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IDENTIFICATION OF NEUROCYSTICERCOSIS DNA BY PCR IN SERUM AND URINE SAMPLES: A REVIEW

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ABSTRACT

Neurocysticercosis (NCC) stands as a neglected zoonotic ailment characterized by a substantial prevalence and disease burden. It frequently contributes to seizures in regions where it is endemic. The timely identification and treatment of NCC play a crucial role in diminishing morbidity and the loss of daily adjusted life years (DALYs). The definitive diagnosis of NCC still relies on the neuroimaging recognition of the scolex or histopathological investigation. The emergence of molecular methods such as PCR offers a promising avenue to supplement or complement these gold-standard diagnostic approaches. The PCR assay, in particular, demonstrates potential as an adjunct for diagnosing neurocysticercosis, particularly in individuals with ambiguity, providing a relatively swift and non-invasive diagnostic modality.

Keywords: Neurocysticercosis (NCC), PCR, Serum and Urine Samples

INTRODUCTION

Neurocysticercosis (NCC) is a parasitic disorder that affects the central nervous system, resulting from the occurrence of larval stages of *Taenia solium* **[1]**. This disorder poses a significant health challenge in emerging nations, and its incidence is on the rise in developed nations, attributed to factors such as immigration and tourism. Common manifestations of Neurocysticercosis include seizures observed in approximately 78% of individuals, headaches in 37.9% of cases, as well as elevated intracranial pressure in 11.7% of individuals **[2]**.

Neuroimaging remains the favoured technique for identifying neurocysticercosis. The Scientific Practice Guidelines provided by the American Society of Tropical Medicine and Hygiene (ASTMH) and the Infectious Disorder Society of America (IDSA) in 2018 advocate for the use of both MRI of the brain and CT scan of the head in the analysis of neurocysticercosis. Specifically, MRI is noted for its higher sensitivity in characterizing both parenchymal and extra-parenchymal lesions, while CT head is more effective in detecting calcifications within lesions **[3]**. These diagnostic procedures are generally accessible, but in certain developing nations, their availability is restricted owing to financial constraints and limited access. Additionally, MRI and CT scans are not suitable for use in epidemiological studies.

Parasite life cycle

Neurocysticercosis results from *T. solium* larvae in humans, with surgical complications potentially leading to fatal outcomes **[4, 5]**. The life cycle involves humans as primary hosts with adult parasites in the intestine, and pigs as secondary hosts for larval stages. Infected humans release eggs in feces, consumed by pigs in areas with poor hygiene. Eggs progress to metacestodes in the intermediate host's tissues, maturing into cysticerci. Consuming undercooked pork introduces them into the gastrointestinal tract. Cysticerci mature into adult worms, producing eggs expelled in human feces, completing the cycle **[6]**. *T. solium* exhibits high fecundity and fertility, emphasizing interventions targeting the tapeworm carrier for control efforts **[6]**.

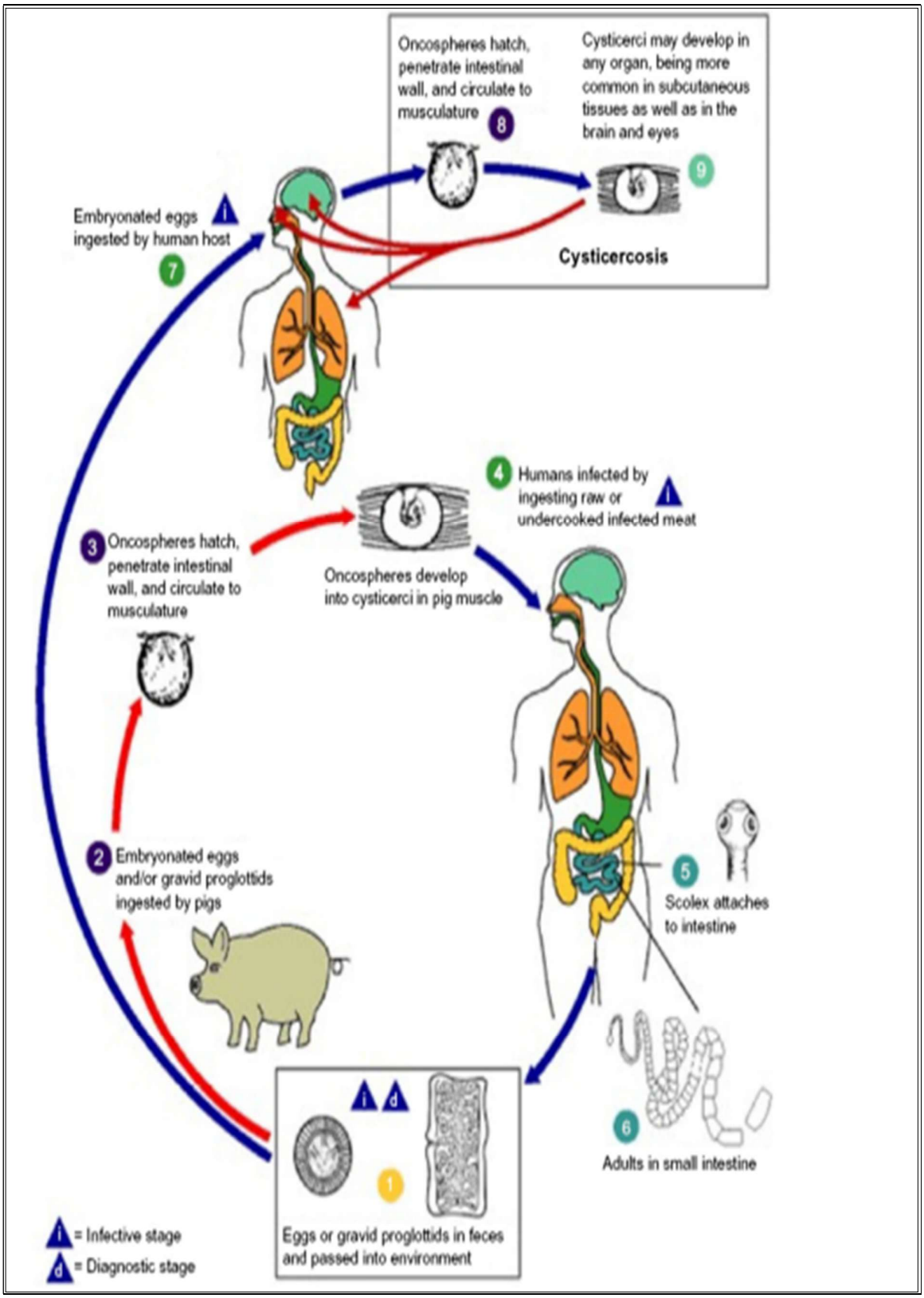

Figure 1: illustrates the life cycle of *Taenia solium* cysticerci, and this depiction has been reproduced by the Centers for Disorder Control and Prevention. The source for these details is the Centers for Disorder Control and Prevention, Atlanta, GA. For further details, the original content can be accessed at http://www.cdc.gov/parasites/cysticercosis/biology.html.

Epidemiology

NCC is globally distributed, predominantly affecting economically disadvantaged regions with free-roaming pigs having access to human feces **[7, 8]** Endemic areas include Latin America, parts of Asia, and other countries. While the global frequency of neurocysticercosis remains unclear, continuous investigations endeavor to ascertain the burden in both endemic and developed countries. Comprehensive data link NCC with epilepsy, estimating significant cases **[7]**. In rural Peru, a survey found a 24% seroprevalence, correlating with seizures at an odds ratio of 2.1 **[9]** Additionally, 13% with negative serology showed NCC calcifications, resulting in an overall 37% cysticercosis prevalence. Latin American prevalence ranges from 15% to 38%. A global review found that 29% of epilepsy cases are linked to neurocysticercosis (NCC) **[10]**. Surveys in Vellore (southern India) found NCC in 28% with active epilepsy, and in Puducherry, seroprevalence was 16% in epileptic patients and 6% in blood donors **[11, 12]**. In Chandigarh (northern India), the overall seroprevalence was 17%, with varying rates in different areas **[13]**. A study in north India's rural pig farming community indicated a high NCC prevalence, with 48% of those with active epilepsy meeting diagnostic criteria, emphasizing family history and pig housing conditions as major risk factors **[14]**.

PCR

Numerous techniques have been devised to identify parasite DNA with species specificity using polymerase chain reaction (PCR). PCR-based methods offer a notable advantage, extending their utility to stool samples, known as coproPCR. This approach surpasses the limitations of microscopy alone, allowing for the detection of a greater number of individuals. The integration of coproPCR with microscopy enhances diagnostic sensitivity. A multiplex PCR, employing the amplification of cox1, facilitates species-specific analysis and has been successfully implemented at the community level **[15]**.

Diagnosis by PCR

Molecular diagnosis

In recent advancements, specific PCR methods have been developed for the discovery of Taenia DNA in fecal samples **[16]**. Nevertheless, these methods are yet to undergo comprehensive field validation. Various molecular approaches have been established to detect and differentiate Taenia species, utilizing both genomic DNA (gDNA) and mitochondrial DNA (mtDNA). These methodologies include Dot Blot analysis, Multiplex PCR **[17]** PCR-RFLP [18] thymine-base reader analysis based on excision sequences, and Random Amplified

Polymorphic DNA (RAPD) **[19]**. DNA probes present an attractive option for a non-invasive, sensitive, and rapid investigative method that distinguishes *T. solium* and *T. saginata* tapeworms. Chapman *et al*. created highly sensitive DNA probes that are specific to particular species by incorporating labeling techniques. [32P] cystidine triphosphate (dCTP) and random primer extension, isolating and characterizing recombinant clones containing repetitive sequences from *T. solium* cysticerci, adult *T. solium* and *T. saginata* . The generated probes, namely HDP1 and HDP2, from a *T. saginata* genomic library were employed in a Multiplex-PCR, demonstrating specificity for *T. saginata* gDNA and differential amplification of gDNA from *T. solium*, *T. saginata* and Echinococcus granulosus **[20]**. A comparative assay using a 1300bp segment in the 5.8S ribosomal region to distinguish *T. solium* and *T. saginata*, showed clear differences with enzymes **[21]**. However, this method had suboptimal outcomes with fecal specimens enriched with Taenia eggs. Alternatively, a reliable PCR-RFLP assay targeting the 12s rRNA mitochondrial gene even with advanced proglottid disintegration **[18]**.

The extraction and purification of Taenia DNA from feces samples are complicated by the diverse nature of DNA sources and the occurrence of PCR inhibitors, such as hemoglobin, etc **[22]**. Novel techniques for detecting Taenia DNA in fecal samples have been introduced, including a Multiplex-PCR assay employing HDP2 gDNA outlined with primers to identify *T. saginata* in feces artificially contaminated with eggs, demonstrating a lower limit of detection of 137 eggs per gram **[23]**. However, only 11 out of 23 specimens positive by the coproantigen finding test positive using this method, probably influenced by the duration of sample storage, potentially affecting test sensitivity. Therefore, a nested PCR approach was employed to reduce the likelihood of amplifying unwanted DNA (non-specific bands) and enhance the detection of target DNA copies in cases where the target DNA is only sometimes readily apparent **[24]**.

In the realm of Neurocysticercosis (NCC) diagnosis, molecular techniques were detecting *T. solium* DNA in the CSF of 29 out of 30 individuals using pTsol9-specific PCR. Another study, using HDP2 primers, revealed cross-reactivity with *T. solium*, detecting parasite DNA in human CSF, with higher sensitivity in extraparenchymal NCC cases. A recent investigation compared pTsol9 PCR with antigen and antibody capture techniques, showing superior sensitivity in 150 CSFs, albeit with 80% specificity **[25]**. Unexpectedly, PCR

detected *T. solium* DNA in 28 out of 31 patients exclusively with calcified NCC. In porcine cysticercosis, a single PCR study demonstrated sensitivities of 23% and 32%, improving to 64% with nested PCR, maintaining 100% specificity **[26]**.

Diagnosis using sample

Serologic methods enable the identification of particular antibodies against *T. solium* or its antigens in blood, urine, and the central nervous system (CNS) **[27, 28]**. However, evaluating *T. solium* -specific antibodies does not distinguish between ongoing contamination and past exposure **[29]**. The enzyme-linked immunoelectrotransfer blot detects precise antibodies to lentil lectin-purified glycoprotein antigens of *T. solium*. In individuals with multiple parenchymal cysts or subarachnoid NCC, EITB exhibits an almost 100% sensitivity **[30, 31]**. Nevertheless, in individuals with only calcified cysts or single parenchymal lesions, the test achieves only 60–70% sensitivity **[31]**. Enzyme-linked immunosorbent assay for *T. solium* antibodies, employing either crude or purified parasitic antigen extracts, targets IgG but generally demonstrates lower specificity and sensitivity compared to EITB **[32-34]**.

The identification of circulating cysticercus antigens is achievable through a monoclonal antibody-based Ag-ELISA **[35]**. This method specifically recognizes the existence of actively viable cysts. When employed alongside antibody-detection assays, Ag-ELISA can distinguish between live parasite infections and deteriorating cysts. Elevated antigen levels are correlated with extraparenchymal neurocysticercosis **[35]**.

In environments lacking neuroimaging capabilities, serological assays play a pivotal role in diagnosing either intraparenchymal or intraparenchymal neurocysticercosis (NCC). Nevertheless, there is a pressing need for cost-effective diagnostic instruments in low- and middle-income countries (LMICs) grappling with endemic infections **[36, 37]**.

In Neurocysticercosis (NCC) antibody detection, various methods and antigens, such as total metacestode soluble antigens, metacestode membrane extracts, and semi-purified proteins, have been explored, showing varying sensitivity and specificity **[38]**. Tests using crude antigens exhibit moderate sensitivity and specificity. The enzyme-linked immune-electro transfer blot, using glycoprotein antigens purified through affinity chromatography, is recognized for its high sensitivity and specificity, with 100% specificity for detecting antibodies in serum and CSF. However, its sensitivity drops to 50-62% with single or calcified lesions, compared to 90% in patients with more than two lesions.

It's crucial to note that many cured patients remain seropositive for up to one year after anti-parasitic treatment due to the persistence of antibodies post-resolution of the active infection. Cross-reactions with antibodies from extraneural cysticercosis and other cestodes or helminths can also lead to false-positive outcomes when serum antibodies are used for diagnosis. An EITB assay based on two-dimensional polyacrylamide gel electrophoresis has shown high sensitivity (100%) in seropositive NCC cases and 60% sensitivity in suspected NCC cases where patients were seronegative for NCC. While promising for serodiagnosis in endemic areas, this method requires expertise and specialized facilities. As an alternative to the EITB, several researchers have developed enzyme-linked immunosorbent assays (ELISA) that utilize either crude or purified antigens from *T. solium* cysticerci or synthetic peptides **[39]**. These ELISA tests exhibit varying degrees of sensitivity and specificity. For the recognition of antigens, sandwich-ELISAs based on monoclonal antibodies have been developed by researchers. These tests allow the detection of excretory/secretory products from *Taenia spp*. Cysticerci in serum. However, it is essential to note that a positive antigen detection test does not definitively confirm neurocysticercosis, as cysts can be located outside the central nervous system. These tests are constrained to identifying the existence of viable larvae and deteriorated or calcified cysts remain undetectable. The precision of these examinations is at the genus level, enabling their application in diagnosing *T. saginata* in cattle and *T. solium* in humans and swine **[38]**.

False positive serology results can also occur due to past infections with *T. solium*. Antibody-ELISAs were found to be comparable to EITB in terms of both sensitivity and specificity for the differential serodiagnosis of NC. EITB has been used in pigs to detect circulating antibodies with high sensitivity and specificity. The sensitivity and specificity of Antigen-ELISA (Ag-ELISA) have been estimated at around 85% and 97%, respectively. However, one limitation is that Ag-ELISA cannot differentiate between infections with *T. solium*, T. s. asiatica, and Taenia hydatigna when multiple Taenia species coexist in the same host, such as in pigs. In bovines, the sensitivity and specificity of antigen detection ELISA are reported to be 98.7% and 92.3%, respectively, but these high values are observed in animals with more than 50 viable cysticerci. However, in cases where the bovine carcass contains fewer than 20 larvae, the sensitivity of this method decreases significantly, dropping to 12% **[38]**.

Despite the development of using various parasite antigens in serodiagnostic assays, none achieve 100% sensitivity and specificity, particularly for single-lesion parenchymal NCC. Dot blots have been utilized for serodiagnosis of several parasitic infections, offering high sensitivities similar to ELISA while being rapid, user-friendly, and easy to interpret. A study demonstrated that in-house ELISA and dot-blot assays could achieve good sensitivity in detecting anti-cysticercal IgG, particularly among pediatric NCC cases with multiple brain lesions **[38]**.

The feasibility of identifying circulating parasite antigens in the sera of individuals experiencing hydrocephalus secondary to neurocysticercosis (NCC) has been investigated. Positive outcomes were observed in 48% of hydrocephalus individuals but consistently negative in individuals with calcifications. A study in Peru showcased an 86% sensitivity for antigen detection in CSF through ELISA, with negative outcomes predominantly observed in individuals with either a single live cyst or only enhancing lesions. Consequently, while the sensitivity proved high in cases with multiple cystic lesions, its diagnostic utility was limited in individuals with a solitary cyst. It is important to emphasize that many of the current immunodiagnostic tests necessitate comprehensive standardization and may require more efficient implementation in rural and underdeveloped areas with a substantial disease burden **[40]**.

This emphasizes the necessity to investigate simpler tests that may or may not depend on invasive specimens, enabling point-of-care diagnosis. Despite these advancements, such tests have yet to be widely accessible for individual care in large-scale settings. For instance, an uncomplicated and swift latex-based agglutination assay has been assessed for neurocysticercosis (NCC) diagnosis by identifying *T. solium* metacestode antigen in cerebrospinal fluid and serum samples. It demonstrated sensitivities and specificities of 64% and 85% in cerebrospinal fluid and 52% and 96% in serum samples, respectively. Non-invasive specimens like urine and saliva have also been explored for antigen identification **[40]**.

However, there is limited literature concerning the efficacy of molecular analysis for neurocysticercosis in individuals with solitary lesions, in serum samples, and whether assessing DNA load in cerebrospinal fluid can be utilized for monitoring neurocysticercosis individuals. Identifying single neurocysticercosis lesions is often problematic with serological technology, making the discovery of parasite DNA by PCR a valuable tool for

diagnosing such instances. This approach can supplement existing radiological and immunological tests, confirming the presence of NCC in a broader range of clinical scenarios. It is essential to highlight that while PCR-based techniques offer substantial advantages in sensitivity and specificity, they also necessitate well-equipped laboratories and technical expertise, which may not be readily available in resource-limited settings. Nevertheless, these molecular methods show promise in enhancing NCC diagnosis, particularly in challenging cases, and further research is required to explore their full potential in clinical practice **[41]**.

Literature review

In a 2023 study led by **K. Satyaprakash *et al***. in Nagpur, Maharashtra, India, researchers aimed to detect neurocysticercosis (NCC) in epileptic patients (n=26). They used IgG-ELISA and EITB assay with various antigens from *T. solium* metacestodes. Results showed varying seroreactivity, with CFA, SA, and ESA equally present in ring-enhancing lesions. EITB indicated immunodominance of protein bands with lower and medium molecular weights in SA and ESA. PCR revealed positivity in 34.6% of patients, marking the first report of NCC detection in Nagpur using both serological and molecular tools **[42]**.

In their 2020 study, **Gunjan Goyal *et al***. investigated the applicability of Polymerase Chain Reaction in identifying *Taenia solium* DNA in Neurocysticercosis individuals. The research involved blood samples (100) and blood samples (58) from neurocysticercosis individuals, alongside control samples. Targeting the repetitive element PTsol9 of *Taenia solium*, the PCR assay exhibited a sensitivity of 57% and 64%, and specificity of 94% and 87% in blood and urine samples, respectively. The findings suggest that PCR can serve as an adjunct diagnostic tool for NCC, particularly in challenging cases, providing a relatively swift and non-invasive method for accurate diagnosis **[43]**.

In their 2017 study, **Arturo Carpio *et al***. performed a prospective case-control investigation to assess the validity of a PCR examination in CSF for diagnosing neurocysticercosis (NC). The multicentre study, carried out in five hospitals in Cuenca, Ecuador, from January 2015 to February 2016, involved 36 cases meeting validated NC diagnostic criteria and 36 controls. CT and MRI scans, along with CSF samples, were collected from both groups, and diagnostic criteria were used as a reference standard. The PCR assay exhibited an overall sensitivity of 72.2% and a specificity of 100.0%. For parenchymal NC, sensitivity was 42.9%, and for extraparenchymal NC, sensitivity reached

90.9%. The findings underscored the utility of the CSF-based PCR assay, particularly in diagnosing extra parenchymal NC cases when conventional neuroimaging techniques proved insufficient **[44]**.

Elise M. O'Connell and team conducted a 2020 study addressing challenges in subarachnoid neurocysticercosis (NCC) treatment. They developed a quantitative polymerase chain reaction (qPCR) test targeting the TsolR13 repeat from the *Taenia solium* genome. The qPCR test demonstrated high sensitivity (97.3%) and specificity (100%), detecting active disease in 100% of CSF and 81.3% of plasma samples from symptomatic patients. The test's predictive ability for distinguishing active from cured disorder was better in CSF (94.4%) than in plasma (86.7%). The qPCR test performed equivalently to *T. solium* antigen finding, offering a highly sensitive and specific tool for NCC diagnosis and cure assessment in both CSF and plasma **[45]**.

CONCLUSION

In conclusion, the use of Polymerase Chain Reaction (PCR) to identify neurocysticercosis (NCC) DNA in serum and urine samples represents a significant advancement in diagnostic methodologies. PCR facilitates non-invasive and accessible screening, enabling broader population-based studies and early identification of potential NCC cases. This approach enhances large-scale screening feasibility, aiding in the identification of NCC across diverse demographic settings and contributing to our understanding of its prevalence and distribution in the general population. The non-invasiveness of sample collection, especially with urine samples, addresses practical challenges associated with traditional diagnostic methods, potentially leading to earlier intervention and improved patient outcomes. However, acknowledging limitations such as the need for well-equipped laboratories and technical expertise is crucial. Additionally, thorough validation of PCR-based techniques is essential for accurate and reliable results.

In summary, PCR's application in serum and urine samples offers a promising avenue for improving NCC diagnosis, contributing to early detection, and potentially enhancing public health outcomes as technology advances.

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