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Research Article

Microbiological Quality of Roasted Cowhide Meat ('Ponmo') Processed and Sold in Some Abattoirs in Bayelsa and Rivers States

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Abstract

Pathogenic microorganisms associated with food are one of the major causes of food-borne infections. The microbiological quality of cowhide meat from different abattoirs in Rivers and Bayelsa States were investigated using standard microbiological methods. The mean range of the total heterotrophic bacteria, coliform, *Vibrio*, heterotrophic fungal, total hydrocarbon utilizing bacterial and total hydrocarbon utilizing fungal counts of the cowhide from the different abattoirs were: 1.8×10^6 to 4.8×10^6 cfu/g, 1.7×10^5 to 3.6×10^5 cfu/g, 0 to 4.0×10^3 cfu/g, 1.0×10^4 to 4.0×10^4 cfu/g, 1.0×10^4 to 3.3×10^4 cfu/g, and 1.6×10^3 to 4.3×10^3 cfu/g respectively. Nine (9) bacteria identified and their frequencies are: *Bacillus* sp (20.39%), *Staphylococcus* sp (17.91%), *Escherichia coli* (13.03%), *Pseudomonas* sp. (11.44%), *Streptococcus* sp. (10.44%), *Micrococcus* sp. (9.45%), *Aeromonas* sp. (8.45%), *Klebsiella* sp (5.47%), and *Vibrio* sp (2.9%). Percentages of occurrence of hydrocarbon utilizing bacteria and fungi were also reported. Kruskal Walis H test showed that there was no significant difference ($P > 0.05$) in the total heterotrophic bacteria and total hydrocarbon utilizing bacterial counts obtained in all the locations. However, there was significant difference in the heterotrophic fungal counts across the locations while analysis showed no significant difference in the hydrocarbon utilizing fungal counts across the abattoirs ($p < 0.01$). The high microbial counts in the cowhide meat sampled and the isolation of potential pathogens as well as mycotoxin producing fungi are indicative of possible contamination during processing. Thus, the public should be aware of the dangers associated with consumption of cowhide meat and the need for proper post purchase preparation before consumption.

Keywords: Cowhide Meat; Abattoir; Coliform; Mycotoxin; Fungi; Hydrocarbon Utilizing Potentials

Introduction

There is a constant rising demand for meat and dairy products such as milk, yoghurt, and eggs in human diet which has been reflected in the global livestock business' response to meeting this demand [1]. Furthermore, livestock are critical to the economy of a country since the industry offers jobs, income as well as protein for the populace and industrial raw materials. The use of processed cow skins (cowhide) as meat for human consumption is a popular delicacy in various African countries [2]. Cowhide meat is known as Ponmo (Yoruba), Kanda (Hausa), Akpupoanu (Igbos), Ano (Igala), and Ohian (Edo) in Nigeria [3]. Raw cowskin is largely transported from the North to the West in Nigeria to augment the demand from the cowskin harvested from locally slaughtered animals [1]. Numerous methods are used in dressing cowhide or ponmo for human consumption in order to remove the animal hair/fur from the cow skin. Drying, roasting, scraping, boiling, and washing are examples of such processes. The resultant pulp referred to as 'cowhide meat' is used to as an alternative to

meat in cooking. Depending on the type of animal skinned and the processing procedure, processed cowhide or Ponmo is often off-white or brownish in colour.

Ponmo is a popular delicacy in Nigeria that is used to make a variety of stews, soups, and other delicacies, especially at ceremonies and/or public gatherings [4]. Cowhide meat (ponmo) eating has been ascribed to the lower classes of society since it is regarded to be less costly than ordinary meat [1]. As the demands for cowhide meat increases, the tendency to process large quantities to satisfy consumer demands has resulted in the use of many materials as fuel sources for the roasting technique. The roasting or burning of cowhide is a typical activity adopted in many abattoirs by ponmo processors to remove the fur/hair on the animal's skin. Due to a recent scarcity of firewood, local butchers have been using plastics, expired automobile tires, and used engine oil with firewood for roasting [4]. Furthermore, butchers claim that using these alternate fuels allows for better and faster roasting. The use of these materials as fuel for processing beef for human consumption may expose processed cowhide to chemicals and biological contaminants, endangering consumers. In a prior investigation, it was discovered that the usage of these materials contaminated hides with potentially dangerous organic compounds such as benzene, polycyclic, aromatic hydrocarbon, furans, lead, and dioxin, making them unfit for human consumption and causing disorders [5]. Microbial contamination of fresh and dried cowhides by microorganisms has been reported. According to Keta., *et al*. [2] *Staphylococcus aureus, Salmonella* spp*, Shigella dysenteria, Escherichia coli, Bacillus subtilis, Klebsiela* spp *and Pseudomonas aeruginosa,* from processed cowhide meat examined in Birnin Kebbi while Enterobacteriaceae bacteria are mostly found on the dried hides. Grilled, smoked and roasted foods present an elevated health risk to the consumer, due to higher levels of microorganisms and other toxic smoke components. This study is aimed at determining the microbial contaminants of roasted and processed cowhide meat available for sale to consumers at different abattoirs in Bayelsa and Rivers State, Nigeria. Thus, the study will provide relevant protection information to the larger consuming population about the possible health risk associated with the consumption of roasted cowhide as well as assisting relevant agencies to enact policies and guidelines that will protect the people and the environment.

Materials and Methods

Sampling location

Cowhide meat samples (500g) were randomly sampled and purchased from four abattoirs in Yenagoa Local Government Area of Bayelsa State and from an abattoir in Obio-Akpor Local Government Area of Rivers State. The abattoirs in Yenagoa are located at Igbogene, Tombia, Opolo and Swale while Rumuokoro abattoir is located in Obio-Akpor Local Government Area of Rivers State. The samples were collected into sterile containers and transported in ice-packed coolers to the Microbiology laboratory of the University of Port Harcourt for microbiological analyses immediately.

Sample preparation

Samples were homogenized by blending to smoothness in an alcohol sterilized Marlex blender and packaged into sterile medium sized plastic bowl containers for analyses [6].

Isolation, enumeration and characterization of microorganisms

Total heterotrophic bacteria (THB)

The pour plate technique for determining heterotrophic bacteria count using nutrient agar as described by Prescott., *et al*. [7] was employed for the determination. To make a 10^{-1} dilution, ten grammes (10g) of cowhide sample was weighed into a 250 ml conical flask containing 90 ml sterile normal saline. To create a 10^{-2} dilution, a sterile pipette was used to transfer 1 ml from this dilution into a test tube containing 9 ml of sterile normal saline. This procedure was carried out until a dilution of 10^{-6} was attained. 1 ml of the appropriate dilution was put aseptically onto sterile petri dishes, and 10 ml of molten sterile nutritional agar was added aseptically, stirred, and allowed to solidify. Samples were plated in duplicates. Plates were incubated in inverted position for 24 hours inverted at 35 ± 2°C, following which live colonies were counted.

Total coliform bacteria count

Prescott., *et al*. [7] described the spread plate technique for determining coliform bacteria using MacConkey Agar. Ten grammes (10g) of mixed cowhide was weighed into a 250 ml conical flask containing 90 ml sterile normal saline to generate a 10^{-1} dilution. One ml of this dilution was transferred to a test tube containing 9 ml of sterile normal saline to make a 10^{-2} dilution. This method

was repeated until a 10^{-6} dilution was achieved. A 0.1 ml aliquot of 10^{-4} dilution was aseptically applied to the surface of pre-dried sterile MacConkey Agar dish. Plates were distributed using a sterile bent glass rod and incubated at 35 ± 2°C for 24 hours in an inverted position, following which live colonies were counted.

Total *Vibrio* count (TVC)

Prescott., *et al*. [7] described the spread plate method for determining Vibrio count using Thiosulphate Citrate Bile Salt (TCBS) Agar. To achieve a 10^{-1} dilution, ten grammes (10g) of fresh minced cowhide sample was weighed into a 250 ml conical flask containing 90 ml sterile normal saline. To achieve the 10^{-2} dilution, 1 ml was pipetted from the original dilution and transferred to a test tube containing 9 ml of sterile normal saline using a sterile 5 ml pipette. This procedure was carried out aseptically until a dilution of 10^{-6} was reached. A 0.1 ml aliquot of 10^{-4} dilution was aseptically poured onto the surface of pre-dried sterile TCBS Agar plates. The inoculum was spread evenly using a sterile bent glass rod and incubated at 35 ± 2°C for 24 hours in an inverted location, following which colonies were counted.

Total hydrocarbon utilizing bacteria (THUB)

The vapour phase transfer method of Mills and Colwell [8] was adopted to determine the population of bacteria with hydrocarbon utilization potentials. Using the spread plate method described by Prescott [7], aliquots (0.1 ml) of serially diluted samples were inoculated on mineral salt agar media (2003). The inoculated agar plates were covered aseptically with sterile filter paper discs soaked in filter-sterilized crude oil, which served as the only carbon source in the mineral salt agar. Viable colonies were counted after 7 days of incubation at 35 ± 2°C.

Total heterotrophic fungi count (THF)

Using the spread plate approach, aliquots of appropriate dilutions of samples were plated on Potato Dextrose Agar (PDA) onto which 1% lactic acid was added [7,9]. The plates were incubated in inverted position at 25 ± 2°C for 7 days after which the colonies were counted and the mean of the count recorded accordingly [10].

Total hydrocarbon utilizing fungi (THUF)

The Vapour Phase Transfer method of Mills and Colwell [8] was adopted to determine the population of hydrocarbon utilizing fungi. Aliquots (0.1 ml) of the serially diluted samples were inoculated on mineral salt agar media added with 1% lactic acid to suppress bacterial growth [9], using the spread plate technique as described by Prescott [7]. Sterile filter paper discs soaked in filter-sterilized crude oil which served as the sole carbon source in the mineral salt agar was placed aseptically unto the cover of the inoculated agar plates. The plates were incubated at 25 ± 2°C for about 5 - 7 days [11]. After the incubation period, mean of the colonies were recorded.

Purification of isolates

After the incubation periods, morphologically distinct and discreet colonies were streaked on respective agar plates to obtain pure isolates. Pure isolates of heterotrophic bacteria and hydrocarbon utilizing bacteria were subcultured on pre-dried NA plates while fungal isolates were subcultured on pre-dried PDA plates. Plates for bacteria were incubated at 35 ± 2°C for 24 hours while the fungal plates were incubated at 25 ± 2°C for 7 days.

Identification of bacterial isolates

Pure bacteria isolates were identified by the method described by Cheesbrough [12]. Pure bacterial isolates were characterized using microscopic procedures and biochemical tests. Procedure for the respective biochemical tests were carried out according to standards [12]. The Bergey's Manual of Determinative Bacteriology was used to identify bacterial isolates [13].

Identification of fungal isolates

Pure mould isolates were identified using morphological characteristics, microscopic examination of their wet mounts prepared with lactophenol-cotton, blue and reference made to Barnett and Hunter fungal identification atlas [14]. Yeast isolates were also identified using their morphological features, followed by microscopic examination of their wet mount prepared with normal saline, Gram-staining, Sugar fermentation, oxidation and fermentation tests. Reference was further made to the fungal identification atlas by Barnett and Hunter [14].

Statistical analysis

The mean and standard deviations of the microbial counts were computed. The log counts were used in plotting the graph. Also, the frequencies of microorganisms across the abattoir sites were determined. The Analysis of variance (ANOVA) was used in

checking for significant difference in the microbial counts across the abattoir samples while Kruskal Walis H test was used in separating the means. All analysis was done using SPSS (version 27).

Results

The results of the total heterotrophic bacterial, total coliform counts, total *Vibrio* counts, total heterotrophic fungal counts, total hydrocarbon utilizing bacterial and fungal counts of cowhide from all abattoirs examined is presented in figure 1.

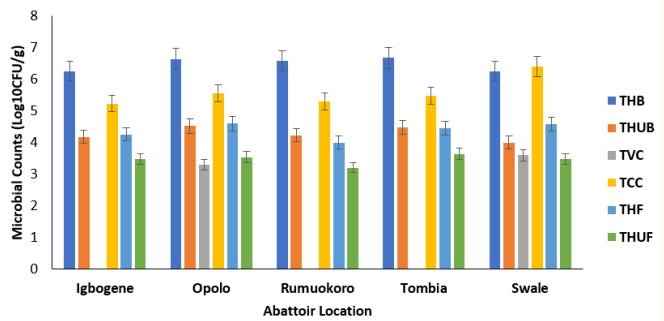

Figure 1: Microbial load of cowhide meat samples sold in the vicinity of the Abattoirs.

Key: THB = Total Heterotrophic Bacteria; THUB = Total Hydrocarbon Utilizing Bacteria; TF = Total Fungi; THUF = Total Hydrocarbon Utilizing Fungi; TCC = Total Coliform; TVC = Total *Vibrio* Count.

The mean range of the total heterotrophic bacterial counts, total coliform counts, total *Vibrio* counts, total heterotrophic fungal counts, total hydrocarbon utilizing bacterial counts and total hydrocarbon utilizing fungal counts of the cowhide from the different abattoir are 1.8×10^6 to 4.8×10^6 cfu/g, $1.7.0\times10^5$ to 3.6×10^5cfu/g, 0 to 4.0×10^3 cfu/g, 1.0×10^4 to 4.0×10^4 cfu/g, 1.0×10^4 to 3.3×10^4 cfu/g and 1.6×10^3 to 4.3×10^3 cfu/g respectively. Results showed that the total heterotrophic bacterial counts were highest in cowhides obtained from Tombia abattoir ($4.0x10^6$ cfu/g) while cowhides obtained from Igbogene and Swale had the least heterotrophic bacterial counts of 1.8×10^6 cfu/g. The cowhide samples from Opolo had the highest coliform counts (3.6×10^6 cfu/g) followed by those obtained from Tombia (3.0×10^6 cfu/g), while cowhide samples obtained from Igbogene had the least coliform counts (1.7×10^5 cfu/g). Results for the *Vibrio* counts showed that there was no detectable *Vibrio* in cowhide samples obtained from Igbogene, Rumuokoro and Tombia abattoirs. Results of the hydrocarbon utilizing bacteria showed that samples from Opolo had the highest counts while samples obtained from Swale abattoirs had the least counts. The Kruskal Walis H test showed that there was no significant difference ($P > 0.05$) in the THB and THUB load obtained in all the locations.

The mean counts of total heterotrophic and hydrocarbon utilizing fungi from cowhide samples collected from all the abattoirs showed that cowhide samples from Opolo recorded the highest count of 4.0×10^4 cfu/g while cowhide samples collected from Rumuokoro had the least heterotrophic fungal counts (1.0×10^4 cfu/g). Results for the total hydrocarbon utilizing fungal counts indicate that, cowhides from the Tombia abattoir had the highest while the least counts were recorded from those obtained in Swale abattoir. There was a statistically significant difference in the heterotrophic fungal counts across the locations while statistical analysis showed no significant difference in the hydrocarbon utilizing fungal counts across the abattoirs ($p < 0.01$).

The distribution of the characterized bacterial isolates found in cowhide samples from the various abattoirs is represented in figure 2.

A total of nine (9) bacteria were identified from the samples and they are: *Bacillus* sp (20.39%), *Staphylococcus sp* (17.91%), *Escherichia coli (13.03%), Pseudomonas sp.* (11.44%), *Streptococcus* sp. (10.44%), *Micrococcus sp.* (9.45%), *Aeromonas sp.* (8.45%), *Klebsiella* sp (5.47%), and *Vibrio sp* (2.9%). The bacteria that occurred in samples from all the stations include *Escherichia coli, Bacillus sp. Micrococcus* sp, *Pseudomonas* sp, *Staphylococcus* sp and *Vibrio* sp. *Klebsiella* sp., *Micrococcus* sp, *Pseudomonas* sp, *Streptococcus* sp., *Staphylococcus* sp., and *Vibrio* sp. *Klebsiella* sp was present in cowhide samples from Rumuokoro, Tombia and Swale abattoirs. *Vibrio* sp was found in cowhide samples from Opolo and Swale abattoirs. The percentages of occurrence of the organisms are: *Aeromonas* sp (8.45%), *Bacillus* sp (20.39%), *Escherichia coli* (13.03%), *Klebsiella* sp (5.47%), *Micrococcus* sp (9.45%), *Pseudomonas* sp (11.44%), *Staphylococcus* sp (17.91%), *Streptococcus* sp (10.44%), and *Vibrio* sp (2.9%). *Bacillus* sp occurred most while *Vibrio* sp was the least occurring isolates.

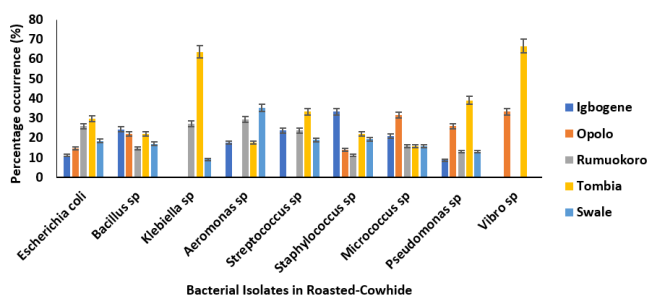

Figure 2: Frequency of occurrence of bacteria isolated from cowhide meat.

The percentages of occurrence of bacteria with hydrocarbon utilizing potentials isolated from cowhide were: *Aeromonas* sp (8.51%), *Bacillus* sp (29.28%), *Klebsiella* sp (6.38%), *Micrococcus* sp (17.02%), *Pseudomonas* sp (25.53%) and *Staphylococcus* sp (12.76%). Kruskal Walis H test showed that there was no significant difference ($P > 0.05$) in the THB and THUB load obtained in all the locations. However, there was significant difference in the heterotrophic fungal counts across the locations while analysis showed no significant difference in the hydrocarbon utilizing fungal counts across the abattoirs ($p < 0.01$).

The distribution of the fungal isolates of roasted cowhide is illustrated in figure 3.

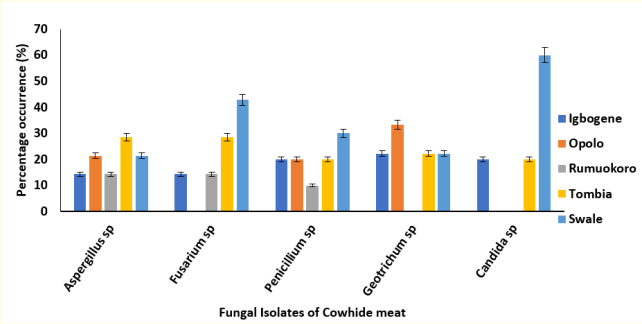

Figure 3: Frequency of occurrence of Fungi isolated from cowhide meat.

Moulds isolated from the cowhide samples were *Aspergillus* sp, *Penicillium* sp, *Fusarium* sp, *Geotrichum* sp and the yeast – *Candida* sp. *Aspergillus* sp was the most occurring and it was isolated in all the samples with the highest count in samples from Tombia and the least count in Igbogene and Rumuokoro samples. *Penicillium* sp was obtained in samples from all the locations with the highest count in Swale and the lowest in Rumuokoro. The mould *Geotrichum* sp was not isolated from samples obtained in Rumuokoro abattoir. More so, *Candida* sp were isolated from samples obtained from Igbogene, Tombia and Swale, respectively.

The percentages of occurrence of the fungal isolates are: *Aspergillus* sp (31.11%), *Penicillium* sp (22.22%), *Geotrichum* sp (20%), *Fusarium* sp (15.66%), and *Candida* sp (11.11%). The percentage of occurrence of fungal isolates with the ability to utilize hydrocarbon potentials are: *Aspergillus* sp (25%), *Penicillium* sp (42.14%), *Fusarium* sp (17.85%), *Geotrichum* sp (14.28%) and *Candida* sp (10.71%).

Discussion

Because of the health hazards associated with microbial contamination of foods, microbiological safety of foods intended for human consumption is critical. The microbial loads found in this study's analyzed cowhide meat were typically high, indicating probable contamination during processing, handling, and/or display of items in open trays for sale. Furthermore, the bacterial counts obtained in this study are higher than those reported in previous studies by Keta., *et al*. [2] who reported bacterial counts of 6.2×10^4 cfu/g, and Olukitibi., *et al*. [1], who reported the highest microbial load of 3.2×10^6 cfu/g from unprocessed ponmo with fur, and the lowest counts of 2.0×10^6 cfu/g from processed ponmo.

A total of nine (9) bacterial species were identified and they are: *Bacillus* sp, *Staphylococcus sp*, *Escherichia coli, Pseudomonas sp, Streptococcus sp, Micrococcus sp, Aeromonas sp, Klebsiella sp* and *Vibrio sp*. These bacteria were not evenly distributed across the various abattoir locations. While some organisms were dominant and occurred in all the samples, others were not isolated. For instance, *Escherichia coli, Bacillus* sp. *Micrococcus* sp, *Pseudomonas* sp and *Staphylococcus* sp were present in all samples across the locations, *Klebsiella* sp was present in samples obtained from Rumuokoro, Tombia and Swale sample and Vibrio sp was obtained in Tombia and Opolo samples only. *Aeromonas* sp and *Streptococcus* sp were obtained in all the cowhide samples except in Opolo abattoir cowhides. The bacteria found in this study corroborates the findings of Keta., *et al*. [2], who reported *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermis, Streptococcus mitis, Micrococcus leteus, Escherichia coli, Shigella*

dysenteriae, and *Salmonella typhimurium* in processed and unprocessed ponmo samples from Ogbese market. The presence of the members of the Enterobacteriaceae in cowhide could be an indication of probable contamination from the huge excreta (faecal source) deposits always dumped on the bare abattoir floor where these cowhides are usually dressed during processing. More specifically, contamination from handlers, contamination from processing on bare polluted slaughterhouse grounds, and contamination from water used in washing the cowhide might all contribute to the presence of these organisms in the cowhide. This current study conforms with the reports in a previous study were bacterial isolates such as *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermis, Streptococcus mitis, Micrococcus leteus, Escherichia coli, Shigella dysenteriae* and *Salmonella typhimurium* were reported from processed and unprocessed cowhides except for *Shigella* and *Salmonella* sp which were not isolated in this current study [1]. Although *Vibrio sp* was found only in samples from two abattoirs (Opolo and Tombia), nevertheless, the organism is of medical significance in that it can vibriosis and cholera illnesses. Worthy of note is the fact that these two abattoirs wash their products in confined containers with limited freshwater supplies. Limited data are available from other researchers on the microbial load and distribution of organisms on cowhide.

Furthermore, some of these bacteria also exhibited the potentials for hydrocarbon utilization as carbon source. The fungi such as *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Geotrichum* sp., and *Candida* sp., isolated from the cowhides could also be as a result of contamination during processing. *Aspergillus* species were the most abundant fungi which confirm its ubiquitous nature. It was also observed in all the abattoirs that roasted cowhide were being dressed on the bare abattoir floor. Thus, this may account for the proliferation and abundance of soil fungi on the cowhide meat. Adesemoye., *et al*. [7] reported the fungi *Aspergillus* sp., *Penicillium* and *Fusarium* from contaminated abattoir soils. Obire and Deeyah [10] also reported *Aspergillus* sp., *Penicillium*, *Fusarium* and *Saccharomyces cerevisiae* from cassava contaminated soils The lack of proper quality control measures in handling dressing and processing of the cowhide in the different abattoir as well as the unhygienic practices of handlers such as talking, coughing and sneezing on the processed cow hide by the butchers, sellers and buyers could all be sources of contamination of the product [2]. Also, most of these handlers have little or no education about quality control and good manufacturing practices. Summarily, indiscriminate discharge of untreated wastes in the abattoir environment and unwholesome processing techniques as well as the materials employed during processing and display of products for sale plays vital role in the type of microorganisms that would contaminate the cowhides.

Conclusion

The high level of microbial contamination of processed cowhide as obtained in this study confirms the unsanitary handling and processing of this highly consumed meat product. The handlers carry out this vocation with bare hand on contaminated abattoir soils whilst washing is also done in unsanitary containers and water. The findings of this study clearly showed that cowhides could be reservoirs of pathogenic gram-positive and negative bacterial isolates as well as reservoir for toxigenic fungal isolates. The suggested approach to reducing microbial contamination would be to provide portable clean water, provide modern abattoirs facilities and train the meat handlers on the need for wholesome processing procedures in order to improve the sanitary quality of processed cowhide available for public consumption. Furthermore, the public should be aware of the dangers associated with consumption of cowhides and the need for proper post purchase preparation before consumption.

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