Prevalence of anthelmintic resistance on sheep and goat farms in the southeastern United States

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Objective—To determine prevalence of anthelmintic resistance on sheep and goat farms in the southeastern United States.

Design—Cross-sectional study.

Animals—Sheep and goats from 46 farms in 8 southern states, Puerto Rico, and St Croix in the US Virgin Islands.

Procedures—Parasite eggs were isolated from fecal samples, and susceptibility to benzimidazole, imidathiazole, and avermectin-milbemycin anthelmintics was evaluated with a commercial larval development assay.

Results—Haemonchus contortus was the most common parasite on 44 of 46 farms; Trichostrongylus colubriformis was the second most commonly identified parasite. Haemonchus contortus from 45 (98%), 25 (54%), 35 (76%), and 11 (24%) farms were resistant to benzimidazole, levamisole, ivermectin, and moxidectin, respectively. Resistance to all 3 classes of anthelmintics was detected on 22 (48%) farms, and resistance to all 3 classes plus moxidectin was detected on 8 farms (17%).

Conclusions and Clinical Relevance—Findings provided strong evidence that anthelmintic resistance is a serious problem on small ruminant farms throughout the southeastern United States. Owing to the frequent movement of animals among regions, the prevalence of resistance in other regions of the United States is likely to also be high. Consequently, testing of parasite eggs for anthelmintic resistance should be a routine part of parasite management on small ruminant farms. (J Am Vet Med Assoc 2008;233:1913–1919)

According to the US Census of Agriculture, goat numbers in the United States doubled between 2002 and 2006. A substantial portion of this growth was in the southeastern region of the country, with growth in the goat industry attributable to a variety of factors, such as low cost of breeding stock, high reproduction rates, profitability of meat and by-products, and ability of goats to thrive on native pastures and brush. The number of sheep in the southeastern United States has also risen in recent years, in part because of increased availability of hair sheep, which are well adapted to southern climates.

As the numbers of goats and sheep in the southeastern United States have grown, so have concerns that drug treatments for parasitism are beginning to fail because of the development of anthelmintic resistance in parasites. Anthelmintic resistance among gastrointestinal tract nematodes of small ruminants has been documented in many countries, with multidrug resistance becoming a major concern for small ruminant producers worldwide. There is little information on the prevalence of anthelmintic resistance in the United States, although high prevalences of multidrug-resistant gastrointestinal tract nematodes were reported in a study involving goat farms in Georgia and in a separate study involving sheep farms in Louisiana. To our knowledge, however, no region-wide studies have been conducted. Therefore, the purpose of the study reported here was to determine prevalence of anthelmintic resistance on sheep and goat farms in the southeastern United States.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>L₃</td>
<td>Infective third-stage larvae</td>
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</tbody>
</table>

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Several methods for detecting anthelmintic resistance in gastrointestinal tract nematodes of small ruminants have been described. The most commonly used method is the fecal egg count reduction test, which involves determining fecal egg counts in animals that have and have not been treated with anthelmintics and calculating the percentage reduction in fecal egg count among treated animals. This method is the procedure of choice for field surveys. However, it requires testing of a large number of animals on a farm if multiple anthelmintics are being evaluated, making it both labor intensive and expensive.

As a result, various in vitro methods for detecting anthelmintic resistance have been developed as alternatives to the fecal egg count reduction test. Of these, the most commonly used is the larval development assay, which involves culturing parasite eggs isolated from pooled fecal samples to L₃, in the presence of various concentrations of anthelmintics. The larval development assay has several advantages over the fecal egg count reduction test, in that it provides results more quickly, at less cost, and with less effort. In addition, sample submission is less complicated because a single pooled sample can be mailed or transported to the diagnostic laboratory at ambient temperature.

**Materials and Methods**

**Farms**—Forty-six sheep (n = 26) and goat (20) farms in Alabama (1 sheep and 1 goat farm), Arkansas (6 sheep and 3 goat farms), Florida (3 sheep and 2 goat farms), Georgia (6 sheep and 4 goat farms), Kentucky (1 sheep and 2 goat farms), Louisiana (3 sheep and 5 goat farms), Maryland (1 sheep farm), Virginia (2 sheep and 1 goat farm), Puerto Rico (2 sheep farms), and St Croix in the US Virgin Islands (1 sheep and 2 goat farms) were included in the study. Eight of the sheep and 11 of the goat farms had ≤50 animals, 10 of the sheep and 2 of the goat farms had 51 to 100 animals, and 3 of the sheep and 1 goat farm had >100 animals (information on animal numbers was not available for 11 farms). Ten sheep farms had Katahdin or Katahdin crosses as their primary breed, with the remaining sheep farms having an assortment of breeds, including Hampshire, Dorper, Suffolk, Texas Doll, Gulf Coast Native, St Croix, Santa Cruz, Royal White, and various crosses of these breeds. The most common goat breeds were Boer and Boer crosses. Other goat breeds represented included Nubian, Spanish, Saanen, and Myotonic.

**Fecal sample collection and analysis**—Individual fecal samples were collected rectally from approximately 5 to 10 animals/farm and pooled to make a single composite sample. The composite samples were rolled tightly in plastic wrap to exclude air the day of collection and then shipped by express mail at ambient temperature for overnight delivery to the University of Georgia College of Veterinary Medicine. All samples were received and processed within 72 hours after collection with the exception of 4 samples that were received and processed between 4 and 6 days after collection.

Fecal samples from an additional 10 farms were excluded from the study because of an insufficient number of eggs in the sample or poor condition of the sample when received at the laboratory (n = 7), poor larval development in control wells making proper interpretation of results impossible (2), or errors associated with handling of the assay plate (1).

Samples were weighed when received at the laboratory, fecal pellets were crushed, and an equivalent volume of water was added to create a fecal slurry. A fecal egg count was then performed on 4 g of the slurry with a modified McMaster technique. A volume of slurry needed to obtain 50,000 eggs was used for egg isolation. If the fecal egg count was too low to meet this target, the total available sample was used.

Coprocultures were prepared from each sample with 20 to 30 g of slurry and an approximately equal volume of vermiculite. Cultures were incubated for 10 to 14 days at room temperature, and L₃ were recovered with the Baermann technique and identified to the genus level. One hundred L₃ were identified, unless <100 larvae were recovered, in which case, all L₃ that were recovered were identified.

**Determination of anthelmintic resistance**—A commercial larval development assay performed in accordance with manufacturer’s directions with minor modifications was used to identify anthelmintic resistance. Nematode eggs were concentrated by filtering the fecal slurry through a series of sieves (425-µm, 180-µm, 85-µm, and 30-µm pore sizes). Material retained in the 30-µm sieve was added to a sucrose gradient and centrifuged at 1,300 × g for 7 minutes at 4°C with slow acceleration and deceleration. The egg layer was retrieved from the sucrose gradient and rinsed with deionized water to remove sucrose residue. The volume of water was then adjusted to yield a final concentration of approximately 3.5 eggs/µL, and amphotericin B was added (90 µL/mL).

Ninety-six well plates consisting of 8 rows with 12 wells in each row were used for the larval development assay. The first well in each row was designated as a control well and contained only agar. Subsequent wells in each row contained anthelmintic (thiabendazole, levamisole, or ivermectin) in agar, with each well having twice the concentration of the previous well. Importantly, drugs used in the larval development assay were not the exact same drugs present in commercial products administered to animals. Rather, with the exception of levamisole, drugs used in the assay were closely related analogs of the commercial anthelmintic products. These specific analogs were selected on the basis of previous research demonstrating optimal dose-response activities for detecting parasite drug resistance in vitro. Because drug resistance in parasites is broadly expressed toward the entire anthelmintic class and not to a specific individual drug analog, results of the larval development assay for a given drug analog are applicable to the entire anthelmintic class that drug represents.

Assay plates were warmed to room temperature, and 20 µL of deionized water was added to each well to ensure adequate moisture content on the agar surface. Frequently, an additional 20 µL of deionized water was added to the outer wells on each plate because of excessive drying of agar during storage. Twenty microliters of the egg suspension was then added to each of the wells.

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Plates were sealed with a laboratory film to prevent drying and placed in a 25°C humidifying incubator. Nutritive medium supplied with the larval development assay plates was diluted 50% with deionized water, and 20 µL was added to each well after plates had been incubated for 24 hours (ie, long enough for eggs to hatch). Plates were again sealed and returned to the incubator for 6 more days. Plates were examined every 2 to 3 days to ensure that a thin layer of moisture remained on the agar surface, and 20 µL of deionized water was added to any wells that appeared dry. Assays were terminated after 7 days by adding 20 µL of 50% Lugols iodine to each well. Larvae were then transferred to a clean flat-bottom, 96-well plate for counting and identification with an inverted compound microscope.

Plates were evaluated to determine the critical well, defined as the number of the well in which 50% of the eggs were inhibited from developing to L₁ (interpolation between 2 wells was allowed if necessary) and previously shown to be approximately equal to the 50% lethal concentration. In addition, for wells containing ivermectin, assay plates were evaluated to determine the 5% discriminating concentration (also known as the delineating dose), defined as the number of the well containing the highest concentration in which ≥5% of eggs developed to L₁ and considered equivalent to the 95% lethal concentration. In brief, plates were examined to estimate the critical well, and all larvae in these wells and in several wells above and below the estimated critical well were counted, along with all larvae in the control wells. In addition, L₁ in all wells above the critical well were counted and identified. Larvae were counted at 100X magnification and identified to the genus level.

Critical well values were the means by which drug susceptibility and resistance were evaluated in the larval development assay. However, to make clinical inferences on resistance, critical well values must be correlated with an in vivo measure of resistance, such as results of the fecal egg count reduction test. Thus, tables supplied by the manufacturer of the larval development assay showing the correlation between critical well values and percentage fecal egg count reduction were used to establish cutoffs for declaring resistance. Because of the inherent variability associated with bioassays such as the larval development assay, prior to making the conversion from critical well to expected in vivo efficacy, we added 0.5 to each critical well value (equivalent to a half well or a 100% increase in the anthelmintic concentration) so that estimates of resistant status were conservative.

For Haemonchus contortus, resistance status was classified as susceptible if estimated percentage fecal egg count reduction was ≥95% (ie, a critical well value ≤4.0), suspected resistant if estimated fecal egg count reduction was 90% to 94% (ie, a critical well value of 4.5 for thiabendazole and 4.5 or 5.0 for levamisole), low resistant if estimated fecal egg count reduction was ≥70% to 89% (ie, a critical well value of 5.0 to 6.0 for thiabendazole and 5.5 to 6.5 for levamisole), and resistant if fecal egg count reduction was <70% (ie, a critical well value ≥6.5 for thiabendazole and ≥7.0 for levamisole). Because of the low numbers of Trichostrongylus colubriformis larvae that were isolated from most fecal samples, resistance status was classified only as susceptible (estimated fecal egg count reduction ≥93%, or a critical well value ≤3.5 for thiabendazole and ≤4.5 for levamisole), suspected resistant (estimated fecal egg count reduction between 90% and 94%, or a critical well value of 4 for thiabendazole or 5 for levamisole), or resistant (estimated fecal egg count reduction <90%, or a critical well value ≥4.5 for thiabendazole or ≥5.5 for levamisole).

For H contortus, resistance status for ivermectin was classified as susceptible if the critical well value was ≤4.0, suspected resistant if the critical well value was 4.5, low resistant if the critical well value was 5.0 to 6.0, and resistant if the critical well value was ≥6.5. For T colubriformis, resistance status was classified as susceptible if the critical well value was ≤5.0, suspected resistant if the critical well value was 5.5, and resistant if the critical well value was ≥6.0. These criteria were shown to be relatively accurate in making determinations of ivermectin resistance in a previous study in which the fecal egg count reduction test was performed in concert with the larval development assay.

Both ivermectin and moxidectin are in the same anthelmintic class (avermectin-milbemycin), but the greater potency of moxidectin produces high in vivo efficacy against ivermectin-resistant parasites of sheep and goats. However, because resistance is a characteristic of the drug class and not the specific drug, ivermectin can be used in the larval development assay to determine resistance to moxidectin. Therefore, we used data obtained from ivermectin wells to make inferences on susceptibility and resistance of H contortus to moxidectin, as described. In brief, resistance status was classified as susceptible if the critical well value was ≤7.0 and the well corresponding to the 5% discriminating concentration was ≤10.0, low resistant if the critical well value was 7.5 to 9.0 and the 5% discriminating concentration value was 10.5 to 11.0, and resistant if the critical well value was >8.5 and the 5% discriminating concentration value was ≥11.5. Both criteria had to be met to assign a resistance status. If critical well and discriminating concentration values did not match the same resistance status category, resistance status of the farm was considered to be between the 2 categories. However, because farms classified as low resistant had unequivocal signs of early moxidectin resistance, all farms categorized as low resistant or resistant were classified as having H contortus that were resistant to moxidectin. No attempts were made to determine whether T colubriformis were resistant to moxidectin because no criteria have been established for this species.

Statistical analysis—Percentages of farms with H contortus and T colubriformis resistant to benzimidazole, levamisole, ivermectin, and moxidectin were calculated, and 95% CIs were determined by use of tabulated values for binomial proportions. Unpaired t tests were used to compare critical well values for thiabendazole, levamisole, and ivermectin and discriminating concentration values for ivermectin between sheep and goat farms. The χ² test was used to compare percentages of H contortus and T colubriformis resistant to benzimidazole, levamisole, and ivermectin between sheep and goat farms. All analyses were performed with standard software. For all analyses, values of P < 0.05 were considered significant.
Results

Parasite identification—The most commonly identified larvae in fecal samples from the 46 farms were *H* contortus and *T* colubriformis. For 44 of the 46 (96%) farms, ≥50% of the larvae identified following coproculture were *H* contortus, and for 30 (65%) farms, >80% of the larvae were *H* contortus (Figure 1). For 14 of the 46 (30%) farms, >20% of the larvae identified were *T* colubriformis. Other genera present in smaller numbers in fecal samples from sheep farms included Cooperia spp and Oesophagostomum spp. Genera detected in small numbers in fecal samples from some goat farms included Bunostomum spp and Teladorsagia spp.

Determination of anthelmintic resistance—Anthelmintic resistance status was determined only for those nematode species that represented ≥20% of all L3 identified following coproculture. Therefore, anthelmintic resistance status was determined for *H* contortus on all 46 farms and for *T* colubriformis on 14 farms (2 sheep and 12 goat farms). Because of the low numbers of farms for which anthelmintic resistance status of *T* colubriformis was evaluated, statistical analyses were not performed.

Development of larvae in the control wells was used as an indicator of the integrity of the fecal sample and validity of the assay. For all assays, mean percentage of eggs that developed to L3 in the control wells was approximately 81%.

For 45 of the 46 farms (98%; 95% CI, 88.5% to 99.9%), *H* contortus recovered from fecal samples was classified as resistant or low resistant to benzimidazole anthelmintics (Table 1). Mean critical well values for thiabendazole were not significantly (P = 0.099) different between sheep and goat farms, and percentage of farms with *H* contortus resistant or low resistant to benzimidazole did not differ significantly (P > 0.099) between sheep and goat farms (Figure 2). For all 14 farms tested, *T* colubriformis recovered from fecal samples was classified as resistant to benzimidazole anthelmintics.

For 25 farms (54%; 95% CI, 39.0% to 69.1%), *H* contortus was classified as resistant (fecal samples from 1 sheep and 3 goat farms) or low resistant (fecal samples from 13 sheep and 8 goat farms) to levamisole (Table 1). Mean critical well values for levamisole were not significantly (P = 0.436) different between sheep and goat farms, and percentages of farms with *H* contortus resistant or low resistant to levamisole did not differ significantly (P > 0.099) between sheep and goat farms (Figure 2). For 8 (1 sheep and 7 goat farms) of the 14 farms, *T* colubriformis recovered from fecal samples was classified as resistant to levamisole.

For 35 farms (76%; 95% CI, 61.2% to 87.4%), *H* contortus recovered from fecal samples was classified as resistant (fecal samples from 11 sheep and 13 goat farms) or low resistant (fecal samples from 6 sheep and 5 goat farms) to ivermectin (Table 1). Mean critical well value for ivermectin

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Table 1—Anthelmintic resistance status of *Haemonchus contortus* and *Trichostrongylus colubriformis* parasites recovered from pooled fecal samples from 26 sheep and 20 goat farms in the southeastern United States.

<table>
<thead>
<tr>
<th>Resistance status</th>
<th><em>H</em> contortus</th>
<th><em>T</em> colubriformis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BZ</td>
<td>LEV</td>
</tr>
<tr>
<td>All farms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Susceptible</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Suspected resistant</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Low resistant</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>Resistant</td>
<td>43</td>
<td>4</td>
</tr>
<tr>
<td>Sheep farms</td>
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<td>Resistant</td>
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</tr>
<tr>
<td>Goat farms</td>
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<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Resistant</td>
<td>20</td>
<td>3</td>
</tr>
</tbody>
</table>

*Haemonchus contortus* parasites from all 46 farms and *Trichostrongylus colubriformis* parasites from 2 sheep and 12 goat farms were tested for anthelmintic resistance with a larval development assay.

BZ = Benzimidazole, LEV = Levamisole, VM = Ivermectin, MOX = Moxidectin. NA = Not applicable (this resistance category was not used for *T* colubriformis).

*Farms with status between susceptible and low resistant are included in the suspected resistant category. Farms with status between low resistant and resistant are included in the low resistant category.*
was significantly ($P = 0.034$) higher on goat than on sheep farms, but percentages of farms with $H$ contortus resistant or low resistant to ivermectin did not differ significantly ($P = 0.082$) between sheep and goat farms (Figure 3). For 7 farms (2 sheep and 5 goat farms), $T$ colubriformis recovered from fecal samples was classified as resistant to ivermectin.

Multiple resistance to all 3 anthelmintic classes (benzimidazole, levamisole, and ivermectin) was detected in $H$ contortus recovered from 22 (11 sheep and 11 goat) of the 46 (48%) farms.

For 32 (19 sheep and 13 goat) farms, both critical well and discriminating concentration values indicated that $H$ contortus was susceptible to moxidectin (Figure 3). For 3 sheep farms, critical well and discriminating concentration values did not fall into the same moxidectin resistance categories, and these farms were classified as having a resistance status between susceptible and low resistant. The remaining 11 (4 sheep and 7 goat) farms (24%; 95% CI, 12.6% to 38.8%) were classified as resistant to moxidectin. Discriminating concentration values did not differ significantly ($P = 0.184$) between sheep and goat farms, nor did percentages of farms with $H$ contortus resistant to moxidectin ($P = 0.169$).

**Discussion**

Results of the present study suggested that high percentages of $H$ contortus and $T$ colubriformis parasites from sheep and goat farms in the southeastern United States were resistant to commonly used anthelmintics. Numerous factors were likely responsible for the high percentages of anthelmintic resistance identified in the present study. However, factors that were of the greatest importance likely included indiscriminate use and overuse of anthelmintics, a general lack of biosecurity on the farm, frequent movement of animals off of and onto the farm, insufficient quarantine procedures for new arrivals, and a failure...
to treat new arrivals with effective anthelmintics during the quarantine period. Such factors would promote the dissemination and spread of anthelmintic-resistant parasites.

None of the 46 farms in the present study had *H. contortus* that were susceptible to all 3 classes of anthelmintics tested. The best result was for a single sheep farm in Virginia, for which *H. contortus* was classified as susceptible to benzimidazole, ivermectin, and moxidectin and suspected resistant to levamisole.

A finding of particular concern was that *H. contortus* that was resistant to all 3 classes of anthelmintics was recovered from 22 of the 46 (48%) farms in the present study. By contrast, a previous study performed several years earlier detected multidrug resistance by means of the fecal egg count reduction test on 33% of the goat farms tested. An additional serious and even greater concern was our finding that 8 of the 46 (17%) farms had *H. contortus* that was resistant to all 3 drug classes plus moxidectin.

An interesting finding in the present study was that for *H. contortus*, critical well values for ivermectin were significantly different between sheep and goat farms, even though percentages of farms with *H. contortus* resistant to ivermectin did not differ significantly between farms. This suggests that parasites on the goat farms may have evolved a higher level of resistance to avermectin-milbemycin anthelmintics. It is possible that because of long-standing problems with anthelmintic resistance, goat producers started using moxidectin sooner, and therefore, there has been more time for parasites to develop higher levels of avermectin-milbemycin resistance. A recent study in Australia found that moxidectin use was associated with a higher prevalence of resistance to avermectin-milbemycin anthelmintics on sheep farms. It is also likely that differences between sheep and goats in regard to dosages and pharmacokinetics of moxidectin may have contributed.

Phenotypic differences among parasite isolates may cause individual isolates to show a nominal difference in drug response, compared with the standard used by the manufacturer of the larval development assay to establish criteria for defining anthelmintic resistance. For this reason, we were conservative in assigning cut-offs for resistance and included a borderline category (suspected resistant) to avoid designating a farm resistant when it was, in fact, susceptible. Cutoffs for assigning a status of low resistant represented critical well values 1.5 to 2.0 higher than the cutoff for assigning a status of susceptible, which corresponded to a 3- to 4-fold increase in drug concentration. Thus, we believe that low resistant represented an important shift towards resistance. Cutoffs for assigning a status of resistant represented critical well values ≥ 2.5 higher than the cutoff for assigning a status of susceptible, which corresponded to a 5-fold increase in drug concentration. It is possible that a few farms in the present study may have been incorrectly classified because critical well values were near the cutoff between anthelmintic resistance statuses, and it is possible that slightly different results might have been obtained if the larval development assay had been repeated. Nevertheless, when the data are viewed as a whole, the results and clinical implications are clear.

Results of the present study make it apparent that small ruminant producers and veterinarians can no longer rely solely on anthelmintics for parasite control. To preserve the few drugs that are still effective, veterinarians and producers must change their attitudes and approaches to parasite control. Anthelmintics should be thought of as extremely valuable, limited resources that should be used less frequently and only in conjunction with non-anthelmintic parasite-control measures. The term smart drenching has been used to refer to strategies designed to maximize the effectiveness of anthelmintics while reducing the development of resistance. One component of smart drenching involves selectively treating only those animals that require anthelmintic treatment. For *H. contortus*, the FAMACHA method has proven to be effective in identifying animals that are anemic and thus in most need of treatment. Monitoring changes in body condition, body weight, and milk yields in dairy goats can also be used to assist in making selective treatment decisions.

Alternatives to anthelmintic treatment that are being investigated include the use of copper oxide wire particles, feeding of forages that contain condensed tannins, administering nematode-trapping fungi, and employing principles of sound pasture management. Our findings provide clear and unequivocal evidence that multidrug-resistant parasites threaten the viability of small ruminant production in the southeastern United States. It is also likely that similar patterns of resistance exist elsewhere in the United States. Consequently, routine testing of anthelmintic efficacy with the fecal egg count reduction test or the larval development assay should be part of all herd health and parasite-control programs. In addition, strict quarantine procedures should be instituted for all new herd additions to prevent introduction of resistant parasites from newly acquired animals. Alone, any one of the alternative strategies discussed is unlikely to substantially reduce parasite burdens. However, when used together in an integrated system, these approaches can greatly reduce production and death losses associated with *H. contortus* and reduce the need for anthelmintic treatment thus slowing the development of anthelmintic resistance.

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