ORIGINAL ARTICLE

Prevalence of Mastitis and Brucellosis in Cattle in Awassa and the Peri-Urban Areas of Two Smaller Towns

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Impacts

- Brucellosis was detected in the peri-urban but not in the urban location. Whether artificial insemination and/or more controlled use of sires played a major role to eliminate brucellosis in the studied city needs further confirmation. As long as raw milk from peri-urban areas is consumed, it may pose a health hazard to consumers. This not only occurs through brucellosis, but also from faecal contaminations resulting from poor hygienic conditions during handling the milk.
- A low percentage of sub-clinical mastitis was found in crossbred cattle in the city and in one peri-urban location. This might be attributed to better hygiene and health care that is provided to crossbreds, and this practice not only helps to sustain higher milk yields but also provides a wholesome product to consumers.
- California mastitis test proved useful in detecting strongly positive and negative cases of sub-clinical mastitis at the farm. As the method is simple to use, producers and/or livestock extension agents need to be encouraged to make it a routine practice.

Keywords:
Mastitis; brucellosis; risk factors; urban dairying; Ethiopia

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Summary

The prevalence of mastitis and brucellosis in urban and peri-urban settings was studied in Awassa and two smaller nearby towns in southern Ethiopia, because milk-born diseases are causing a risk for human health, besides direct impacts on animal production. Mastitis was investigated by examining 80 cows (320 udder quarters) using California mastitis test (CMT) and somatic cell count (SCC). The prevalence of brucellosis was assessed by sampling 177 cattle in Awassa and its peri-urban areas using serological methods. Logistic regression was used to analyse risk factors associated with mastitis. Prevalence of clinical mastitis on quarter level was 0.9%, and 1.9% of quarters were non-functional or blocked. Prevalence of sub-clinical mastitis at quarter level in urban and peri-urban areas was significantly different ($P < 0.05$). Cows in large herds and at advanced lactation number were associated with higher risk of infection. The percentage of quarters positive on CMT (42.5%) was close to the percentage-positive detected by SCC (41.2%). Prevalence of brucellosis was 3.9% in the peri-urban area, while no brucellosis cases were detected in Awassa. More frequent use of artificial insemination in the urban than in peri-urban area might have contributed to the absence of brucellosis in the urban location. The extent of mastitis is, however, a threat to the dairy enterprise in and around Awassa. Pasteurization of milk and milk products is indicated in some parts of the area because of the danger of brucellosis.

Introduction

Urban and peri-urban dairy production plays an important role in meeting the increasing demand for milk and milk products in urban areas of developing countries. Urban dairy production in Ethiopia differs from that in peri-urban areas by being characterized by zero-grazing, no support by projects, more crossbred instead of local breeds, higher percentage of milking cows in the herd, higher milk yield per cow, shorter calving interval, frequent use of hired labour and higher importance of milk sales for income generation (Ike, 2002). While many factors are known to affect the productivity of the dairy herd in these areas, diseases such as mastitis and brucellosis are of special relevance, the former for increasing impact on animal health and performance, the latter for bearing a threat to human health. The majority of the dairy producers in and around Addis Ababa (Ethiopia) identified mastitis as a constraint for production [International Livestock Centre for Africa (ILCA), 1994]. Kassa et al. (1999) reported a prevalence of sub-clinical mastitis (SCM) at quarter level in over 40% in urban and peri-urban areas of the capital's dairy enterprises and 25% in urban areas of secondary towns.

Although mastitis can result from a wide variety of causes, mastitis in dairy cattle is generally a result of microbial infection, bacteria being the most implicated microbial agents. Geressu (1989) reported that the major bacterial species identified in mastitis cases in Ethiopia are *Streptococcus* spp., *Staphylococcus* spp. and *Corynebacterium* spp. The inflammation arising from mastitis usually led to a rise in the somatic cell count (SCC) in the milk (Kehrli and Shuster, 1994). Factors that can pre-dispose to mastitis include inherited susceptibility like weak sphincter muscles that leave the teat canal continuously open and deep pendulous udders, wet and dampness of grasses and beddings, lack of ventilation in the stable, poor milking hygiene and lack of sanitation in the dairy yard and building (Chamberlain, 1989). In the central highland of Ethiopia, Mungube et al. (2004) identified previous udder infection and leaking udder to have a significant effect on the presence of mastitis. Mastitis decreases milk yield and affects milk quality (Shook, 1989), resulting in economic losses (Philpot, 1984).

Human cases of brucellosis were reported in the Afar region of Ethiopia in December 2002, and the same report suggests that brucellosis may be a permanent issue in the region (UN-EUE, 2003). Existence of human cases of brucellosis in Ethiopia was again reported by OIE for 2004 (Pappas et al., 2006). Brucellosis has also been reported from neighbouring countries including Somalia (Wernery et al., 1979), Eritrea (Omer et al., 2000) and Sudan (McDermott et al., 1987). Several serological methods are available for the diagnosis of brucellosis, however, differing in sensitivity and specificity; the only confirmation of a current brucellosis is by the isolation of the causative agent.

Brucellosis remains a public and animal health problem in many regions of the world (Robinson, 2003). Apart from Mauritius, no country in Africa has reported the eradication of brucellosis. Among sampled cattle herds in southern Sudan, a prevalence of 20.2% was found for brucellosis (McDermott et al., 1987). In dairy cattle under intensive management in Asmara, Eritrea, 1.6% of male animals and 8.5% of female animals were found to be seropositive for brucellosis (Omer et al., 2000). In intra- and peri-urban dairy production systems in Addis Ababa, an overall prevalence of 8.1% was reported and the prevalence has been shown to be related to herd size and age of animals (Asfaw et al., 1998). The re-emergence of brucellosis in some countries that had reported eradication has made the prospect of total eradication even more remote (Amato Gauci, 1995).

Mastitis and brucellosis are two diseases with high economic importance. Most of the work in Ethiopia on urban and peri-urban dairy production have concentrated in the capital city, i.e. Addis Ababa and surrounding towns or on large dairy herds owned by institutions (Abdella, 1996). To our knowledge, this is the first study that is based on these two diseases in the study area targeting private dairy farmers. It was hypothesized that the occurrence of mastitis and brucellosis differ between peri-urban and urban locations, with both diseases occurring more frequently in the peri-urban areas.

Materials and Methods

Study area

Awassa is the capital of the Southern Nations, Nationalities and Peoples region of Ethiopia. The study area lies between 6.75°N and 38.47°E with Awassa at 7.06°N and 38.47°E. Awassa city represents the urban area in this study, which was contrasted to the peri-urban areas of two smaller towns, namely Leku and Yirg'Alem for the mastitis study. The prevalence of brucellosis was studied in Awassa and its immediate peri-urban area, namely Loke.

Selection of farms and cows for mastitis test and SCC

Of 124 interviews on production conditions on dairy farms in the region (Ike, 2002), 49 farms involved in market-oriented milk production were selected for mastitis examination. From those, 26 were from Awassa (urban area) and 23 from the peri-urban areas (Table 1). In Awassa, 50 cows were randomly selected from 153 lactating cows (33%) and in the peri-urban area, 30 cows were

Table 1. Number of herds and cows in the mastitis sample by location and herd size

Location	Herd size (average number of cows)	Herds selected for mastitis test (*n*)	Lactating cows selected for mastitis test (*n*)
Urban	1–3	6	7
	4–9	13	26
	>10	7	17
Peri-urban	1–3	9	10
	4–9	10	14
	>10	4	6

selected from a total of 92 lactating cows (33%), resulting in 320 udder quarters for further examination. All examined cows were crossbreds between Friesian and local breeds. Based on the initial survey (Ike, 2002), farms were categorized into three classes of herd size viz. small (1–3 cows), medium (4–9 cows) and relatively large (≥10 cows). Cows having lactation numbers from 1 to 3 were grouped in an early lactation group and all others in a late lactation group.

Collection of milk samples

Milk samples were collected just before the regular hand-milking by the farmers. The udders were washed with warm water and dried; the first stream of milk was discarded. About 2 ml of foremilk was collected from each of the functional quarters of the cow for the California mastitis test (CMT). Another 10 ml of milk sample from each quarter of the udder was collected in a clean tightly closed container. This was preserved at 4°C in a refrigerator until the following day for SCC.

California mastitis test

All 320 udder quarters were examined for abnormal changes in the gland and its secretion. Clinical mastitis (CM) and non-functional or blocked quarters (NFBQ) were diagnosed by conducting clinical examination in the four quarters of the cows. CM was diagnosed when there were signs of inflammation and macroscopic abnormal changes in milk such as purulent discharge or presence of blood. No CMT was performed on these quarters. In NFBQ, no milk was produced from the quarters.

California mastitis test was employed in the diagnosis of SCM. The 'eimü-MILCHTEST-NEU' kit by Eimermacher (Nordwalde, Germany) was used. The milk sample was collected into different wells of the CMT paddle. An equal volume of CMT reagent was added. The mixture was rotated for a few seconds and the result observed. The reaction was visually scored as 'negative' (0), 'trace' (+), 'weak positive' (++), 'distinct positive' (+++) and 'strong positive' (++++) according to the amount of gel formation. One microbiologist did all the tests and scoring throughout the study.

Somatic cell count

Somatic cell count was carried out by a direct microscopic cell count on the 310 samples that were tested for SCM by CMT. The milk was removed from the refrigerator and allowed to attain room temperature. It was then diluted 1 : 20 with 18 drops of whey and one drop of light methylene blue and left to stand for about 10 min to allow the cells to be stained with the dye. The improved Neubauer counting chamber (Laboroptik GmbH, Friedrichsdorf, Germany) was charged and left to stand for about 2 min to allow the cells to settle. The cells were counted using an ×40 objective lens in two diagonal large squares of the counting chamber (Cheesbrough, 1984). The number of cells in the sample was obtained by multiplying the counted cells by 50 000.

Brucella antibody test

Brucella antibodies were tested in 177 cows, of which 75 were from Awassa (22 farms), and 102 from the peri-urban area Loke (from three places where several owners herded their animals together). Most of the crossbred cattle tested (62 of 67) were from the urban area, and most of the local cattle (97 of 110) were from the peri-urban area. As this test involved bleeding of animals, the willingness of the farmers was decisive for the selection of animals and therefore they were not necessarily identical with those tested for mastitis. Consequently, the sampling is not claiming to be representative for the whole region.

About 10 ml of blood was collected by jugular bleeding using the pressure of the thumb according to Ryley (1983). This was after restricting the animal and swabbing the skin with pharmaceutical (70%) alcohol. The blood was collected in a plain test tube and kept in the refrigerator overnight for the serum to separate.

Brucella antibodies were screened in the serum using *Brucella abortus* Rose Bengal test antigen provided by the Veterinary Laboratories Agency (Weybridge, UK). The antigen was prepared from *B. abortus* (Strain 99) stained with a 1% Rose Bengal dye and suspended in lactic acid buffer with pH 3.65 ± 0.05. A rapid slide agglutination test was used to screen the samples by mixing a drop of the serum with a drop of the antigen on a slide and shaken for a few minutes. In addition, the reagent was positively controlled with the one usually used for screening animals before slaughter by veterinarians in the town of Awassa.

Statistical analysis

Descriptive statistics were performed using Microsoft Excel. A logistic regression analysis was carried out for detecting risk factors using the GLIMMIX procedure of SAS software 9.1 (SAS Institute, Cary, NC, USA). The CMT status at quarter level was the response variable with two possible outcomes, negative (0) or positive (1), representing CMT values + to ++++, and the explanatory variables were location (one urban and two peri-urban sites), herd size (three classes) and lactation number (early and late). The variable 'cow' was included as a random effect in the model to remove biases that could arise from correlations among the four quarters of a cow.

Somatic cell count values were log-transformed and an analysis of variance (PROC GLM, SAS 9.1) was used to identify factors that most affected them. Factors tested in this multivariate model were location, herd size and lactation number.

The discriminant analysis of SAS (PROC DISCRIM), including the cross-validate option, was used to investigate if SCC (after log transformation) would help in detecting SCM compared with CMT values (all five categories). Only moderate departure from normality was detected for the log-transformed SCC data. The inverse of the apparent error count estimates was considered as apparent success rate of CMT scores being in accordance with SCC results.

Results

Mastitis prevalence

More than half of the quarters were mastitis free (negative reaction of CMT, 54.7% of all quarters) and 1.9% of quarters fell under the category NFBQ (Table 2). Of the infected quarters, the major part showed SCM whereas CM was detected in only 2.1% of infected cases. Clinical mastitis was detected exclusively in the urban area.

The overall percentage of quarters positive for SCM according to the CMT test was 42.5%. Among the two urban areas considered, Leku exhibited a higher prevalence of mastitis. Cows in Leku had a higher rate of infection as compared with all others. Figure 1 shows the distribution of various degrees of CMT for all locations studied.

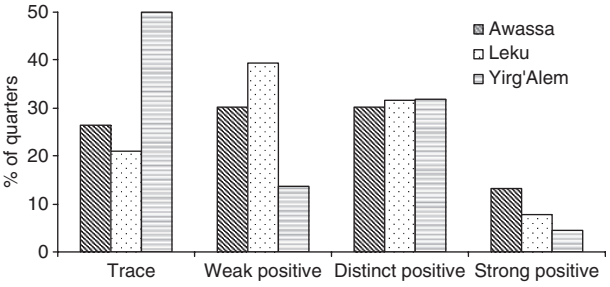

Fig. 1. Distribution of California mastitis test scores of infected quarters in urban Awassa and two peri-urban locations.

Cows in the peri-urban areas of Yirg'Alem were characterized by a higher percentage of 'trace' scores while in Leku, scores of 'weak positives' were more frequent as compared with the others. The percentage of 'distinct positive' was almost the same for all locations. The 'strongly positive' quarters were most frequent in Awassa.

Risk factors of having mastitis

In Leku (peri-urban), the odds of having mastitis were 3.1 times higher than those of cows in the urban place (Table 3). However, the lower confidence limit of Leku was just above 1, indicating that some farms at Leku were quite similar to the urban mean whereas others were very distant as shown by the upper limit of 8.7. One of the peri-urban locations (Yirg'Alem) did not differ from the urban location.

Large herd size was also associated with higher risk in comparison with the medium herd size; however, there was no significant difference with the small farms. Cows in the fourth and above lactation had a higher risk than those in early lactation.

Table 2. Prevalence of udder mastitis according to California mastitis test

Location	Quarters (*n*)	Non-functional and blocked (%)	Clinical mastitis* (%)	Sub-clinical mastitis† (%)	Free (negative reaction) (%)
Urban	200	1.5	1.5	38.0	59.0
Periurban	120	2.5	0.0	50.0	47.5
Leku	60	3.3	0.0	63.3	33.3
Yirg'Alem	60	1.7	0.0	36.7	61.7
Total	320	1.9	0.9	42.5	54.7

*Defined as abnormal appearance of the milk and clinical signs of inflammation of the udder.
†Defined as no clinical signs observable but gel formation in California mastitis test of at least trace score.

Table 3. Importance of risk factors for mastitis according to least squares means of California mastitis test results

Risk factor	Level	Odds ratio	*P*-value	Lower confidence limit	Upper confidence limit
Location	Leku	3.10	0.04	1.106	8.671
	Yirg'Alem	0.82	0.03	0.273	2.461
	Awassa	1			
Herd size	1–3 cows	0.60	0.39	0.188	1.898
	4–9 cows	0.32	0.02	0.125	0.823
	≥10 cows	1			
Lactation number	Early	0.21	0.001	0.089	0.482
	Late (≥4 lactations)	1			

Somatic cell count

Factors considered in the general linear model analysis, i.e. location, herd size and lactation number, proved not to be decisive as the model explained only a small part of the variation (R^2 = 8%). Location had the highest impact of the three factors considered. Milk from cows in the peri-urban area had significantly higher counts than milk samples obtained from the urban location ($P < 0.01$). This result was, however, because of disproportional poor conditions in only one of the peri-urban areas studied, which outweighed the above average standard of a second peri-urban area. Furthermore, small herd size was associated with higher count ($P < 0.01$) as compared with large herd sizes. Cows in late lactation number (indicative of old age) had significantly higher counts ($P < 0.01$) as compared with cows in their first to third lactation.

Detecting mastitis with CMT

Matching SCC with CMT data on quarter level revealed that about 48% of the counts could be classified properly into different scores of CMT. As it is shown in Table 4, a higher proportion of cases were correctly classified to 'negative' (CMT score = 0) and 'strong positive' cases (CMT score = ++++), namely 65% and 69%, respectively. However, the assignment of quarters by CMT to the scores 'trace' and 'weak positive' could only be sustained by SCC data by about 25% of the cases.

Table 4. Success rates in predicting CMT scores from SCC counts as simulated by discriminant analysis

Substitute tested	CMT score					
	0	+	++	+++	++++	Overall
SCC (%)	65	24	25	57	69	48
n	143	53	31	57	26	310

0, negative; +, trace; ++, weak positive; +++, distinctive positive; ++++, strong positive; SCC, somatic cell count; CMT, California mastitis test.

The SCC medians (untransformed data) corresponding to the five CMT groups were: 'negative' 150 000 cells/ml, 'trace' 250 000 cells/ml, 'weak positive' 350 000 cells/ml, 'distinct positive' 700 000 cells/ml and 'strong positive' 1 300 000 cells/ml milk.

Brucellosis

Of the 75 blood samples from cows tested for *Brucella* antibodies in Awassa, none was positive. There was a 3.9% positive reaction from the 102 samples from the peri-urban areas. None of the 67 crossbred cattle tested was positive.

Discussion

The study partly confirmed the hypothesis that mastitis and brucellosis occurred more frequently in the peri-urban than in the urban location. The findings of this study show that CM and NFBQ occurred at lower percentages than SCM in the entire study area. This is in accordance with the findings of other studies (Maina and Mulei, 1993; Kassa et al., 1999). The prevalence of NFBQ, 1.9% in this study, was clearly lower than the prevalence of 16.9% reported by Maina and Mulei (1993) for Kenya. Prevalences of CM and SCM were slightly lower than figures given by Kassa et al. (1999) for Addis Ababa.

The higher percentage of SCM in the peri-urban area (50%) than in the urban area (38%) in this study was in contrast with the results obtained in Addis Ababa, where no significant difference was found between the peri-urban and the urban study locations (Kassa et al., 1999). In Leku, a very high prevalence of SCM of 67.2% was identified. This may be attributed to the observed lower level of hygiene in this area pre-disposing cows to mastitis. High prevalence of SCM causes reductions in milk production (Mungube et al., 2005), reducing competitiveness of the peri-urban area and this means lost income for the dairy farmers.

There was an apparent contradiction in the results of SCC and risk factors of mastitis with regard to herd size. When taking SCC as an indicator of poor hygiene, then this explains why higher counts are evident in small herds. Small herds are constituted of local zebu cows and the attention given to them is little as compared with exotics. On the other hand, large herds in urban areas are dominated by crossbreds with exotics (Friesians), which are less adapted to the local conditions and may be more prone to mastitis. Breed as an effect has not been tested in the analyses because of a wide overlap with location,

especially for the urban site where 98% of the milking cows were crossbreds, while in the peri-urban sites, about 60% were crossbreds. Moreover, the low goodness of fit (R^2) of the analysis of variance for SCC suggests that major causes of differentiation between farms could not be considered in this study. Further investigations about the hygienic conditions and milking practices of the farmers and their workers are necessary before drawing final conclusions.

In most cases, SCC is used for the identification of SCM rather than CMT. The results of discriminant analysis show that it is possible to use CMT to predict at least the strongly positive and the negative results of SCC with a reasonable degree of accuracy. Farmers can rely on CMT in general, and the CMT-kit used for the study in particular, in monitoring the mastitis status of their cows, thus being able to take the necessary steps early enough, provided that access to the kits is given.

The very high SCC obtained in some cases in this study (>5 000 000 cells/ml) (result not shown) may be indicative of bacterial infections. According to Kehrli and Shuster (1994), an elevated bulk milk SCC is indicative of the types of pathogens present within a given herd. Pyogenic bacteria like *Staphylococcus* spp. and *Streptococcus* spp. are known to stimulate leucocytes more than other non-pyogenic organisms. No attempt was made to isolate causative agents in this study; however, a previous study conducted on large dairy farms owned by institutions in the same region identified *Staphylococcus aureus* (47%), *Streptococcus uberis* (31%) and *Streptococcus agalactiae* (15%) as the major bacteria causing mastitis (Abdella, 1996). Many of the bacteria that cause intramammary infections are also the causative agents of human diseases (e.g. *Escherichia coli*, *S. aureus*, *S. agalactiae*). In many of the developing countries, where pasteurization is not a standard process, these organisms can have a devastating result on human health. It is thus crucial for consumers in such countries to boil milk before consumption. The health risk for milk consumers further increased with hazards of milk contaminated with faecal material or unsanitary equipment during or after the milking process, especially where low levels of hygiene were observed.

The different prevalence of brucellosis between the urban (no case of *Brucella*) and peri-urban location suggests that there was limited or no contact between the animals in the two locations. Furthermore, it was noted that urban producers use artificial insemination as a breeding method more frequently than peri-urban producers do (Ike, 2002) and this might have contributed to the control of this disease.

The individual animal prevalence of brucellosis obtained in this study was below the high ranges given by Berman (1981) for East Africa (15%) and Newton et al. (1974) for Uganda (18%) and for intra- (5.2%) and peri-urban (9.6%) dairy production systems in Addis Ababa, Ethiopia (Asfaw et al., 1998). We cannot exclude that the real prevalence in our study area might have been underestimated, given that the sampling depended on voluntary participation of farmers and no confirmatory test but only a screening could be performed because of unavailability of more sophisticated tests and financial restrictions. However, literature also presents low rates comparable with the finding of this study (Bedard et al., 1993 for Malawi and Mustafa, 1984 for Bangladesh). Our finding of brucellosis among local cattle under extensive management system is in agreement with the finding of Bedard et al. (1993), who attributed the infection to the fact that local breeds are usually allowed to graze together, which was also the situation in our study. However, it contrasts with the findings of Wernery et al. (1979), who reported higher prevalence among cattle kept under an intensive system than among the nomadic herds in pastoral areas. It seems that in the case of our study, the close contact between the animals outweighed the advantage of the solar radiation destroying the infective agent on the pasture (Wernery et al., 1979). While the nomads moved from place to place, allowing the animals to avoid infected sites, in the case of the peri-urban areas in our study, the animals were grazed on the same pasture repeatedly. As the crossbreds in the study were the non-infected and also the cows predominately found in the urban area, threats from, e.g. non-adequate handling of afterbirths in a densely populated area were reduced.

Measures being taken towards control and eradication of brucellosis in the study area included carrying out regular screening for antibodies in animals, particularly cattle, by the Ministry of Agriculture and persuading owners of affected animals to cull them. Our results only indicate the presence of antibodies in animals as confirmation of results by isolation was not carried out. There was also no contemporaneous testing for *Brucella* antibodies in humans in the study area. However, of 240 respondents (farming families who usually consume raw milk from cows) interviewed in the course of this study, none reported any evident signs of human infection (Ike, 2002). According to WHO (1997), there is generally a high underreporting of *Brucella* cases in humans and true incidence is estimated to be between 10 and 25 times higher than reported figures. This is partly because of the initial symptoms of the disease in humans being quite similar to symptoms of other diseases. Pappas et al. (2006) reported that most febrile patients are initially diagnosed as suffering from malaria and only few may be further tested for brucellosis. Moreover, it is difficult to find official reports on the incidence of brucellosis in

humans as diseases related to abortions are usually hidden.

This study can give a first insight into the occurrence of mastitis and brucellosis in urban and peri-urban cow milk production in Southern Ethiopia, the latter posing considerable threat on human health. Follow-up studies should focus on the specific critical control points of the animals and the related human health situation at the producer level, e.g. in terms of disposal of afterbirths, and at the consumer side, e.g. with regard to pasteurization of milk and its derivatives.

Conclusion

This study has demonstrated that mastitis is a threat for dairy farms in and around Awassa. The farmers should be encouraged and empowered to regularly check their cows and seek for veterinary help. The case of Leku (one of the peri-urban areas studied) clearly showed the importance of improving hygiene in dairy farming.

The fact that *Brucella* antibodies were detected suggests a potential threat to human health. As detection occurred only in local cattle, which are predominately found in peri-urban areas, but not in crossbreds, which predominate in the urban area, it may be useful to separate the local cattle from the crossbreds specifically used for dairy production. In local cattle, the ones probably more resilient under the climatic conditions, it would be useful to optimize hygienic conditions and milking techniques. As raw cow milk consumption is common in the study area, adequate pasteurization or other inactivation and sanitary measures to be applied before consumption of milk and milk products from potentially infected animals is highly recommended.

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