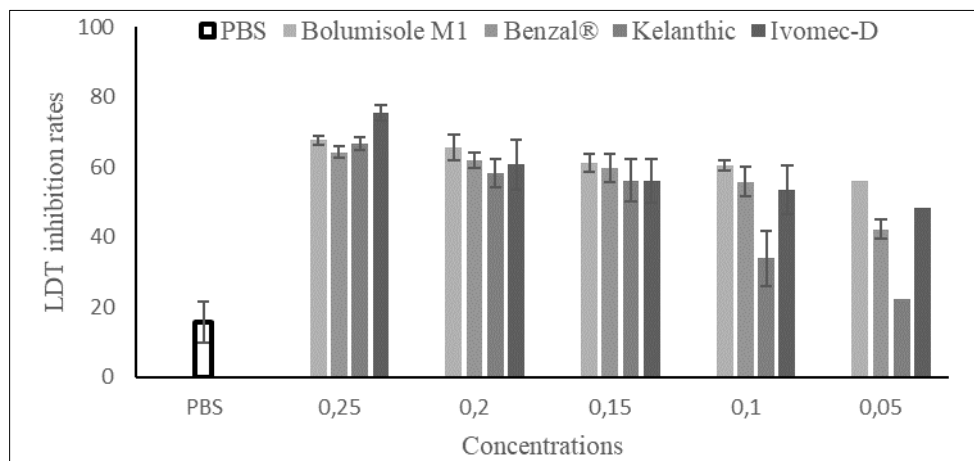


Figure 3 Profil of the concentration-response effect of *H. contortus* larval development inhibition at different concentrations.

3.2.3. Determination of inhibition concentrations 50 (IC₅₀)

Table 1 presents the inhibition concentrations (IC₅₀) for egg hatching and larval development. The IC₅₀ varied depending on the tests and AHs used. For the EHA, the inhibition concentration (IC₅₀) was higher for *Bolusisole* M1 (IC₅₀: 0.122 $\mu\text{g/mL}$) and *Ivomec-D* (IC₅₀: 0.130 $\mu\text{g/mL}$) compared to *Kelanthic* (IC₅₀: 0.032 $\mu\text{g/mL}$) and *Benzal**300 (IC₅₀: 0.091 $\mu\text{g/mL}$). In the LDA, *Bolusisole* M1 (IC₅₀: 0.024 $\mu\text{g/mL}$), *Benzal**300 (IC₅₀: 0.079 $\mu\text{g/mL}$) and *Ivomec-D* (IC₅₀: 0.070 $\mu\text{g/mL}$) showed low concentrations to induce 50% inhibition of larval development compared to *Kelanthic* (IC₅₀: 0.144 $\mu\text{g/mL}$).

Since their discovery, the AHs serve as a means to fight against digestive strongles of small ruminants. Almost the AHs families, *Benzimidazoles*, *Imidathiazoles* and Lactones *macrocylic* are the most used in the world in veterinary medicine [15,16]. In our study, the survey effectuated with 12 veterinary establishments in Ouagadougou city showed that three high AHs families are prescribed by veterinary establishments for fight against digestive parasitics of SR. Almost this AHs, the most used are *Benzal**300 and *Kelanthic* which belong to *Benzimidazoles* family, *Ivomec-D* Lactones macrocylics family and *Bolusisole* M1 to *Imidathiazoles* family. Our results are in accordance with those obtained by Kaboré et al. [3] who had showed that central plateau farmers of Burkina Faso use the same AHs to treat sick animals.

For *In vitro* assay, all AHs used recorded higher inhibition rates than the control (PBS = 12%) in the two biological tests, thus indicating the presence of a molecule active against the *H. contortus* parasite. These efficacy show that the four AHs tested have ovicidal and larvicidal properties on *H. contortus* parasite. The mean inhibition rates showed a high ovicidal activity of *Kelanthic* for EHA and a high larvicidal activity of *Bolumisole* M1 and *Ivomec*-D for LDA with low concentrations. Similarly, these AHs showed a concentration-dependent effect. The same results were obtained with *Thiabendazole*, *Ivermectin* and *Tetramisole* on LDA [17]. According to IC_{50} of each AHs and referring to minimal inhibition concentrations (MIC), there is a suspicion of *H. contortus* resistance to *Bolumisole* M1, *Kelanthic* and to *Ivomec*-D depending on the tests realized. Indeed, a resistance were suspected with *Bolumisole* M1 and *Ivomec*-D with a $IC_{50} > 0.1 \mu\text{g/mL}$ for EHA and LDA. Indeed, many studies showed a resistance to this drugs on nematodes eggs [18- 19- 20] and larvals [21]. Also, Mickiewicz et al. [22] suspected a resistance to *Ivomec*-D with an $IC_{50} = 0.17862 \mu\text{g/mL}$ and to *Bolumisole* M1 with an $IC_{50} = 0.94 \mu\text{g/mL}$ for larval development. Similary, a resistance to *Bolumisole* M1 and *Ivomec*-D were suspected by Farias et al. [23] and Varady and Corba [24]. A suspicion of *H. contortus* resistance to *Kelanthic* was recorded in the LDT with an $IC_{50} = 0.144 \mu\text{g/mL}$. Our results are in accordance with those obtained by Vernerova et al. [12] and Amulya et al. [25] who recorded resistances on *H. contortus* larvals development.

Table 1 Inhibition Concentrations 50 (IC_{50}) of *H. contortus* eggs hatching and larval development

Anthelmintics	Inhibition Concentrations 50 (IC_{50})	
	EHT	LDT
Bolumisole M1	0.111 < 0.122 < 0.133	0.003 < 0.024 < 0.045
Benzal*300	0.082 < 0.091 < 0.100	0.060 < 0.079 < 0.095
Kelanthic	0.021 < 0.032 < 0.043	0.130 < 0.144 < 0.161
Ivomec-D	0.106 < 0.130 < 0.158	0.042 < 0.070 < 0.092

EHT: eggs hatch test;. LDT: larval development test

Bolumisole M1 and *Ivomec*-D act on ion channels [26]. *Ivomec*-D causes flaccid paralysis of the parasite by increasing membrane permeability to chloride ions [27] and *Bolumisole* M1 causes spastic paralysis [28,29]. As for *Benzal**300 and *Kelanthic*, they act by binding to β -tubulins, thus disrupting metabolism energy and strongyles embryogenesis by inhibiting the production of microtubules of the parasite without altering those of the host [30,31]. Consequently, the mechanism of resistance of these AHs would probably be a mutation or an alteration of the receptor which is the target of the products, thus leading to a dysfunction of the mechanism of action of the AHs [6]. The bad practices of AH treatments would be the basis of this resistance [32].

4. Conclusion

At the end of the antiparasitic tests, a resistance of *H. contortus* to *Kelanthic* (*Benzimidazoles*), *Bolumisole* M1 (*Imidathiazoles*) and *Ivomec*-D (*Macrocyclic lactones*) was suspected in Burkina Faso. These resistances may be due to the bad practice of treatments such as: non-compliance with the frequency of treatment, the dosage, the total absence of rotation in the use of prescribed molecules. For a deepening and a better clarification of the results obtained, *in vivo* tests on sheep or goats in natural or artificial infestations could be necessary.

Compliance with ethical standards

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Author should write about third party like funding agencies, institution where experiment is carried out or who help in the experiment apart from authors.

Disclosure of Conflict of interest

All the authors declare that they have no competing interests.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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