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Research Note

Prevalence of Antimicrobial Residues in Table Eggs in Trinidad

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ABSTRACT

The prevalence of antimicrobial residues in pooled table eggs from layer farms, shopping malls, and supermarkets in Trinidad was determined. A total of 23 layer farms and 14 shopping malls were sampled twice, 1 month apart, whereas 102 supermarkets were each sampled once. For each farm, 25 eggs were randomly collected and pooled to constitute a composite sample, whereas six eggs from each farm source available at sale outlets were randomly sampled from malls and supermarkets to constitute a composite sample. Questionnaires were administered at the farms to determine the occurrence of risk factors for contamination of antimicrobial residues in eggs and at sale outlets to determine storage conditions. The Charm II test was used to qualitatively detect antimicrobial residues (β-lactams, macrolides, sulfonamides, and tetracyclines). Of 46 composite eggs tested from farms, 3 (6.5%) were contaminated with residues compared with 5 (16.1%) of 31 and 16 (15.0%) of 107 mall and supermarket eggs, respectively, but the difference was not statistically significant ($P > 0.05$). The residues detected were as follows: sulfonamides, 12 (6.5%) of 184; macrolides, 7 (3.8%) of 184; tetracycline, 5 (2.7%) of 184; and β-lactam, 0 (0.0%) of 184. The difference was statistically significant ($P < 0.05$). The use of medicated feeds on farm, claim of adherence to the antimicrobial withdrawal period, and temperature of egg storage did not significantly ($P > 0.05$) affect the prevalence of residues in eggs. It was concluded that the presence of antimicrobial residues, particularly sulfonamides, in table eggs could be of public health significance to the consumer.

In the livestock industry, antimicrobial agents are used in chemoprophylaxis, in chemotherapy, and as feed additives, particularly as growth promoters *(7, 12, 20)*. Use of these antimicrobial agents in food animals results in the excretion of their metabolites in body fluids, as well as their accumulation in the body tissues or products such as eggs *(4, 11, 20)*. It has been established that antimicrobial residues may cause side effects such as direct toxicity, involve allergic reactions, and aid the development of antimicrobial resistance among bacterial pathogens *(9)*.

Studies have determined the metabolic profiles of antimicrobial residues in chickens with the detection of residues in the liver, muscles, and eggs *(3, 6, 14)*. In an effort to reduce the risk of contamination of eggs by antimicrobial residues, maximum residue levels were established for various antimicrobial agents in food *(5, 8, 15)*.

In Trinidad and Tobago, antimicrobial residues have been detected in raw and processed milk products *(1, 2)*. It is known that antimicrobial agents are widely used in the poultry industry, but to date the prevalence of residues in table eggs consumed in the population is unknown. The present study was therefore conducted to determine the prevalence of four antimicrobial residues in table eggs sold in Trinidad and to relate farm practices to contamination of eggs by antimicrobial residues.

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MATERIALS AND METHODS

Study design. The study design was to sample table eggs from layer farms and sale outlets (shopping malls and supermarkets) across Trinidad. At the farms, 25 eggs were randomly collected from each farm, with samples proportionally distributed based on bird population and the number of poultry houses. Each farm was sampled twice, 1 month apart. At the shopping malls and supermarkets, six eggs were randomly sampled for eggs from different producers available for sale during the visit. The shopping malls were sampled twice, 1 month apart, whereas the supermarkets were sampled once.

Source of samples. A questionnaire was administered to address management practices and production on each farm. Twenty-three layer farms in operation at the time of the study served as sources of table eggs from farms. For farms, following two visits, 46 composite egg samples were processed. Fourteen large shopping malls across Trinidad served as mall samples of table eggs, accounting for a total of 31 composite eggs. A total of 102 supermarkets or kiosks (small, medium, and large) were the sources of supermarket table eggs across Trinidad that accounted for a total of 107 composite egg samples from this source. Overall, for the three sources studied, 1,978 table eggs were pooled and processed.

Sample collection. All egg samples from the malls and supermarkets were collected in the crates used for their sale to the consumer. The temperature of storage at the sale outlet (room temperature, ambient temperature, or refrigeration temperature) was noted, and the eggs were kept at that temperature until pro-

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TABLE 1. *Management practices on layer farms studied*

Parameter	No. (%) of farms
Daily egg production	
0–1,000	8 (34.8)
1,001–5,000	5 (21.7)
5,001–10,000	2 (8.7)
>10,000	4 (17.4)
No response	4 (17.4)
No. of pens	
1–5	17 (73.9)
6–10	4 (17.4)
>10	2 (8.7)
Type of poultry house	
Floor	19 (82.6)
Cage	1 (4.3)
Both	3 (13.0)
Use of medicated feeds	
Yes	20 (87.0)
No	3 (13.0)
Adherence to withdrawal period	
Yes	6 (26.1)
No	11 (47.8)
Occasionally	3 (13.0)
Not applicable	3 (13.0)

cessed. For farm samples, eggs were collected in crates and kept at the same temperature before processing. The investigators could not personally collect eggs from the farms because of restrictions by farm owners. Egg samples were therefore collected by the farmers or their assistants. For all sources, eggs were processed within 24 h of collection.

Detection of antimicrobial residues. To prepare eggs, 6 or 25 eggs from each source were weighed to determine the mean egg weight per source. Scalpel blade was then used to break a small hole at the pointed end of each egg through which the egg content (albumen and yolk) was emptied into a stomacher bag. The pooled eggs (6 or 25) were then blended for 30 s at normal speed in the stomacher bag using a stomacher 400 (Seward, London, England).

To process the eggs for residue, 10 ml of homogenized egg was poured into a 50-ml centrifuge tube and heated in boiling water for 6 min. The boiled solid was emptied into a stomacher bag, and 30 ml of MSU extraction buffer (Charm Scientific Inc., Lawrence, Mass.) was added. This was homogenized for 30 s at normal speed in a stomacher, poured back into the same 50-ml centrifuge tube, and centrifuged at 1,750 $\times$ g for 5 min. The opaque extract (supernatant) was poured into a test tube for the detection of β-lactams, macrolides, sulfonamides, and tetracyclines using appropriate test kits and the Charm II protocol provided by the manufacturer (Charm Scientific).

The range of detection levels for members of the four groups of antimicrobial agent residues in eggs assayed by Charm II are as follows: β-lactams (40 ppb for cephapirin to 500 ppb for oxacillin and cloxacillin), sulfonamides (20 ppb for sulfadimethoxine and sulfamerazine to 50 ppb for sulfamethazine), tetracyclines (20 ppb for tetracycline to 100 ppb for oxytetracycline and chlortetracycline), and macrolides (200 ppb for erythromycin and tilmicisin to 800 ppb for pirlimycin). All antimicrobial agents were detected qualitatively.

TABLE 2. *Sources and mean weights of table eggs tested for antimicrobial residues*

Source	No. of outlets tested	No. of composite egg samples tested[a]	Total no. of eggs tested	Mean ± SD weight per egg (g)[b]
Farm	23	46[c]	1,150	61.0 ± 4.2
Mall	14	31[c]	186	61.0 ± 7.1
Supermarket	102	107[d]	642	61.0 ± 6.4

[a] For farms, 25 eggs were randomly sampled per visit, whereas for malls and supermarkets, 6 eggs were randomly sampled per source and pooled as composite samples.
[b] Differences in mean weight of eggs across sources were not statistically significant ($P > 0.05$).
[c] Two visits each, 1 month apart, made to each of the 23 farms and 14 malls.
[d] A visit per outlet during which eggs from different producers were sampled.

Statistical analysis. The frequency of detection and type of antimicrobial agents in table eggs were compared for the various sources (farm, malls, and supermarkets) and farm practices by the χ^2 test using the Statistical Package for Social Sciences, version 10 (SPSS Inc., Chicago, Ill.). All statistical analyses were two-sided and interpreted at the 0.05 level of significance.

RESULTS

The management practices on layer farms from where table eggs were sampled are shown in Table 1. Most layer farms have daily egg production that ranges from 0 to 1,000 (34.8%), most (73.9%) kept hens in one to five pens, and 19 (82.6%) of 23 farms used only the floor-type management system. Twenty (87.0%) of the farms used medicated feeds, which usually contained tylosin, nitrofurantoin, or chlortetracycline, and only 6 farms (26.1%) claimed adherence to withdrawal periods following the use of antimicrobial agents. For therapeutic purposes, the practice is for the farmer to inform the local feed manufacturer of health problems on the farms after which any of the above-mentioned antimicrobial agents are incorporated in the feed. At the time of the visits to the farms, only 2 farms (8.7%) admitted that the birds were undergoing therapy using medicated feeds, one containing tylosin and the other chlortetracycline.

The mean weight of table eggs from farm, shopping mall, and supermarket sources was 61.0 ± 4.2 g, 61.0 ± 7.1 g, and 61.0 ± 6.4 g, respectively (Table 2). Overall, 1,978 table eggs were pooled and processed.

The frequency of detecting antimicrobial residues was highest in eggs from mall outlets (35.7%) and lowest in eggs sampled directly from farms (13.0%), but the difference was not statistically significant ($P > 0.05$) (Table 3). Similarly, of the composite egg samples processed, the highest prevalence of residue was obtained from mall eggs (16.1%) compared with 15.0% for eggs from supermarkets and 6.5% for farm eggs. Again the difference was not statistically significant ($P > 0.05$).

Table 4 shows that the most prevalent antimicrobial agent detected in table eggs from all sources was sulfon-

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TABLE 3. *Frequency of detection of antimicrobial residues in table eggs by source or purchase*

Source	Outlets		Composite samples	
	No. of outlets tested	No. (%) positive for residues[a]	No. of composite eggs tested	No. (%) positive for residues[b]
Farm	23	3 (13.0)	46	3 (6.5)
Mall	14	5 (35.7)	31	5 (16.1)
Supermarket	102	16 (15.7)	107	16 (15.0)

[a] Difference in the frequency of outlets positive for antimicrobial residues was not statistically significant ($P > 0.05$).
[b] Difference in the frequency of pooled composite eggs positive for antimicrobial residues by source was not statistically significant ($P > 0.05$).

amides (6.5%), whereas the least was β-lactam (0.0%). The difference in prevalence was statistically significant ($P < 0.05$).

The frequency of antimicrobial residues in eggs sampled from malls and supermarkets by farm source is shown in Table 5. Four (40.0%) of the 10 known farm sources had table eggs positive for residues. Sulfonamides were detected in eggs from all known farm outlets positive for antimicrobial residues. Of a total of 94 composite egg samples tested from known farm outlets, 11 (11.7%) were positive for antimicrobial residues.

Table 6 shows the frequency of detection of antimicrobial agents in eggs by egg weight, farm, and sale outlet practices. Three (7.5%) of 40 composite egg samples from farms using medicated feeds were positive for antimicrobial residues compared with 0 (0.0%) of 6 samples from farms that did not ($P > 0.05$). Two (16.7%) of 12 samples from farms that claimed to adhere to withdrawal periods were positive for antimicrobial residues compared with 1 (4.5%) of 22 samples from farms that did not. The mean weight of eggs and the temperature of storage of eggs did not statistically significantly ($P > 0.05$) affect the prevalence of antimicrobial residues in eggs.

DISCUSSION

The finding that 13.0% of 184 composite egg samples were positive for antimicrobial residues coupled with the fact that 17.3% of the sale outlets (farms, shopping malls, and supermarkets) across Trinidad were contaminated with residues of sulfonamides, macrolides, and tetracyclines did not come as a surprise. As many as 87.0% of the layer farms admitted the use of medicated feeds that contained nitrofurantoin, tylosin, or chlortetracycline, only 26.1% claimed to adhere to stipulated withdrawal periods, and there is neither control from the poultry industry or the government of the types of antimicrobial agents to be used in layers nor a monitoring system for antimicrobial residues in eggs. To our knowledge, this is the first documented demonstration of antimicrobial residues in table eggs in the country, although residues have been demonstrated in preprocessed and processed milk *(1, 2)*.

In a study in the eastern province of Saudi Arabia, Al-Ghamdi et al. *(3)* reported that 60.0% of the layer farms studied had eggs contaminated with tetracycline and the amount detected exceeded the maximum residual level in 14.4% of raw eggs tested. In the present study, only 3 (13.0%) of 23 layer farms sampled had antimicrobial residue-contaminated eggs. Variable prevalence of antimicrobial residues have been reported in table eggs from naturally and experimentally exposed layers elsewhere *(10, 18, 21)*. Although the 23 farms studied constituted most of the table egg sources to the sale outlets in Trinidad, only 3 (6.5%) of 46 composite eggs sampled directly from farms were contaminated with antimicrobial residues compared with a prevalence of 16.1 and 15.0% found in composite eggs sampled from shopping malls and supermarkets, respectively. Furthermore, 11 (11.7%) of 94 composite eggs sampled from both types of outlets, where the farm sources were indicated, also had antimicrobial residues. The difference in the findings detected in eggs from farms and those from malls and supermarkets could be explained, in part, by the possibility that being a cross-sectional study some detected residue-positive eggs may have originated from birds that had before the farm visit been treated or were undergoing treatment at the time of the visit that was undeclared by the farmer. Although it is expected that birds being treated are more important sources of antimicrobial residues than the use of medicated feeds, it is pertinent to mention that tylosin (macrolide) and chlortetracycline (tetracycline) detected in the eggs tested are added to feeds in Trinidad. Use of medicated feeds appears to have contrib-

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TABLE 4. *Frequency of detection of various antimicrobial residues in table eggs by source*

Source	No. of composite eggs tested	No. (%) positive for residues[a]		
		Sulfonamides	Macrolides	Tetracyclines
Farm	46	1 (2.2)[b]	1 (2.2)[b]	1 (2.2)[b]
Mall	31	2 (6.5)	2 (6.5)	1 (3.2)
Supermarket	107	9 (8.4)	4 (3.7)	3 (2.8)
Total	184	12 (6.5)[c]	7 (3.8)[c]	5 (2.7)[c]

[a] All samples were negative for β-lactams.
[b] Three different farms (13.0%) of the 23 tested yielded the three residue-positive samples.
[c] Overall, regardless of source, sulfonamides were detected at a statistically significantly ($P < 0.05$) higher frequency than the other three groups of antimicrobial agents (macrolides, tetracyclines, and β-lactams).

TABLE 5. *Frequency of detection of antimicrobial residues in table eggs from supermarkets and malls by farm source*

Identification of farm outlet	No. of samples tested[a]	No. (%) positive for residues	Type (no. of samples) of antimicrobial residues detected
A	32	4 (12.5)	Sulfonamides (3), macrolides (1)
B	31	3 (9.7)	Sulfonamides (1), tetracyclines (2)
C	9	2 (22.2)	Sulfonamides (1), macrolides (1)
D	5	2 (40.0)	Sulfonamides (1), tetracyclines (1)
E	5	0 (0.0)	—
F	4	0 (0.0)	—
G	4	0 (0.0)	—
H	2	0 (0.0)	—
I	1	0 (0.0)	—
J	1	0 (0.0)	—
K[b]	44	10 (23.8)	Sulfonamides (5), tetracyclines (1), macrolides (4)

[a] Number of composite samples consisting of six pooled eggs.
[b] Farm sources could not be ascertained.

uted to contamination of table eggs with antimicrobial residues, because 3 (7.5%) of 40 users of medicated feeds had residue-positive eggs, whereas all eggs from nonusers were negative. Use of medicated feeds in layers and contamination of feeds with antimicrobial agents at the feed mills have been reported to contribute to a spillover of antimicrobial agents into the yolk or albumen of eggs *(13, 14, 17, 20)*.

Side effects in humans, including allergy and development of resistance among strains of bacteria, have been documented for the presence of antimicrobial residue(s) in foods such as eggs *(9, 20)*. The risk posed by the con-

TABLE 6. *Frequency of detection of antimicrobial residues in eggs by farm and outlet practices*

Parameter	No. of samples tested[a]	No. (%) positive for residues[b]	*P* value
Farm practices			
Use of medicated feeds			
Yes	40	3 (7.5)	0.65
No	6	0 (0.0)	
Adherence to withdrawal period			
Yes	12	2 (16.7)	0.39
No	22	1 (4.5)	
Occasionally	6	0 (0.0)	
Not applicable	6	0 (0.0)	
Fate of eggs during withdrawal period			
Disposal	10	2 (20.0)	0.26
No practice change	6	0 (0.0)	
No response	24	1 (4.2)	
Not applicable	6	0 (0.0)	
Farm and sale outlet			
Egg mean weight (g)			
$<$50	10	3 (30.0)	0.30
51–60	81	12 (14.8)	
61–70	81	8 (9.9)	
70	12	1 (8.3)	
Temperature of storage			
Room temperature	78	13 (16.7)	0.45
Ambient temperature[c]	60	6 (10.0)	
Refrigeration temperature	46	5 (10.9)	

[a] Pooled eggs (6 or 25) constituting a composite sample.
[b] Positive for any of the four antimicrobial residues tested.
[c] Temperature of kiosks, open vans, or other outlets where table eggs are sold.

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sumption of antimicrobial residues in foods has resulted in the establishment of maximum residual levels for various antimicrobial agents in many food products *(5, 8, 15)*.

Although the levels of antimicrobial residues found in the present study was not quantified, the potential health risk their presence poses to the consumer cannot be ignored, especially because there is no existing monitoring system for residues in table eggs in the country and because a number of food products consumed locally use raw or partially cooked eggs. Heat treatment has been demonstrated to degrade some antimicrobial agents, making the metabolite undetectable by microbiological methods *(16)*, but some metabolites, although not detectable, may possess allergic or toxicological properties and thereby pose health risks to the consumer *(14, 19)*.

The detection of residues of sulfonamides, macrolides, and tetracyclines in table eggs in Trinidad was not unexpected. Sulfonamides, erythromycin, and tylosin (macrolide group) and chlortetracycline, oxytetracycline and doxycycline (tetracycline group) are readily available to the poultry farmers from agricultural stores and readily applied for prophylaxis and chemotherapy (Poultry Surveillance Unit, Trinidad and Tobago, personal communication). The fact that sulfonamide residues were detected at a significantly higher frequency than the two other residues detected agrees with the knowledge that it is most easily available, soluble, and readily applied in water and feeds by the local poultry farmers. It is known that 3.0% of the general population exhibits allergic response to sulfa drugs *(6)*.

In conclusion, the presence of antimicrobial residues in raw table eggs in Trinidad could pose a health hazard to consumers. It may be important for the Ministry of Agriculture, Land, and Marine Resources to consider measures to regulate antimicrobial residues in table eggs with accompanying penalties for noncompliance.

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