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## Bibliographic Synthesis of Sero-Epidemiological Studies on Brucellosis in Domestic Ruminants (Cattle, Sheep and Goats) in Cameroon

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### ABSTRACT

Brucellosis is a neglected zoonosis in Cameroon but it remains enzootic in some agro-ecological zones of the country. This review aims to describe the current status of this disease in domestic ruminants in Cameroon. In order to know the current status of this disease, a systematic and synthetic review was conducted on brucellosis in domestic ruminants in Cameroon. Different types of studies with serological results were reported and considered. A total of 16 studies from 1980 to 2020 were included in this synthetic review of the literature. Most of the studies were cross-sectional descriptive studies ( $p = 0.12$ ). However, there were also studies with simplified survey methods, modelling approaches, stratified non-probabilistic and probabilistic surveys. 5095 cases of brucellosis infection were identified in the literature, for which the laboratory diagnosis allowed confirmation of brucellosis in the different species (indirect or direct Elisa in the majority of studies (41%), followed by rose Bengal (26%)). In most cases cattle represent 80% of the most studied species. Associated with this, the northern part of Cameroon was the preferred study area at 70% followed by the west (27%) and the south (3%). The biological material of choice for the studies was serum (75%). In addition, other biological materials (15%) were used. This systematic review has identified the tools used over the last 40 years to diagnose brucella infection in ruminants in Cameroon. However, it highlights the need for continuous monitoring of the spatio-temporal evolution of abortive microorganisms on farms.

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### Background

Brucellosis is a bacterial disease of socio-economic importance in both animal and human medicine [1]. Because of its threefold importance (Medical, Sanitary and Economic) it is listed in the Terrestrial Animal Health Code of the World Organization for Animal Health (OIE) and must be notified to the OIE. Transmission to humans most often occurs through ingestion of raw milk from animals infected with the bacterium, which causes a severe and debilitating disease [2]. A notifiable disease, it represents a neglected cause of mortality in Cameroon, due to a lack of notification on the part of farmers. This lack of information limits the government's decision making and in turn forces the country to work in under production.

Brucellosis is a major cause of reduced sheep, goat, and cattle production, with serious repercussions on the livelihoods of livestock farmers [3]. Generally, the disease is mild, with the infected animal showing few signs before abortion [4]. Surveillance with serological tests as well as milk tests such as the color ring test can be used to detect the disease and are important in campaigns

to eliminate it. Similarly, individual animals can be tested for both prophylactic and commercial purposes [5]. Livestock rearing is now seen in Cameroon as a means of developing agricultural resources with people having an agro-pastoral vocation. For some groups, it is the only production that gives rise to regular marketing and with a capital that is growing among farmer-breeders, whereas it seems to have reached a ceiling for traditional breeders [6]. It goes without saying that, in parallel with this socio-economic professionalism, the development of productive and sustainable livestock production systems is essential, and it is necessary to broaden the scope for identifying action levers.

Controlling infection in domestic ruminants is the best way to prevent human brucellosis and to increase the income of livestock farmers. Monitoring the transmission and spread of brucellosis is essential for estimating the disease burden, planning control strategies and evaluating the impact of interventions. However, surveillance systems can assist programmed managers in reducing the transmission and spread of brucellosis by providing information on the animal populations in which incidence is highest and, therefore, to which resources should be targeted. A few studies on the seroprevalence of brucellosis in domestic ruminants have been identified [7-10]. These studies point to the multiplicity and

complexity of abortifacient infections either at the individual or herd level. This result allowed them to propose, in order to reduce the impact of these microorganisms in farms, to carry out a continuous monitoring of the spatio-temporal evolution of microorganisms in farms. Therefore, the overall objective of this study is to collect data from serological surveys on brucellosis in Cameroon through a strategic and systematic search of published papers on PubMed and Google Scholar. The specific objectives of this study include: (a) description of representative studies on the seroprevalence of brucellosis in Cameroon; (b) listing of serological methods used; (c) identification of limitations in knowledge, attitude and practice for serological investigation of brucellosis in Cameroon.

**Methods**

**The Research Strategy**

It considered different types of studies (cross-sectional surveys and longitudinal studies) with serological and molecular results in well-defined ruminant populations (Cattle, Sheep and Goats). Articles included in this review were searched using PubMed and Google Scholar databases published up to August 2020. The search was conducted using the following search terms: “Brucellosis, Ruminants and Cameroon”.

**Selection criterion**

This review included studies reporting data from serological and molecular surveys and simplified survey methods on brucellosis in Cameroon. This review included articles written in English and

French. To be included:

- Studies should have provided a description of the laboratory methods used for sample analysis;
- The statistical methods and the description of the sample strategy must be notified;
- The studies must have provided a description of the biological material used.

**Data extraction**

Data extraction was based on the inclusion criteria to identify potential studies based on titles and abstracts. Relevant information from the selected articles was extracted and entered into a standardized Excel spreadsheet. Data were extracted using a predefined form, which included the following information: Publication data (journal, authors, study period, year of publication), Study area in Cameroon; Targets (Sheep, Goats, Cattle), Type of study, biological material, Sample size, Diagnostic methods and Prevalence of pathology.

**Critical Assessment**

The critical appraisal was based on a weighted tool developed by Folegatti et al. 2017 based on the modified checklists proposed by Downs and Black and the NOS (Table 1). Articles were evaluated against a score-based system that combined elements of both scales, giving weight to studies with samples that were truly representative of the population. Articles with a score > 70% were included in this review [11].

**Table 1: Critical appraisal tool used in this review[11].**

<b>Critical Appraisal Tool</b>	
1. Was the research question or objective in this paper clearly stated?	1 point
2. The study clearly describes the exposures and outcomes	1 point
3. The study clearly describes the basic characteristics of the participants	1 point
4. Results were adjusted for potential confounding variables by stratification or multivariate analysis	1 point
5. The statistical test used to analyze the data is clearly described and appropriate, and the measure of association is presented	1 point
6. The study provides information on the characteristics of sight loss: numbers and reasons	1 point
7. Participants were followed for the same time period or the study was adjusted for different follow-up times	1 point
8. The measures used for the main results were accurate: description of the diagnostic technique for Brucellosis	1 point
9. The demographic characteristics were comparable or adjusted: geographical area of breeding, speculation...	1 point
10. Participants from different groups were recruited during the same period	1 point
11. Representativeness of the sample	
i. Representative of the average of the target population: all subjects or random sampling	1 point
ii. Somewhat representative of the average target population: non-random sampling	1 point
12. Sample size Justified and adequate	1 point
13. Verification of exposure (risk factor)	
i. Validated measurement tool or non-validated measurement tool, but the tool is available or described	1 point
ii. No description of the measurement tool	

Cross-sectional studies receive a maximum of 12 points. Cohort studies receive a maximum of 14 points.

**Results**

**Description of included studies**

The first cases of brucellosis in Cameroon were described during clinical and epidemiological studies in semi-intensive cattle farms and published in the 1980s [12]. The search produced 25 articles in the two different databases (PubMed and Google Scholar). 16/25 articles scored 80% when the critical appraisal tool was applied and were therefore excluded. Therefore, 16 studies were included in this study for the qualitative synthesis of the published literature (Table 2). The studies were published between 1980 and 2020, with a greater proportion of these after the year 2000. Only 1 (One) study was published in the 1990s and 4 in the 1980s.

**Table 2: Summary of published cases of brucellosis in ruminants in Cameroon**

Year	Authors	Target(s)	Study area in Cameroon	Type of study	Biological materials	Sample size	Diagnostic methods	Number of positive	Prevalence (%)
1980	Domenech et al,(12)	Cattle	North, Lake Chad, Adamaoua	Clinical and epidemiological study	Serums	5500	E.A.T. serology	1620	29,5
1982	Domenech et al, (13)	Cattle	North	Clinical and epidemiological study	Serums	7665	E.A.T. serology	2361	30,8
1982	Domenech J. et al, (25)	Cattle	North, Lake Chad, Adamaoua	Simplified survey method	Hygroma	NA	Correlation	>NA	85,2
1983	Domenech J. et al, (24)	Cattle	North	Clinical and epidemiological study	Liquid-hygromas, vaginal mucus, abortions, placentas, milks	44 strains of brucella	PCR	42	92,5
1987	Akakpo, Bornarel, (14)	Zebu, bulls	North, Far North, Adamaoua	Descriptive	Serums	962	ELISA, SAW, RB, FC	120	12,5
1993	Martrenchar et al, (15)	Zebu	North, Far North	Descriptive	Serums	607	RB, FC	51	8,4
2005	Shey-Njila et al, (16)	Cattle	Dschang	Descriptive	Serums	840	Elisa indirect, RB	122	14,52
2009	Bayemi et al, (17)	Holstein Cattle	Kutaba, Bamenda, nkwe Finge	Descriptive	Serums	192	ELISA	14	8,4
2010	Scolamacchia et al, (18)	Cattle	Adamaoua	The modeling approach	Serums	1377	ELISA	275	20
2014	Nicolas Houli B (19)	Cattle	North, Adamaoua	Laminate type survey	Serums	1031	RB, i-ELISA	199	19.3
2015	Bayemi et al,(1)	Cattle	North West Region	Descriptive	Serums	689	ELISA	36	5,2
2016	Kong, A., et al, (20)	Cattle	Western Highlands	Descriptive	Serums	198	C-ELISA	8	4,04
2018	Awah-Ndukum et al (21)	Cattle	Ngaoundéré	Descriptive	Serums	590	RB, ELISA	20	3.40
2018	Awah-Ndukum et al (22)	Cattle	Adamaoua and North Regions	Cross-sectional seroprevalence	Serums	1031	Rose Bengal and i-ELISA	55.7	5.4
2019	Musallam et al,(23)	Cattle	Bamenda, Ngaoundéré	Probabilistic	Milk	242	Indirect ELISA	18	16,8
2020	Kamga et al, (10)	Cattle, sheep, goats,	South	Cross-sectional studies	Plasma	1873	Rose Bengal and ELISA	118	6,35

EAT = Buffered Antigen Test; SAW= Wright's serum agglutination; RB= Rose Bengal; FC= Complement Fixation; ELISA = Enzyme-linked immunosorbent assay; PCR= Polymerase Chain Reaction

Most of the studies carried out from 1980 to 2020 on brucellosis infections in Cameroon are of the descriptive type  $V_{inter} = 764258.97$ ,  $V_{intra} = 313353.83$ ,  $F = 2.44$ ,  $p = 0.015$ . However, there are also studies with simplified survey methods (correlation), modelling approaches, non-probabilistic and probabilistic stratified type surveys. Associated with this, the northern part of Cameroon was the preferred study area at 70% followed by the west (27%) and the south (3%). The biological material of choice for the studies was serum. However, the prevalence of brucella infection has been high when some authors have used other biological materials including hygromas, vaginal mucus, abortus, placenta and milk [10,23,25]. In most cases, the authors describe clinical signs of brucella infection, but do not specify the germ found, as the diagnostic method of choice used by these authors has been serology. Indirect or direct Elisa in the majority of studies (69%), followed by Rose Bengal or buffered antigen test (64%). Studies carried out from 1980 to 2020 show that this disease is diagnosed in a minority of cases in other animal species (sheep, goats, pigs, dogs) [10].

### Studies on the seroprevalence of brucellosis in Cameroon

The first paper on the seroprevalence of brucellosis included in this review was published in 1980 by Domenech et al. representing a pioneering clinical and epidemiological study for future researchers [12]. This study was carried out on 5,500 sera from cattle in semi-intensive farms in the North, Lake Chad and Adamaoua. 1620 sera were positive for brucellosis by the *buffered antigen* test. In view of the importance of this disease in these regions of Cameroon, the author decided in 1993 to identify the species of brucella

circulating in the northern region. He identified 42 strains of brucella in Northern Cameroon. Subsequently, the authors sampled from 1987 to 1993 to take into account the variations of the modes of transmission during the rainy season or the dry season. But also, to highlight the presence of antibodies by various serological methods. In particular, indirect or direct Elisa in the majority of studies (69%), followed by the Rose Bengal or buffered antigen test (63%). However, we also note the use by the authors of Wright's seroagglutination test, the Complement Fixation and the Polymerase Chain Reaction. Cases of brucella infection are more frequent in the northern part of Cameroon. The Far North was first with 2889 cases in 14 years of study, followed by the Adamaoua region with 1139 cases and the Northern region with 755 cases of brucella infection. The overall seroprevalence varied from 3.40 to 30.8% in the clinical and epidemiological studies. In the studies using a simplified survey method, the prevalence was 85.2%.

The prevalence of this disease has increased over the last 40 years due to the effect of the breeding method and the persistence of the infection in the farms by healthy carriers. Indeed, the correlation of the number of positive animals according to the year's highlights with a significant difference the evolution of the number of cases according to the years with a Correlation Coefficient of -0.61 (Number of positive explains 37% of the variance of the year) and a Standard Deviation of the regression coefficient = 0.01. This difference was also observed with the sample size with a Correlation Coefficient of +0.98 and a Standard Deviation of the regression coefficient of 0.02.

#### **Serological methods used**

Several diagnostic methods have been used over the past 40 years for the detection of brucellosis in ruminant livestock in Cameroon. The first was the "buffered antigen test or rose bengal test" used by Domenech et al. in 1980 [12]. This technique enabled him to carry out a clinical and epidemiological study of brucellosis on 5,500 sera collected from semi-intensive farms in Cameroon. This test is a simple rapid agglutination using an antigen stained with Rose Bengal and buffered to a low pH, usually  $3.65 \pm 0.05$  (26). Of 5500 sera collected, 1620 were positive, giving it a prevalence of 29.5%. This technique remains usable to this day [10,22,27]. It should be noted that it was in 1987 that the enzyme-linked immunosorbent assay (ELISA) was used for the serology of brucellosis in Cameroon [14]. In combination with this, the authors used three other serological techniques to test the sera from Senegal and Togo. These were Wright's serum agglutination (SAW) because of its historical interest, rapid slide agglutination with buffered antigen in acidic medium stained with Rose Bengal (RB) because of its ease of performance, simplicity and convenience, and finally complement fixation (CF) because of its specificity and sensitivity [14]. This study by Akakpo & Bornarel in 1987 highlights the possibility of combining serological tests to get a better result. It will serve as a basis for future studies in Cameroon [10,15,16,19,27]. The enzyme-linked immunosorbent assay (ELISA) alone or in combination with other methods was the selected serological test used in 11 (69%) of the included papers. Rose Bengal or buffered antigen test was used in nine studies published between 1980 and 2020. However, molecular biology techniques were used by Domenech et al, 1983 to identify 44 strains of brucella in liquid-hygromas, vaginal mucus, aborta, placentas and milk [24]. This technique has not been used in the last 37 years to identify species circulating in Cameroon.

The diagnostic methods used by the authors significantly influence the detection of positive animals. The equation of the regression

line shows that the number of positives is  $+3.88 *$  and the diagnostic methods are  $-0.00$ . The correlation coefficient is  $-0.94$  (diagnostic methods explain 87% of the variance in the number of positives) with a standard deviation of the regression coefficient of 0.00 (Figure 1).

#### **Cross-sectional study and antibody responses to brucella antigens in domestic ruminants in Cameroon**

A total of 12 cross-sectional studies investigated humoral responses to *Brucella* antigens (1,7,27,10,14-18,20,23) and two (2) of the studies assessed IgG profiles to Brucella blood antigens in domestic ruminants in the South, Adamaoua, North, North West and West Cameroon regions respectively [7,10]. The areas studied are considered hypo-/hyper-endemic with variable and unstable brucellosis transmission. Two different techniques, RB and ELISA, were used to measure total blood IgG in the study populations. The relationship between seroprevalence and exposure to brucellosis was heterogeneous among the studies: one study found an increase in seroprevalence with age while another suggested an increase in seroprevalence with sex and age of the sampled subjects [10]. In cross-sectional studies conducted from December 2016 to August 2018 in five sites in southern Cameroon, blood samples were collected from cattle, sheep, goats. The prevalence of Brucella antibodies using indirect enzyme-linked immunosorbent assay (i-ELISA) was 9.12% (78/855) in cattle; 8.04% (30/373) in sheep; 6.06% (2/33) and 1.1% (5/452) in goats. Between animal species ( $p$ -value  $< .0001$ ,  $x^2 = 33.63$ ) as well as between sampling sites ( $p$ -value =  $.0001$ ,  $x^2 = 18.97$ ), significant differences were observed in the prevalence of Brucella antibodies. This prevalence was significantly higher ( $p = .03$ ,  $x^2 = 1.25$ ) in female cattle than in males. Between adult (16.923%) and young cattle (7.8%), significant difference ( $p = 0.04$ ,  $x^2 = 6.42$ ) was observed in the prevalence of anti-Brucella antibodies [10]. This study shows that the prevalence of Brucella antibodies varies between animal species and localities. It also shows several domestic animals in southern Cameroon that have been in contact with Brucella. It allowed the identification of villages where investigations on the transmission dynamics should be focused for the final objective of developing control measures against this neglected zoonosis [10]. Another cross-sectional study was conducted on the serum of 590 cattle at the slaughterhouse in Adamaoua and the North Cameroon region by Awah-Ndukum et al. in 2018 [7]. Despite the low prevalence of brucellosis in these localities, a considerable overall serological response (5.3%) was reported. Bayesian analysis revealed a sensitivity of 58.3% (26.4-92.7) and 89.6% (80.4-99.4) and specificity of 92.1% (88.7-95.2) and 95.7% (91.1-99.7) for RBPT and i-ELISA, respectively [7]. The study highlights the need for control measures and the need for public awareness of the zoonotic occurrence and transmission of bovine brucellosis in the country [7].

#### **Cohort studies**

Four cohort studies (4) were included in this systematic review. These longitudinal studies resulted in the identification of 44 strains of brucella in the North Cameroon region [12,13,24,28]. A crucial point to mention here is that these four (4) published articles used different periods of monitoring of the bacterium by serology. The first study included was conducted by Domenech et al. in 1980 in the Northern, Lake Chad and Adamaoua regions (12). The authors sampled 5,500 bovine sera, of which 1,620 were positive for brucellosis. Subsequently, in a second study conducted in 1982 in the northern region, the authors sampled 7,665 cattle sera, of which 2,361 were positive by the buffered antigen test [13]. Another study using simplified survey methods

was carried out in the same year (1982) to demonstrate the close correlation between the average annual brucella abortion rate and the percentage of cows showing or having shown knee hygroma during the previous five years. In 1983, the molecular technique (PCR) enabled Domenech et al. to identify and type the strains isolated in Chad and Cameroon. This last article of the series allowed them to conclude that the majority of isolations were *Brucella abortus* biotype 3 (67%), and the homogeneity noted by other researchers was not entirely confirmed. 3% of the strains were *Brucella melitensis* biotype 1, 1% *Brucella abortus* biotype 2, 5% *Brucella abortus* biotype 6 and 14% *Brucella abortus* intermediate biotype 3/6 (24). This study was the only one of a long series of studies carried out in the last 37 years to highlight the main strains circulating in Cameroon.

## Discussion

The search strategy used in this systematic literature review identified 16 studies reporting prevalence data (serological and molecular) of brucellosis in domestic ruminants in Cameroon. Most of the studies conducted between 1980 and 2020 were descriptive. Associated with this, the northern part of Cameroon has been the preferred study area at 70% and the biological material of choice used in most studies has been serum. The studies conducted from 1980 to 2020 show that this disease is diagnosed in a minority of other animal species (Sheep, Goats, Pigs, Dogs) reflecting the epidemiological changes in the distribution of brucellosis in Cameroon. The studies included in this review were very heterogeneous and the reported seroprevalence varied according to the risk of transmission in the area, the serological method used and the target population (cattle, goats, sheep). Most of the authors concentrated their study in the northern part of Cameroon to correct for sample size bias and to ensure that the study was nationally representative. The diagnostic method of choice used by most authors was the ELISA technique [1,7,27,10,14-18,20,23]. It is justified by its high sensitivity and specificity suitable for finding target molecules even at pictogram levels. It is frequently used for high throughput critical testing because of easy and less demanding experimental methods.

Since the study conducted by Domenech J. et al. in 1980, serology has been used to measure exposure to brucellosis in Cameroon and was therefore considered an important method in the attempts to eliminate this epizootic early on as reported in articles published in the 1980s [12-14,24,28]. With good results, this diagnostic method was used in combination or alone in future studies. Recent technological improvements mean that serology has become a much more robust tool for measuring disease transmission, but there is still a need to standardise ELISA protocols and antigens.

The regions of Cameroon that were studied are characterised by a highly variable and unstable transmission of brucellosis. The risk of contracting brucellosis varies considerably in the regions, depending on the type of livestock farming, the occupational activities at risk and the climatic situation in the different regions. These different transmission patterns could explain some of the variation in seroprevalence found in the selected studies. Thus, a comparison of seroprevalence between these populations with variable brucellosis transmission using several different targets would be inappropriate. There appears to be evidence to suggest that higher IgG antibody titres to some (but not all) antigenic targets correlate with the number of years the animal has lived in the enzootic area and not necessarily with age or sex. Sero-epidemiological studies in parts of Cameroon have shown an association of the presence of anti-brucella antibodies with age as well as sex [7,10]. No studies have attempted to investigate the

association between seropositivity and years/months since the first studies of brucellosis cases in Cameroon. However, the cross-sectional design adopted by most authors is subject to recall bias. The criteria used to determine previous brucellosis infections are not clearly defined in any of the reported cross-sectional studies, with the exception of articles that assessed IgG profiles to brucella blood antigens in domestic ruminants in southern, Adamaoua, North, northwestern and western Cameroon respectively [7,10].

This systematic review corroborates the results describing an association between seropositivity and brucellosis exposure parameters (age, sex and husbandry) in cross-sectional seroepidemiological studies. However, it would be important to distinguish between reinfected individuals and new brucella infections serologically. Only one of the studies included in the review identified target antigens but none described differences in antibody levels in relation to reinfected or new infections [24]. Relapses were not distinguished from new infections in any of the reported studies.

The antibodies produced during brucellosis infection are based on cell-mediated immunity that controls bacteremia in the bloodstream, thereby reducing clinical symptoms and life-threatening complications [29]. It consists of activation of bactericidal mechanisms specific to antigen-presenting cells (macrophages and dendritic cells) with subsequent proliferation of antigen-specific CD4+ and CD8+ effector T cell clones. Brucella antigens induce the production of T helper type 1 (TH1) cytokines. This TH1 immune response is crucial for overcoming Brucella infection [29]. The role of its antibodies was highlighted by the work done by Skendros & Boura in 2013 on human and experimentally induced brucellosis. They showed that interferon- $\gamma$  (IFN $\gamma$ ) is the most active cytokine against Brucella infection. That said, Brucella has evolved several evasive strategies during its evolution to circumvent host innate and acquired immunity and establish an intracellular niche to survive for a long time. Disruption of the TH1-like response and anergy have been described in patients with chronic brucellosis and are often associated with an adverse outcome [29].

Brucellosis is the subject of increasing interest in the Cameroonian context, but considerable data are still lacking. Serology has the potential to detect not only ongoing blood stage infections, but also recent infections, symptomatic or not. Despite this potential, the use of serology to target asymptomatic carriers has been rarely reported in Cameroon and the available data provide poor comparative results. Sensitive diagnostic methods, such as PCR, and an appropriate follow-up period are needed to identify asymptomatic brucella infection. These conditions impose significant costs on epidemiological investigations, which may explain the low number of longitudinal sero-epidemiological studies in Cameroon.

## Conclusion

The vast majority of studies in this review used serology as a means of identifying potential targets against brucellosis. This approach poses significant problems of sample representativeness, as most authors have used a convenient sampling method instead of structured sample size calculations and random selection of individuals. Despite the bias introduced by the sampling methods used and the focus on identifiable targets, valuable information on brucellosis transmission can still be extracted from these studies. The systematic review also highlights the need for longitudinal studies to better understand the role of these antibodies in protecting against brucellosis and asymptomatic infections.

### List of abbreviations

OIE: World Organisation for Animal Health

EAT = Buffered Antigen Test

SAW= Wright's serum agglutination

RB= Rose Bengal

FC= Complement Fixation

ELISA = Enzyme-Linked Immunosorbent assay

PCR= Polymerase Chain Reaction

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### Declaration of the authors' contributions

RCN and PAKK participated in the design and planning of the study. RCN, MFD participated in the analysis and interpretation of the data. RCN wrote the first draft of the manuscript. PAKK, MFD and FFDT critically reviewed the manuscript. All authors read and approved the final version of the article.

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