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Review

An Overview of Anthelmintic Resistance in Domestic Ruminants in Brazil

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Abstract: Gastrointestinal nematodes (GIN) significantly, negatively impact livestock worldwide, and their control depends on the use of chemotherapy drugs. However, this approach is unsustainable as anthelmintic resistance (AR) is growing widespread. This article provides a comprehensive overview of the historical and current data published on AR in domestic ruminants in Brazil. Alternative measures of GIN control have been discussed to provide helpful information to prevent the development of AR in the country. This review consisted of a search of technical and scientific publications between January 1960 to January 2023, using online sources such as PubMed, Scielo and Google Scholar. Eighty-three articles published over the last six decades reporting AR in sheep (n = 43), goats (n = 20) and cattle (n = 20) were included. A total of 37.3%, 25.4% and 37.3% evaluated one, two and three or more molecule classes, respectively. Among all studies, 82.1% used fecal egg count reduction test as a method of AR diagnosis. In conclusion, AR is an urgent and emerging issue for ruminant production in Brazil. It is necessary to evaluate on a large scale the distribution and management of anthelmintic drugs and discuss strategies that delay this phenomenon's development.

Keywords: ruminant livestock; macrocyclic lactones; benzimidazoles; nematodes; chemotherapy drugs

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1. Introduction

Gastrointestinal nematodes (GIN) are important pathogens of grazing ruminants, responsible for economic losses in animal production worldwide [1–3]. The controlling of these parasites has been a challenge for producers and there is an emerging need to seek effective alternatives that do not cause animal toxicity [4]. Among Brazilian ruminant livestock, the most common GIN are those belong to the following genera: *Haemonchus* and *Ostertagia* (parasite of abomasum); *Trichostrongylus* (parasite of small intestine and abomasum); *Cooperia* (parasite of small intestine); and *Oesophagostomum*, also known as the nodular worm, which parasitizes the large intestine [5–7]. Infections by these parasites are characterized by lesions in the gastrointestinal mucosa, which impair the absorption of nutrients, reducing body weight gain and milk production. In addition, some species (e.g., *Haemonchus contortus*) are hematophagous [8]. Studies have already been conducted in Brazil to assess the economic impact of GIN infection in ruminants [9,10]. For instance, a reduction of 0.6 kg/cow/day of milk in dairy cattle is estimated, with a potential annual loss of up to USD 1870.48/animal [11]. In sheep, losses may reach approximately USD 400/animal/year [12].

GIN control has been largely achieved by using both broad (benzimidazoles, imidazothiazoles, hydropyrimidines and macrocyclic lactones) and narrow-spectrum (salicy-

lanilides, nitrophenols and organophosphate) anthelmintics [13]. More recently, amino-acetonitrile derivatives have emerged as a new chemical class of synthetic anthelmintics, effective against GIN of sheep [14]. Nevertheless, the excessive dependence on these substances has led to the development of anthelmintic resistance (AR) in all species of domestic ruminants to all classes of anthelmintics [15]. In Europe [16–18], Africa [19–21], Asia [22,23], Oceania [24–26] and the Americas [27–30], this phenomenon is associated with multiple drugs, threatening the viability of ruminant livestock production, especially small ruminants [18,31,32].

In Brazil, the first AR reports were on thiabendazole and ivermectin in sheep in the country's southern region [33,34]. Similarly, benzimidazoles resistance was also detected in goats from the northeast region. In the same period, AR was also detected in goats treated with albendazole, parbendazole and levamisole [35], which raises the discussion about different dosages for small ruminants [36]. Soon after, the resistance of *Haemonchus* spp. to oxfendazole and albendazole was detected in cattle from the southern region [37]. With the recent increase in AR in Brazilian herds, the development of new compounds is pivotal, as well as the integration of rational GIN control [38,39].

No articles have gathered data on AR in domestic ruminants in the country. Few studies have been conducted singly, such as a study on AR in small ruminants [40] and resistance to the class of benzimidazoles [41]. Therefore, this article provides a comprehensive overview of the historical and current data published on AR in domestic ruminants in Brazil. Additionally, alternative measures of GIN control have been discussed to provide helpful information to prevent the development of AR in the country.

2. Methods

Review Procedures and Map Construction

This review consisted of a comprehensive search of technical and scientific reports published from January 1960 to January 2023 using online sources such as PubMed, (US National Library of Medicine National Institutes of Health/National Center for Biotechnology Information Search database), Scielo (Scientific Electronic Library Online) and Google Scholar. Keywords, including "ruminants", "small ruminants", "anthelmintic resistance", "sheep", "goat", "cattle" and "Brazil", were combined for search articles. All articles written in English or Portuguese were included in this review.

Articles were included whether they described AR exclusively through the fecal egg count reduction test (FECRT) or associated with other tests such as the egg hatchability test, larval development test, molecular tests and controlled efficacy tests. Studies that described resistance using necropsy were also included. Finally, they were screened to assess their originality, time of publication, aim, technique employed for diagnosis and reliability in presenting results.

Two maps were constructed to represent graphical data of the distribution of AR and the class of drugs used. Geographic coordinates were used through the Google Maps platform of the municipalities where the studies were carried out; then, these coordinates were incorporated into the QGIS program (version 3.22.10) together with the vector layer (Shapfiles, version 2017) obtained in the database of the Brazilian Institute of Geography and Statistics.

3. Results and Discussion

Eighty-three peer-reviewed articles reporting AR in domestic ruminants (sheep, goats and cattle) were published over the last six decades. Of all these studies, 51.8% (43/83) were reported in sheep, 24.1% (20/83) in goats and 24.1% (20/83) in cattle. Most of the studies were carried out with molecules from different classes. Of all studies, 37.3% (31/83) evaluated only one class, 25.4% (21/83) two drug classes and 37.3% (31/83) three or more drug classes. Figure 1 illustrates the map with a graphical representation of the distribution of the class of drugs used in the considered articles: macrocyclic lactones (24.1%; 20/83), combination of benzimidazole + macrocyclic lactones (14.5%; 12/83) and

benzimidazole + macrocyclic lactones + other combinations (37.3%; 31/83). Most of these studies (83.1%) used FECRT as a method of AR diagnosis. Currently, for the interpretation of FECRT, the recommendations of the World Association for the Advancement of Veterinary Parasitology (WAAVP) are based on classification criteria that outline how the observed data (values for the upper and lower 90% CI or results of separate hypothesis tests) are compared to the expected effectiveness and the lower limit of effectiveness (which serve as the values for the upper and lower limits of the gray zone), accepting a Type 1 error rate of 5% [41].

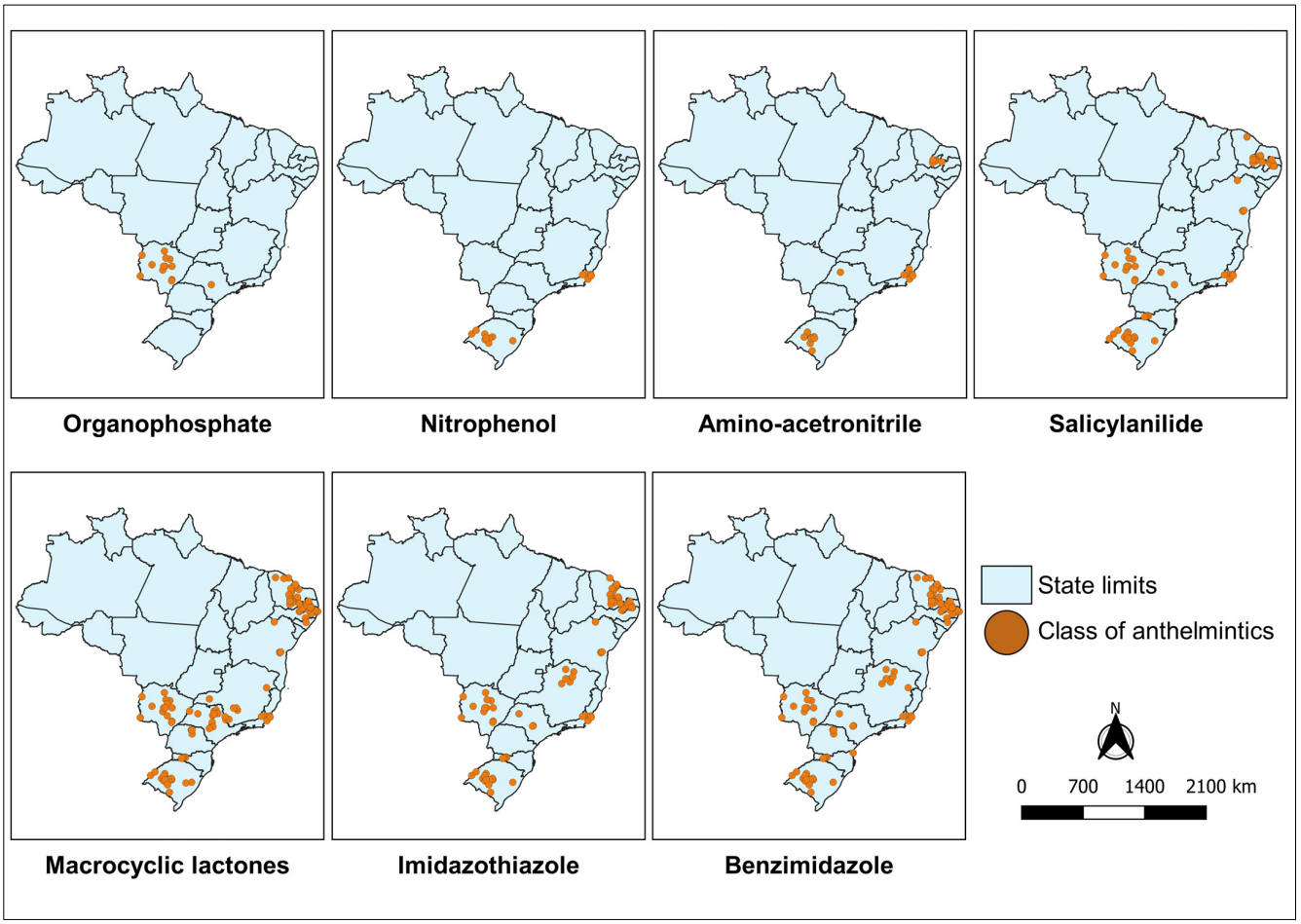

Figure 1. Geographic distribution of anthelmintic resistance according of the class of drugs used in ruminants from Brazil.

From 1960 to 1999, 18 articles (21.7%) reported AR in domestic ruminants, with one in cattle (5.6%; 1/18), three in goats (16.7%; 3/18) and fourteen (77.8%; 15/18) in sheep. From 2000 to 2023, the number of reports tripled, with 65 articles (78.3%) distributed across 14 states and 127 municipalities. Most of the studies were concentrated in the southern (39.8%; 33/83), northeastern (31.3%; 26/83), southeastern (22.9%; 19/83) and midwestern (6.0%; 5/83) regions. Despite lower reliability, some references [33,38,42–73] that do not include animal numbers or locations were considered in this review. Figure 2 illustrates the map with the graphical representation of the distribution of AR in domestic ruminants from Brazil.

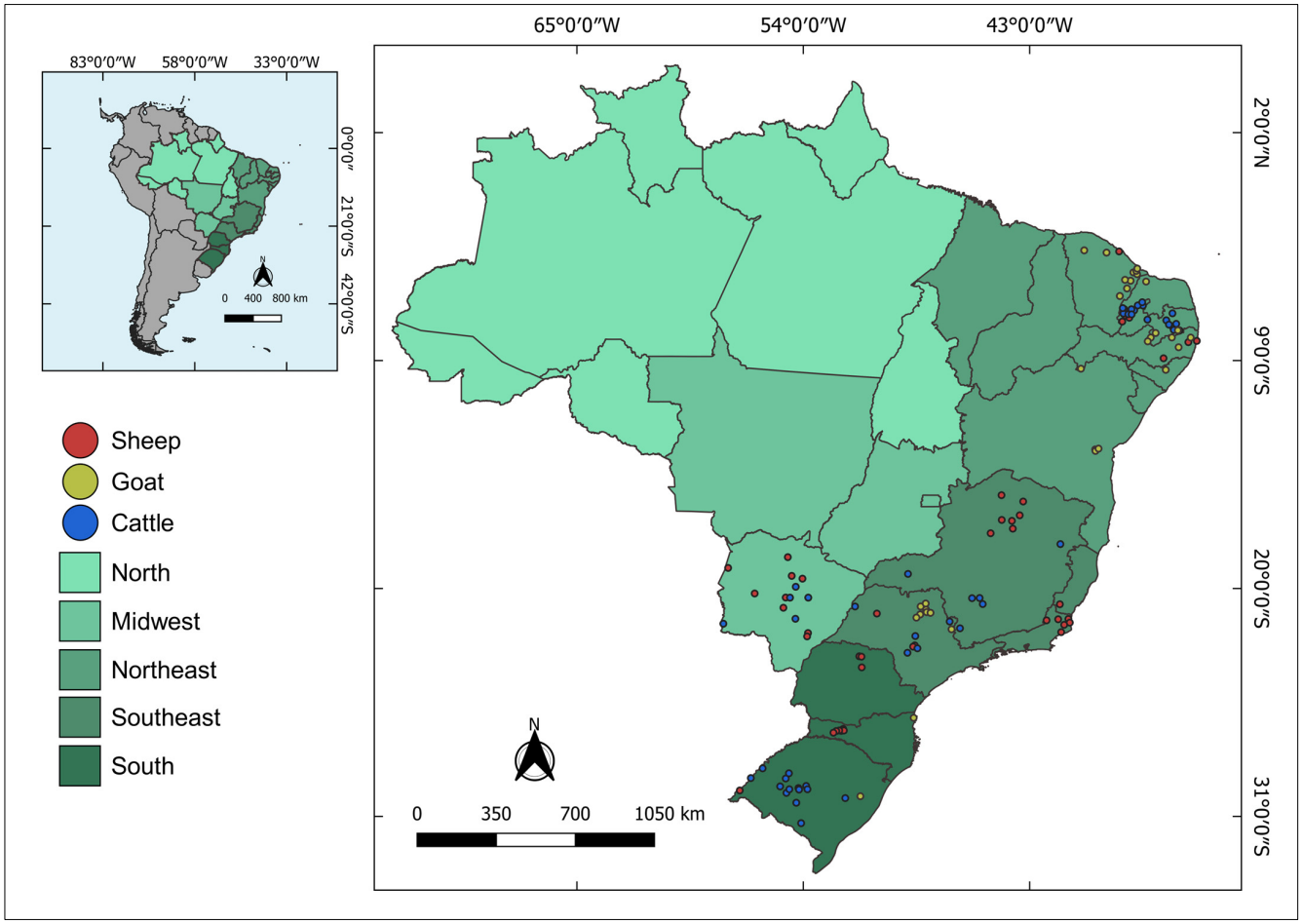

Figure 2. Geographic distribution of anthelmintic resistance according to published records in cattle, goats and sheep from Brazil.

3.1. Anthelmintic Resistance in Small Ruminants

Sixty-three (75.9%) peer-reviewed articles have been published on small ruminant AR, including 51.8% (43/83) in sheep and 24.1% (20/83) in goats. Anthelmintic resistance was reported in 92 municipalities from four regions of the country: (i) south—states of Paraná, Rio Grande do Sul and Santa Catarina; (ii) southwest—states of Minas Gerais, Rio de Janeiro and São Paulo; (iii) midwest—state of Mato Grosso do Sul; and (iv) northeast—states of Ceará, Paraíba, Pernambuco and Rio Grande do Norte (Tables 1 and 2). The lack of information in the north of the country does not mean the absence of AR. However, it is worth mentioning that this region of the country concentrates the smallest number of small ruminants (2.3% of total Brazilian herd—IBGE, 2021), while the northeastern and southern regions possess the vast majority of goats and sheep (91.9%). Additionally, most of the research has been conducted in these regions.

Table 1. Distribution of anthelmintic resistance in sheep from Brazil.

Region/State	Municipality	Anthelmintics	Number of Animals	Diagnostic Method	References
Southern					
RS	NI	THI	308	FECRT	[34]
NI	NI	LEV	NI	NI	[41]
NI	NI	LEV	NI	NI	[42]
NI	NI	LEV	6	Necropsy	[43]
RS	Uruguaina	RAF	9	FECRT	[44]
RS	Bagé	ALB, LEV	NI	FECRT	[45]
RS	Bagé	IVE	89	FECRT	[34]
RS	NI	ALB, LEV, IVE, CLO, ALB + LEV	NI	FECRT	[46]
PR	NI	ALB, CLO, LEV, FEB, IVE, TET, DIS + TET	480	FECRT	[47]
RS	NI	LEV, ALB, FEB, OXF, MEB	870	FECRT	[48]
PR	Cambé, Tamarana and Londrina	IVE, ALB, MOX	850	FECRT	[74]
PR	NI	OXF, IVE, CLO, CLO + OXF, LEV, MOX	NI	FECRT	[49]
SC	NI	IVE, LEV, CLO, ALB	7529	FECRT	[50]
SC	Passos Maia, Vargeão, Ponte Serrada, Faxinal dos Guedes, Xanxere and Xaxim	IVE, ALB, MOX, CLO, LEV	450	FECRT	[75]
PR	NI	CLO + ALB, IVE	120	FECRT	[51]
RS	NI	LEV, MON, ALB, IVE, NIT, DIS, TRI, CLO, IVE + LEV + ALB	500	FECRT	[76]
SC	NI	CLO, LEV, ALB, ALB + LEV	135	FECRT	[52]
PR	NI	MON	50	FECRT/CT	[53]
RS	NI	MOX, FEB	78	FECRT	[54]
RS	NI	ABA, ALB, CLO, LEV, MON, TRI	1540	FECRT	[55]
RS	São Pedro do Sul, São Gabriel and São Martinho da Serra	MON	NI	FECRT	[56]
RS	São Gabriel, São Martinho da Serra, Dilermando de Aguiar, Bagé, Capão do Cipó, São Francisco de Assis and Santa Maria	IVE, DOR, MON, LEV, ALB, CLO	366	FECRT	[57]

Table 1. *Cont.*

Region/State	Municipality	Anthelmintics	Number of Animals	Diagnostic Method	References
Southeastern					
SP	São Manuel	OXF, IVE, LEV	540	FECRT	[58]
SP	Jaboticabal	MOX	24	FECRT	[77]
SP	Pratânia	ALB, LEV, MOX, IVE, TRI, CLO	42	FECRT	[78]
RJ	Campos dos Goytacazes, Cardoso Moreira, Quissamã, São Francisco de Itabapoana, Santo Antonio de Pádua and São João da Barra	ALB, CLO, DIS, FEB, IVE, MON, NIT	770	FECRT	[79]
MG	Montes Claros, Bocaiúva, Janaúba, Pirapora, Francisco Sá, Coração de Jesus and Januária	ALB	252	FECRT	[80]
SP	NI	ALB, CLO, IVE, LEV, MON	1617	FECRT	[81]
SP	Jaboticabal, Viradouro, Pontal, Morro Agudo, Sertãozinho, Ribeirão Preto, Taquaritinga and São João da Boa Vista	IVE, MOX	160	Necropsy	[82]
SP	Araçatuba	ALB, LEV, IVE, MON, CLO, IVE + LEV + ALB	350	FECRT	[59]
SP	NI	ALB, LEV, IVE, MON, THI	245	FECRT/LDT	[83]
ES	Alegre	MON	20	FECRT/Necropsy	[84]
MG	NI	ALB, IVE, LEV	381	FECRT	[60]
Midwest					
MS	Angélica, Camapuã, Campo Grande, Corumbá, Coxim, Ivinhema, Miranda, Porto Murtinho, Ribas do Rio Pardo, São Gabriel do Oeste, Sidrolândia, Terenos, Camapuã, Campo Grande, Miranda and Porto Murtinho	ALB, CLO, IVE, LEV, MOX, TRI, ALB + IVE + LEV	120	FECRT	[85]
Northeastern					
CE	Sobral	NET, IVE	20	FECRT	[61]
CE	Pentecoste	CLO, OXF	38	FECRT	[86]
CE	Limoeiro do Norte, Palhano, Jaguaruana, Itaiçaba, Aracati, Alto Santo, Morada Nova and Jaguaribe	OXF, LEV, IVE	768	FECRT	[87]

Table 1. *Cont.*

Region/State	Municipality	Anthelmintics	Number of Animals	Diagnostic Method	References
CE	Limoeiro do Norte, Aracati e Jaguaribe	OXF	144	FECRT	[88]
RN	NI	ALB, IVE	54	FECRT	[89]
PE	Recife, Vitória de Santo Antão and Garanhuns	ALB	NI	FECRT	[90]
PB	Gado Bravo	IVE, LEV	234	FECRT	[62]
PB	Aparecida, Marizópolis, Patos, Souza, São José da Lagoa Tapada, São josé de Piranhas and São José do Rio do Peixe	ALB, IVE, CLO, LEV, MON	600	FECRT	[63]
CE	Caucaia	ALB, IVE, LEV	74	EHT, FECRT/LDT/qPCR	[91]

ALB: albendazole; OXF: oxfendazole; FEB: febendazole; PYR: pyrantel; RAF: rafoxanide; THI: thiabendazole; TET: tetramisole; LEV: levamisole; DOR: doramectin; IVE: ivermectin; MOX: moxidectin; CLO: closantel; TRI: trichloform; NIT: nitroxynil; DIS: disophenol; NET: netobimin; MEB: mebendazole; MON: monepantel; ABA: abamectin; (+): associations of drugs; (NI): not informed; FECRT: fecal egg count reduction test; CT: controlled efficacy test; LDT: larval development test; EHT: egg hatch test; qPCR: quantitative real-time PCR.

Table 2. Distribution of anthelmintic resistance in goats from Brazil.

Region/State	Municipality	Anthelmintics	Number of Animals	Diagnostic Method	References
Southern					
RS	Gravataí	CLO, LEV	40	FECRT	[92]
RS	Porto Alegre	IVE	12	FECRT	[93]
SC	São Francisco do Sul	ALB, ABA, CLO, NIT, LEV, MOX, IVE, IVER + LEV + ALB	63	FECRT	[94]
PR	NI	MOX	45	FECRT	[95]
Southeastern					
SP	Jaboticabal, Viradouro, Pontal, Morro Agudo, Sertãozinho, Ribeirão Preto, Taquaritinga and São João da Boa Vista	IVE, MOX	160	Necropsy	[82]
Northeastern					
CE	Sobral	OXF, FEB, ALB, THI	25	FECRT	[35]
CE	Pentecoste	IVE, CLO	29	FECRT	[87]
CE	NI	OXF, LEV	1020	FECRT	[96]

Table 2. *Cont.*

Region/State	Municipality	Anthelmintics	Number of Animals	Diagnostic Method	References
CE	Limoeiro do Norte, Palhano, Jaguaruana, Itaiçaba, Aracati, Alto Santo, Morada Nova and Jaguaribe	OXF, LEV, IVE	336	FECRT	[88]
AL	Mar Vermelho	IVE, ALB	40	FECRT	[64]
CE	Sobral	EPR	24	FECRT	[65]
PB	NI	ALB, IVE	120	FECRT	[97]
RN	NI	ALB, IVE	54	FECRT	[89]
RN	Mossoró	ALB, IVE	1350	FECRT	[98]
PB	Monteiro	ALB, IVE, LEV	264	FECRT	[66]
PE	Sertânia, Paudalho, Camocim de São Félix and Taquaritinga do Norte	ALB, IVE	NI	FECRT	[90]
PB	Gado Bravo	IVE	270	FECRT	[62]
PB	Sumé	ALB	40	FECRT	[99]
BA	Santa Inês, Cravolândia and Ubaíra	ALB, IVE, LEV, MOX, CLO	360	FECRT	[100]
PE	Petrolina	ALB, IVE, LEV, MOX, CLO	420	FECRT	[101]

ALB: albendazole; OXF: oxfendazole; FEB: febendazole; PYR: pyrantel; THI: thiabendazole; LEV: levamisole; NIT: nitroxynil; IVE: ivermectin; MOX: moxidectin; CLO: closantel; TRI: trichloform; EPR: eprinomectin; (+): associations of drugs; (NI): not informed; FECRT: fecal egg count reduction test.

Overall, 24,449 animals were assessed primarily through FECRT (85.7%; 54/63). These studies evaluated different classes of drugs but mainly molecules belonging to the class of benzimidazole (i.e., albendazole, thiabendazole, oxfendazole, mebendazole and febendazole), macrocyclic lactones (i.e., ivermectin, doramectin, abamectin and moxidectin) and imidazothiazole (i.e., levamisole and tetramisole).

The AR is more serious than has been documented so far. The gradual growth from its somewhat sporadic occurrence in the early 1960s to the current situation threatens the sustainability of production systems. The primary tool adopted for controlling GIN parasites is the use of anthelmintics, which generally positively impact the well-being and health of domestic and production animals [3]. It is known that AR is an evolutionary process that is unpredictable if anthelmintics are used intensively in a herd [102]. Nonetheless, it is possible to reduce the rate of resistance development by modifying anthelmintic use strategies [15]. Particularly in sheep, resistance of *H. contortus* is associated with economic losses and mortality [3,103]. In addition, there is more research and wider availability of drugs for this animal species (51.8%), reflecting more AR reports compared with goats and cattle. For example, the nematodes of sheep treated intensively with monepantel may show resistance to this drug in three months [104]. Similarly, lambs treated with levamisole every 42 days may present resistant nematode populations after the third treatment [7].

With the decreasing effectiveness of anthelmintics, the prophylaxis of GIN infections in small ruminants has become more challenging. Hence, the rational and integrated use of these compounds with sustainable measures of control is necessary to prevent AR [105]. Unfortunately, GIN of sheep developed resistance to a more recent molecule available commercially, the amino-acetonitrile derivatives [14,106–108].

3.2. Anthelmintic Resistance in Cattle

Twenty articles were found reporting AR in cattle. These reports originated from 51 municipalities, distributed in the southern (Rio Grande do Sul and Santa Catarina), southeastern (Minas Gerais and São Paulo), midwestern (Mato Grosso do Sul) and northeastern (Paraíba) regions of Brazil (Table 3). In total, 6729 individual animals were assessed for AR. Similarly, to small ruminants, most studies used FECRT (70.0%; 14/20) as a method for resistance detection. These studies evaluated different classes of drugs but mainly evaluated drugs from the class of macrocyclic lactones (avermectins and milbemycins).

In cattle, using chemical compounds to control infections by GIN is commonly conducted with broad-spectrum molecules (macrocyclic lactones, benzimidazoles, imidazothiazoles and salicylanilides). Mainly, macrocyclic lactones are used worldwide in ruminant livestock. They are available in different formulations, concentrations and associations, with ivermectin being the predominant chemical compound [31]. Frequently, these drugs are administered without any technical criteria for drug selection, and this empirical and indiscriminate use has favored AR development. This incorrect use has additional implications for the effectiveness of anthelmintic treatments, as it causes the emergence and spread of parasite resistance [56]. Unlike the development of resistance in small ruminants, in cattle, this phenomenon occurred slower; however, in recent decades, there has been a rapid increase in reports of AR in GIN infection of cattle worldwide [29,56,109–111]. It is imperative to address the AR issue in cattle, with the view of a significant threat to cattle productivity [102].

Table 3. Distribution of anthelmintic resistance in cattle from Brazil.

Region/State	Municipality	Anthelmintics	Number of Animals	Diagnostic Method	References
Southern					
RS	NI	ALB, OXF	16	FECRT	[37]
SC	NI	IVE, LEV, ALB	2340	FECRT	[112]
RS	São Pedro do Sul	IVE, DOR, ABA, MOX, ALB	149	FECRT	[113]
RS	Butiá	IVE	144	Necropsy	[67]
RS	São Martinho da Serra, Dilermando de Aguiar, Cacequi, São Gabriel, Itaqui, São Borja, Santiago and São Vicente do Sul	IVE, DOR, EPR, MOX, LEV, ALB, FEB, CLO, NIT, DIS, ALB + CLO, DOR + CLO	1704	FECRT	[114]
RS	São Gabriel, São Martinho da Serra and Dilermando de	IVE, DOR, MON, LEV, ALB, CLO	384	FECRT	[56]
RS	São Gabriel	IVE, DOR, ABA, EPR, MOX	70	FECRT	[115]
Southeastern					
SP	NI	IVE	187	FECRT	[116]
SP	Castilho	MOX	20	FECRT	[117]
MG	NI	IVE	24	Necropsy	[68]
MG	Teófilo Otoni	IVE, ALB, ABA, DOR	84	FECRT	[118]
MG/SP	Candeias, Formiga, Pimenta, Caldas, Prata, Jaboticabal and São José do Rio Pardo	IVE	144	Necropsy	[67]
MG	NI	IVE, MOX	40	FECRT/Necropsy	[69]
SP	Jaú, Botucatu and Avaré	IVE	99	FECRT	[119]
Midwest					
MS	NI	IVE, MOX	NI	FECRT	[70]
MS	Bandeirantes, Campo Grande, Porto Mortinho and Nova Alvorada do Sul	IVE	NI	LMIT	[120]
MS	NI	DOR, IVE	24	FECRT/Necropsy	[71]
MS	Ribas do Rio Pardo	MOX, IVE, ABA, ABA + IVE	300	FECRT	[121]
Northeastern					
PB	NI	IVE, ALB, OXF, LEV, TET, CLO, DIS, PYR, MOR	200	FECRT	[72]
PB	Uiraúna, Aroeira, S. J. Rio do Peixe, Caturité, Barra de Santana, Soledade, Lagoa, Patos, Bom Sucesso, Campina Grande, Santa Cruz, Boa Vista, Gado Bravo, Barra de Santa Rosa, Brejo do Cruz, Joca Claudino, Catolé do Rocha, Belém do Brejo do Cruz, Souza and Aparecida	ALB, IVE, CLO, LEV	800	FECRT	[29]

ALB: albendazole; OXF: oxfendazole; FEB: febendazole; EPR: eprinomectin; PYR: pyrantel; LEV: levamisole; DOR: doramectin; IVE: ivermectin; MOX: moxidectin; CLO: closantel; TRI: trichloform; NIT: nitroxynil; DIS: disophenol; MON: monepantel; MOR: Morantel; ABA: abamectin; (+): associations of drugs; (NI): not informed; FECRT: fecal egg count reduction test; LMIT: larval migration inhibition test.

The most frequent helminths in Brazilian cattle herds are *Cooperia* spp. and *Haemonchus placei* [122], which were identified in several reports of AR [67,71,116]. Resistance of the genus *Cooperia* is also common in countries such as Argentina [73], United Kingdom [123], Mexico [124], Sweden, Belgium, Germany [125], United States [126,127] and Australia [128,129]. Most of these reports are related to resistance to ivermectin. Infection by *Cooperia punctata* can significantly impact productivity by reducing weight gain and decreasing feed intake [127]. In addition, it influences phosphorus kinetics, reducing food intake and altering phosphorus absorption and retention [130].

4. Current Methods for Detection of AR

The primary method for detecting resistance is FECRT, which can be used with all anthelmintic groups. Nematode eggs are counted at pre- and post-treatment times defined according to the anthelmintic group used [131]. However, it is unsuitable for detecting resistance levels below 25% [132]. Several factors must be considered when planning an FECRT (i.e., study design, sample size considerations, choice of fecal egg count (FEC) method, statistical data analysis and interpretation) [133]. Other in vitro tests have been used less, such as the EHT, established to detect drug resistance in the benzimidazole class [134]. In addition, it is possible to use tests evaluating larval development and motility (LDT and LMIT) [15]. Particularly in cattle, most animals in a herd, even the young ones, have lower FEC, making diagnosing AR difficult [20]. For example, the McMaster technique is the most used method in studies of AR detection but it has a detection limit of 50 EPG [135]. The use of methods with higher detection, such as FLOTAC (one EPG) and Mini-FLOTAC (five EPG), might be encouraged in this kind of analysis [136,137]. The consensus is that there is a need for improvements in the AR detection methods, such as more reliable parasitological tests and an increase in the number of animals required for simultaneous testing on several drugs [119].

With the limitations of current in vivo and in vitro resistance tests, molecular tools can potentially improve drug resistance diagnosis [138]. The development of molecular diagnostics for anthelmintic resistance has been one of the leading research topics involving the molecular mechanisms of drug resistance [139]. Thus, developing molecular markers for diagnosing resistance can help develop new anthelmintic drugs [140]. The molecular mechanism of resistance is better understood for benzimidazoles; therefore, it offers a potential opportunity to expand molecular diagnostic tests for drugs of this class [141]. For example, in Brazil, some studies were conducted using the β-tubulin isotype gene, a marker to monitor resistance [141–143]. In addition, molecular characterization is an essential tool for the validation and phylogenetic analysis of nematodes, such as allele-specific polymerase chain reaction, endpoint polymerase chain reaction (PCR), semi-quantitative PCR, quantitative PCR (qPCR), high-resolution melt curve analysis (HRMC) and "Nemabiome" internal transcribed spacer 2 (ITS-2) amplicon sequencing [144,145].

A recent development in large-scale surveillance is the "Nemabiome" approach, which applies deep amplicon sequencing of barcoded PCR products [146]. Although initially developed for species identification and quantification, it has recently been adapted to assess the presence of resistance by benzimidazoles by deep sequencing of β-tubulin amplicons [147]. In general, molecular tests have greater sensitivity and specificity and can provide powerful tools to overcome many of the disadvantages of classical methods of AR. However, it requires further research to be used as a practical universal tool in the field.

5. How to Prevent AR Development?

It has already been proved that the excessive and incorrect use of anthelmintics to control GIN infections has resulted in AR. However, concerns about the use of these products are more comprehensive than studies of AR itself. Recently, with the improvement in awareness about the consumption of organic products, there has been a rise in concern with the potential residual effect of these products in meat and milk, derived products from ruminants that are widely consumed worldwide [148]. Despite reducing the withdrawal

period, the risks associated with residues in milk intended for human consumption and dairy products may be present and should be considered [149]. For example, a study with moxidectin demonstrated that this molecule may be present in goat milk for up to 21 days [150]. Additionally, to the direct consequence of using anthelmintics, the excretion of these by-products may also be considered an essential threat from an environmental perspective [151]. The access of anthelmintic residues into the environment resulting from the direct excretion of the original drugs and metabolites in pastures during grazing, as well as through the dispersion of the manure and slurry containing anthelmintic residues, represents a potential risk for the environment [152].

Hence, studies focusing on controlling GIN but with a rational use of these chemical molecules might be encouraged. Investigating the antiparasitic activity of natural bioproducts can contribute to the development of alternative treatments and a reduction in dependence on conventional chemotherapy [153]. The antiparasitic activity of plants derives mainly from biologically active compounds known as secondary metabolites, which could lead to the detection of new antiparasitic molecules [154]. For example, flavonoids and condensed tannins may have anthelmintic effects, as demonstrated in a study inhibiting in vitro sheathing of larvae (L3) of *H. contortus* [155]. In addition, using nanoparticles can provide good results in the treatment of parasitic infections because they increase the bioavailability and biodistribution of drugs. However, the safety of using nanoparticles from a broader perspective needs to be better investigated [156,157].

So far, most of the studies have been conducted in lab conditions, as they have low cost, repeatability and allow the use of different stages (i.e., eggs and larvae) [152]. Although these plant alternatives can be cheap and accessible, they have limitations. These molecules' potential adverse toxicity effects in vivo are generally controversial or completely unknown [157,158]. In vivo studies consist of oral administration of the leaves (fresh, hay and flour), aqueous or ethanolic extracts and oil of plants to ruminants infected naturally or experimentally with GIN [151,159]. Therefore, the association of standardized in vivo and in vitro methods is paramount for evaluating the effectiveness of plant products, especially for the determination of EC_{50} and EC_{90} (50% and 90% maximal effective concentration, respectively), which allows comparing the activities of different plants [160].

In order to postpone the development of AR, it is necessary to integrate GIN control measures. Therefore, some factors are essential to be considered: (i) good management has a direct effect on the health of animals with feeders and drinkers that avoid waste and contamination [161]; (ii) strategies such as grazing rotation, co-grazing with other appropriate species and manure management are alternatives to reduce the use of anthelmintics [162]; (iii) the improvement of animal resistance through genetic selection to reduce the use of chemoprophylaxis [163]; and (iv) to optimize the effectiveness of anthelmintics in populations of multiresistant nematodes, drug combinations can be used [114]. It is worth emphasizing the importance of carrying out anthelmintic efficacy tests for choosing the chemical groups to be used. The need to develop new anthelmintics for the management of AR is evident; however, it is a slow and expensive process [164]. Furthermore, it is crucial to use existing anthelmintics in a way that minimizes the impact of AR [165].

6. Conclusion Remarks and Perspective for Future Research

The present review demonstrates that AR is an urgent and emerging issue for ruminant production in Brazil, especially in the southern and northeastern regions, where most of the data discussed herein were produced. It is necessary to evaluate on a large scale the distribution and management of anthelmintic drugs currently available and discuss strategies to prevent the development of this phenomenon. Technological advances in diagnostic tools associated with individual management of animals with continuous monitoring are fundamental issues to better guide the control of GIN infections. Several challenges remain, and we hope to enter a new anthelmintic era, including innovative, integrated control approaches and more sensitive and cost-effective diagnostic tools.

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