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Prevalence of *Escherichia coli* O157:H7 and *Salmonella* in Camels, Cattle, Goats, and Sheep Harvested for Meat in Riyadh†

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**ABSTRACT**

*Escherichia coli* O157:H7 and *Salmonella* are significant foodborne pathogens that can be found in the feces and on the hides of meat animals. When hides are removed during the harvest process, the carcass and subsequent meat products can become contaminated. Camels, cattle, sheep, and goats are harvested for meat in Riyadh, Saudi Arabia. The prevalence of *E. coli* O157:H7 and *Salmonella* are unknown in these animals, and it is assumed that if the animals carry the pathogens in their feces or on their hides, meat products are likely to become contaminated. To this end, a minimum of 206 samples each from hides and feces of camels, cattle, goats, and sheep were collected over the course of 8 months and tested for *E. coli* O157:H7 and *Salmonella*. It was found that *E. coli* O157:H7 was present in feces (10.7, 1.4, 2.4, and 2.4%) and on hides (17.9, 8.2, 2.9, and 9.2%) of cattle, goats, camels, and sheep, respectively. The prevalence of *Salmonella* was 11.2, 13.5, 23.2, and 18.8% in feces and 80.2, 51.2, 67.6, and 60.2% on hides of cattle, goats, camels, and sheep, respectively. The prevalence of *E. coli* O157:H7 was nearly zero in all samples collected in June and July, while *Salmonella* did not exhibit any seasonal variation. These results constitute the first comprehensive study of *E. coli* O157:H7 and *Salmonella* prevalence in Saudi Arabian meat animals at harvest.

Foodborne pathogens are a major source of illness around the world caused by consumption of contaminated foods. In reviewing the statistics of foodborne-related outbreaks in the Kingdom of Saudi Arabia (KSA), Al-Mazrous (1) acknowledges the difficulty in assessing the real threat posed by foodborne outbreaks primarily due to inadequate system of data collection and reporting. He cites reports that indicate the number of foodborne disease outbreaks increased from 184 to 482 per year over a span of 11 years, with *Salmonella* as one of the primary foodborne pathogens responsible for these outbreaks. Although specific data are lacking on the occurrence of *E. coli* O157:H7 in KSA, Al-Mazrous cites the emergence of pathogenic *E. coli* as another foodborne threat in KSA.

*Salmonella* is an important foodborne pathogen noted for causing an estimated 1 million cases of food poisoning in the United States each year (38). Of the 19,056 laboratory-confirmed cases of food-related infection in the United States in 2013, 38% were caused by *Salmonella* (11). Salmonellosis is generally a self-limiting disease consisting of diarrhea, fever, and abdominal cramps, with most patients recovering without the need of medical attention. However, in a small percentage of *Salmonella* infections, the diarrhea may be so severe that the patient needs to be hospitalized. In these patients, the *Salmonella* infection may spread from the intestines to the bloodstream, to other body sites, resulting in death unless promptly treated with antibiotics (39).

*E. coli* O157:H7 can cause enterohemorrhagic colitis and hemolytic uremic syndrome. This bacterium is capable of producing large quantities of toxins (Shiga toxins) that severely damage the intestinal lining, causing hemorrhagic colitis (7). The infective dose is unknown, but from a statistical analysis, the infective dose is estimated to be as low as 10 organisms. Some victims, particularly the very young, develop hemolytic uremic syndrome, which is characterized by renal failure and hemolytic anemia (8, 31). Up to 15% of hemolytic uremic syndrome victims may develop hemorrhagic colitis, which can lead to permanent loss of kidney function and have a mortality rate as high as 50% (31).

In the early 1980s, *E. coli* O157:H7 gained recognition as the causative agent for an outbreak of severe bloody diarrhea traced to consumption of improperly prepared hamburgers (21, 36). It is now well established that *E. coli* O157:H7 can be found in healthy animals and that the
organism is associated with meat contaminated during slaughter (24, 25). A study of the prevalence of *E. coli* O157:H7 in feces, hides, and carcasses of beef cattle at U.S. processing plants in the late summer months (July and August 1999) found that 28% of feces and 11% of hides tested positive for the presence of this pathogen (14). The report by Elder et al. (14) found that 45% of pre-evisceration carcasses were positive for *E. coli* O157:H7, due to transfer of the pathogen during the process of hide removal. Subsequent interventions reduced but did not eliminate the pathogen, so that even after a full complement of interventions were applied, 2% remained positive.

The frequency of *E. coli* O157:H7 and *Salmonella* in Saudi Arabian meats is unknown, therefore this research assessed their prevalence in feces and on the hides of animals at harvest. The rationale was that the presence of the microorganism in feces and on hides would be a good indicator of its potential significance on meats.

**MATERIALS AND METHODS**

**Design.** The necessary number of observations to accurately measure a 5% incidence rate of either *E. coli* O157:H7 or *Salmonella*, with 90% confidence and a 2.5% confidence interval, is 206 (Research Dimensions, Inc., Lombard, IL). Therefore a minimum of 206 feces and 206 hide samples for each animal type was collected from camels, cattle, sheep, and goats at a municipal abattoir in Riyadh, Saudi Arabia. Biweekly sample collection began in March 2012, stopped in mid-July for the summer holiday, resumed in September, and ended in October 2012. When samples were collected, the breed, estimated age, and gender of each animal sampled were recorded.

**Sample collection.** Although hide samples and feces samples were not collected from the same animal as a matched set, each month feces and hide samples of each animal species were collected on the same day, but without attempts to collect the samples from the same animal, pen of animals, or lot of animals. Hides and feces samples were obtained from cattle and camels according to previously described methods for cattle (4). Hides and feces samples were obtained from sheep and goat according to previously described methods for sheep (22). Hides and feces were collected from all species immediately after bleeding. Hides were swabbed with Difco buffered peptone water (BD, Sparks, MD) moistened Speci-Sponges in Whirl-Pak bags (Nasco, Fort Atkinson, WI) over an area of 1,000 cm². Feces was obtained by the grab sample technique to obtain a 10- to 100-g portion that was placed into a Whirl-Pak bag. All Whirl-Pak bags were held in a cooler box until transported to the laboratory for analysis.

*E. coli* O157:H7 isolation and characterization. A 90-ml aliquot of Difco tryptic soy broth (BD) was added to each hide sample bag and to a 10-g portion of feces (5, 22). All sample bags were massaged by hand, then incubated at 25°C for 2 h and at 42°C for 6 h, then held at 4°C overnight. One milliliter of each enrichment was added to 20 μl of anti-O157 immunomagnetic beads (Invitrogen Corp., Carlsbad, CA) and subjected to immunomagnetic separation. The bead-bacteria complexes (50 μl) were spread plated onto one CHROMagar O157 plate (DRG International, Mountainside, NJ) supplemented with 5 mg of novobiocin per liter and 2.5 mg of potassium tellurite per liter and one Sorbitol MacConkey (Oxoid, Basingstoke, UK) agar plate supplemented with 50 μg/liter cefixime and 2.5 mg/liter potassium tellurite. After the plates were incubated for 16 to 18 h at 37°C, at least two presumptive colonies from each plate were tested by latex agglutination (DrySpot *E. coli* O157; Oxoid). The presumptive colonies were then confirmed to be *E. coli* O157:H7 using PCR to identify the presence of Shiga toxin genes (*stx*₁ and *stx*₂), intimin (*eaeA*), and fliC-H7 and *rfb*-O157 genes (19).

*Salmonella* isolation and characterization. *Salmonellae* in hides and feces samples were concentrated by immunomagnetic separation from 1 ml of the culture enrichment described above, using 20 μl of anti-*Salmonella* immunomagnetic beads (Invitrogen Corp.) as previously described (3). The bead-bacteria complexes were resuspended in 0.1 ml of phosphate-buffered saline–Tween 20 wash buffer, transferred into 10 ml of Rappaport-Vassiliadis soya broth (Oxoid), and incubated at 42°C for 18 to 24 h. Each Rappaport-Vassiliadis soya broth culture was then streaked for the isolation of individual colonies on to a petri plate of brilliant green agar with sulfadiazine (Oxoid) and a petri plate of Hektoen Enteric agar (Oxoid) supplemented with 15 mg of novobiocin per liter. All plates were incubated at 37°C for 16 to 18 h. Two suspect colonies from each plate (Hektoen Enteric agar and brilliant green agar with sulfadiazine) were picked for confirmation by PCR for the invA gene (41).

**Statistical analysis.** In order to test for pathogen prevalence differences, the DIFFER procedure of PEPI software (USD, Inc., Stone Mountain, GA) was used to calculate the pairwise difference among different sample types, with the probability level of *P < 0.05* considered significantly different.

**RESULTS AND DISCUSSION**

The hides and feces samples from cattle, sheep, goats, and camels were obtained from a municipal abattoir in Riyadh. Per the project design, a minimum of 206 samples of feces and hides were obtained for each of the four species. The results of the *E. coli* O157:H7 prevalence in feces and hides are summarized in Table 1. Cattle feces contained the highest level of *E. coli* O157:H7 (10.7%) while feces from sheep (2.4%), camels (2.4%), and goats (1.4%) were not different (*P > 0.05*). For hide samples, cattle had the highest level of *E. coli* O157:H7 (17.9%), followed by sheep (9.2%), and goats (8.2%), while camel hides (2.9%) had the lowest level (*P < 0.05*) of *E. coli* O157:H7. The cattle were mostly young Friesian steers, and older cull Friesian cows. Likely due to age and rearing practices, the cows had significantly (*P < 0.05*) lower *E. coli* O157:H7 prevalence in feces and on hides than the steers (data not shown). Within the population of camels sampled, young males were predominant and had a significantly (*P < 0.05*) greater level of *E. coli* O157:H7 in feces but not on hides than older female camels (data not shown). Interactions between breed, age, or gender of sheep and goats with *E. coli* O157:H7 prevalence could not be made due to the fact that very few does and ewes were harvested at the slaughterhouse to be sampled.

*E. coli* O157 prevalence in raw beef, camel, sheep, and goat meat purchased from a number of butcher shops in Iran was reported to be 8.2, 2.0, 4.8, and 1.7%, respectively (32). *E. coli* O157:H7 was isolated from 1.1% of final camel carcasses during processing in a major commercial camel...
<table>
<thead>
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<th>Camels</th>
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<td>6 (17.6) A</td>
<td>34</td>
<td>2 (5.9) CD</td>
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<td>207</td>
<td>6 (2.9) Z</td>
<td>207</td>
<td>22 (10.6) Y</td>
<td>207</td>
<td>37 (17.9) X</td>
<td>207</td>
</tr>
</tbody>
</table>

**a** Feces were obtained by the grab sample technique to obtain a 10- to 100-g portion.

**b** Hides were swabbed over an area of 1,000 cm².

**c** Monthly values within a column followed by the same letter are not significantly different ($P > 0.05$).

**d** No samples were collected in August due to summer holiday.

**e** Total values represent total number of feces or hide samples collected in all monthly sample periods. Percent prevalence values in this row followed by the same letter are not significantly different ($P > 0.05$).
slaughterhouse in Iran (33). Since beef, camel, sheep, and goat processing in Iran and Saudi Arabia are similar, the data from meat products shows that if *E. coli* O157 is present on a meat animal, it may end up on the final meat product.

In other parts of the world, Kalchayanand et al. (22) reported that 12.8% of U.S. sheep had *E. coli* O157:H7 on their pelts (hides). In a survey of public premises in England and Wales from 1997 to 2010, the fecal prevalence of *E. coli* O157 was highest in cattle (29.0%) followed by sheep (24.4%) and goats (9.9%). Duffy et al. (13) reported that fecal and fleece *E. coli* O157 prevalence at two Australian slaughterhouses were 5 and 3%, respectively. In a year-long study of the prevalence of *E. coli* O157 in sheep raised in a feedlot setting or on native pasture. Kilonzo et al. (23) reported that 22.7% of fecal samples collected from feedlot sheep and 1.9% in sheep raised on pasture were positive for *E. coli* O157:H7.

Unlike cattle, sheep, and goats, the prevalence of *E. coli* O157 from camels has not been widely studied. El-Sayed et al. (15) failed to detect any positive *E. coli* O157 among 400 camel fecal samples collected from Egypt, Somalia, Djibouti, Kenya, and Sudan. Moore et al. (29) also failed to identify *E. coli* in feces of racing camel calves in the United Arab Emirates. Based on these reports, the presence of *E. coli* O157:H7 has been generally excluded from camels in this part of the world (37). However, these results were based on characterization of a limited number of *E. coli* colonies picked from each sample, rather than specific screening and isolation attempts using tools such as immunomagnetic separation that target *E. coli* O157:H7.

The numbers of feces and hide samples collected from each type of meat animal during each month of our study and the prevalence of *E. coli* O157:H7 are summarized in Table 1. Samples were collected biweekly from March through October, except for the month of August when no samples were collected. A temporal analysis of the prevalence of *E. coli* O157:H7 in the feces and hide samples shows early peaks of about 10% in feces and 20 to 40% on hides in the April to May time period (Table 1). This tapered off to prevalence rates of near zero for both hides and feces in June and July. When sample collection resumed in September, the prevalence rates had returned to levels similar to those of April and May, with cattle *E. coli* O157:H7 prevalence rates spiking to their highest level in September.

Of the factors that have been described to affect the prevalence of *E. coli* O157:H7, only season has been repeatedly identified as a factor. *E. coli* O157:H7 prevalence in cattle feces has been reported to be low in the winter, increase in the spring, reach peak levels during the summer, then taper off in autumn again (10, 18, 40). In a survey of retail meats in the United Kingdom, the majority of *E. coli* O157:H7 positives were found between May and September (9), while in the United States, ground beef samples were three times more likely to be positive for *E. coli* O157 between June and September (42). The seasonal prevalence in cattle and meat products is mirrored in human cases of *E. coli* O157:H7 that predominantly occur in the summer months (7, 34). The seasonal prevalence of *E. coli* O157:H7 is often attributed to factors such as ambient temperature and rainfall, or other seasonal contributing factors like insect populations (27, 35). The warmer summer months may provide more suitable environments outside of the host in soil, feed, and water for *E. coli* O157:H7, resulting in a continual source of infection or reinfection of cattle populations. This is possibly the case in the spring and early autumn months as observed in our studies. However, with the lack of precipitation and intense heat of the Saudi Arabian summer months when daytime temperatures may reach 50°C, the cycle of *E. coli* O157:H7 prevalence appears to decline due to its inability to persist in the environment. The lowest prevalences of *E. coli* O157:H7 in all meat animals were observed in June and July of our study, in contrast to the typically reported seasonal prevalence of this pathogen in other parts of the world.

*E. coli* O157:H7 may possess Shiga toxin genes (type 1 and/or type 2). Strains that carry stx2 rather than stx1 have been associated with greater virulence and human outbreak isolates (8, 12), and stx2 was identified as a key factor in the development of hemolytic uremic syndrome during *E. coli* O157:H7 infections (26). Further, epidemiological data of *E. coli* O157:H7 isolates from human infections have shown a bias toward carrying stx2 rather than stx1 (17). The *E. coli* O157:H7 isolated in our study was confirmed using a PCR that identified the serotype specific rfb-O157 and flIC-H7 genes as well as stx1, stx2, and another essential virulence factor gamma-intimin (16). From the confirmation testing, it was noted that in isolates from cattle, 10 of 37 isolates from hides and 3 of 22 isolates from feces contained both stx1 and stx2 while all others from cattle carried only stx2. The *E. coli* O157:H7 isolates from cattle that carried both stx genes were from samples collected in April and May but not during the later months of the study. In isolates from sheep and goats, isolates containing stx2 alone were most common, only two feces and two hide isolates from sheep contained stx1 and stx2 while all others from cattle carried only stx2. The fewest isolates of *E. coli* O157:H7 were found in camels (six hide and five feces isolates) only one isolate contained both stx1 and stx2. Further, all isolates of *E. coli* O157:H7 from all meat animal species contained gamma-intimin, except one camel isolate was identified that lacked this virulence factor.

The results of *Salmonella* prevalence in feces and hides are summarized in Table 2. The prevalence of *Salmonella* in feces samples was greater in camels (23.2%) than in goats or cattle. Sheep feces had a *Salmonella* prevalence of 18.8%, while feces collected from goats and cattle had the lowest prevalences of *Salmonella*, 13.5 and 11.2%, respectively. For hide samples, cattle had the highest level of *Salmonella* (80.2%), followed by camels (67.6%) and sheep (60.2%), while goat hides had the lowest prevalence (51.2%).

The prevalence of *Salmonella* in cattle feces and hide samples by breed, age, and gender of animal was unremarkable; however, the prevalence of *Salmonella* in the other meat animal types showed some breed-specific effects (data not shown). Ashaal, Bahri, and Baladi camels
TABLE 2. Prevalence of *Salmonella* in feces\(^a\) or on hides\(^b\) for each species of meat animal by month

<table>
<thead>
<tr>
<th></th>
<th>Camels</th>
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<td>No. (%)</td>
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<td>19 (76.0) (AB)</td>
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<td>15 (53.6) (B)</td>
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<td>28</td>
<td>19 (67.9) (BCD)</td>
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<td>48 (23.2) (W)</td>
<td>207</td>
<td>140 (67.6) (Y)</td>
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<td>23 (11.1) (U)</td>
<td>207</td>
<td>166 (80.2) (Z)</td>
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</table>

\(^{a}\) Feces were obtained by the grab sample technique to obtain a 10- to 100-g portion.

\(^{b}\) Hides were swabbed over an area of 1,000 cm\(^2\).

\(^{c}\) Monthly values within a column followed by the same letter are not significantly different (\(P > 0.05\)).

\(^{d}\) No samples were collected in August due to summer holiday.

\(^{e}\) Total values represent total number of feces or hide samples collected in all monthly sample periods. Percent prevalence values in this row followed by the same letter are not significantly different (\(P > 0.05\)).
were a minor proportion of camels sampled, yet were found to have zero prevalence of \textit{Salmonella} in their feces that, as a group, was significantly less ($P < 0.05$) than feces samples from the other camel breeds. Bahri camels had approximately one-half the prevalence of \textit{Salmonella} on their hides (43\%) as the other camel breeds; however, because there were only seven samples collected from this camel breed there was not a difference ($P > 0.05$) from the rest of the breeds that carried \textit{Salmonella} on their hides at a rate of about 70\% (data not shown).

Most samples were collected from Ardi goats, with Barbari the next most common breed, and although Barbari represented 4 to 5\% of the samples collected, this breed was found to have significantly ($P < 0.05$) higher feces and hide prevalence than the Ardi and other breeds (data not shown). Barbari goats had \textit{Salmonella} feces prevalence of 44\% and hide prevalence of 93\%, while Ardi and other breeds had feces prevalence of 12\% and hide prevalence of 53\%. It is possible that the Barbari goats all originated from a single source; however, age estimates showed that samples were collected from Barbari goats of less than 1 to 3 years of age. Further, the \textit{Salmonella}-positive Barbari goat samples were collected at two different time points each, in June and September as well as at one time point in October.

The prevalence of \textit{Salmonella} in feces of sheep by breed ranged from 14\% in Najdi to 33\% in Harri (data not shown). However, these differences in prevalence were not significantly different ($P > 0.05$). Harri sheep had the highest feces prevalence but the lowest hide prevalence (27\%). The low hide prevalence of \textit{Salmonella} on Harri sheep was significantly lower than the prevalence of \textit{Salmonella} on hides of Barbari, Sawakni, and Noaami sheep (71, 60, and 60\%, respectively). The reason for this difference is unclear, but the Harri sheep samples were only collected in April, June, and May, whereas the other breeds were represented throughout the samples collection period of our study.

The monthly prevalence of \textit{Salmonella} was highly variable between and within each meat animal type (Table 2). No general trends were observed for \textit{Salmonella} prevalence in feces. Camel feces prevalence of \textit{Salmonella} increased the initial 2 months of our study then slowly decreased, while sheep and cattle feces prevalence of \textit{Salmonella} varied month-to-month. Goat feces tended to increase monthly, with the exception of a drop following a peak in July. The monthly hide prevalence of \textit{Salmonella} generally followed the feces prevalence except for the goat feces that had a peak in July, and a trough on hides in July. Unlike \textit{E. coli} O157:H7 that appeared to be sensitive to the extreme Saudi Arabian summer, \textit{Salmonella} prevalence was generally unchanged through the hot summer months.

Barkocy-Gallagher et al. (6) reported that feces and hide \textit{Salmonella} prevalence of feedlot cattle were 4.4 and 71\%, respectively. Kallbackyand et al. (22) reported that the (hide) prevalence of \textit{Salmonella} on sheep was 14.4\%. Duffy et al. (13) reported that fetal and fleece \textit{Salmonella} prevalence at two Australian slaughterhouses were 20 and 13\%, respectively. Examining \textit{Salmonella} prevalence at slaughter, Molla et al. (28) reported that the \textit{Salmonella} prevalence was 1.9 and 15.1\% in fecal samples obtained from cattle and camels, respectively.

A number of samples from meat animal hides and feces were found to be positive for both \textit{Salmonella} and \textit{E. coli} O157:H7. Cattle hide samples had 34 dual-positive samples. No breed, age, or gender effect on dual-positive samples was noted, but almost all (92\%) of the \textit{E. coli} O157:H7 positive samples also contained \textit{Salmonella}. In cattle feces the number of dual positives was only five. In camels, however, one-half (50\%) of the \textit{E. coli} O157:H7 feces positives also were positive for \textit{Salmonella}, and four of five hides also were positive for both organisms. The four dual-positive camel hide samples were found in samples collected in September. In samples collected from goats, only hides were found to be positive for both \textit{E. coli} O157:H7 and \textit{Salmonella}. Nine of the 17 \textit{E. coli} O157:H7–positive goat hide samples were positive for \textit{E. coli} O157:H7. Finally, in sheep, one feces sample was positive for both organisms, but nearly all (89\%) of the positive \textit{E. coli} O157:H7 hide samples also contained \textit{Salmonella}.

The study presented here is the most comprehensive examination of the prevalence of \textit{E. coli} O157:H7 and \textit{Salmonella} on four meat animal species presented for harvest in KSA. Although there is a previous report of \textit{Salmonella} among farm animals in Saudi Arabia (30), the samples were collected upon necropsy of cattle, sheep, and goats with \textit{Salmonella} prevalence of 1.5, 18.6, and 18.8\%, respectively. The results presented here are in general agreement with published reports from around the world. However, the hide prevalence is considerably less than the hide prevalence for sheep and cattle in the United States. We speculate that the principal reason for the use of high-density confined animal feeding operations in the United States is where feces from one contaminated animal can cause the hide contamination of many animals (2, 5). And these sorts of high-density operations are not generally used in KSA.

In this study, the hides and feces of meat animals were sampled instead of the meat products themselves because it is well established that if \textit{E. coli} O157:H7 and \textit{Salmonella} are present in feces and on hides (24, 25), then the meat products from these animals may become contaminated. Observations of the current state of animal slaughter and processing in Saudi Arabia suggests that hide to carcass transfer is occurring and no antimicrobial intervention measures to reduce the level of pathogens on carcasses as used in the United States are in place. This is supported by a recent report by Iyer et al. (20) of \textit{E. coli} and \textit{Salmonella} isolated from nonspecified meats collected from market places in Jeddah, KSA.

It is true that unlike the Western world, in Saudi Arabia most meat products are well cooked (i.e., sufficiently high temperatures to eliminate pathogens if present). But this does not reduce the risk posed by cross-contamination to other noncooked items such as fruits and vegetables during preparation. Therefore even though typical cooking in Saudi Arabia would eliminate pathogens present on meat, the likelihood of cross contamination to other foods is a significant risk.
In conclusion, these data were collected to identify the base line of E. coli O157:H7 and Salmonella present in meat animals harvested in Riyadh, KSA. Now that the prevalence is known, further studies are warranted to examine levels on carcasses during and after processing, as well as studies of the most appropriate antimicrobial interventions to reduce levels of these pathogens in the meat processing facility. It is felt that education programs on processing best practices for the RAS and other slaughterhouses throughout KSA are needed to provide some level of protection to Saudi citizens from foodborne pathogens.

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