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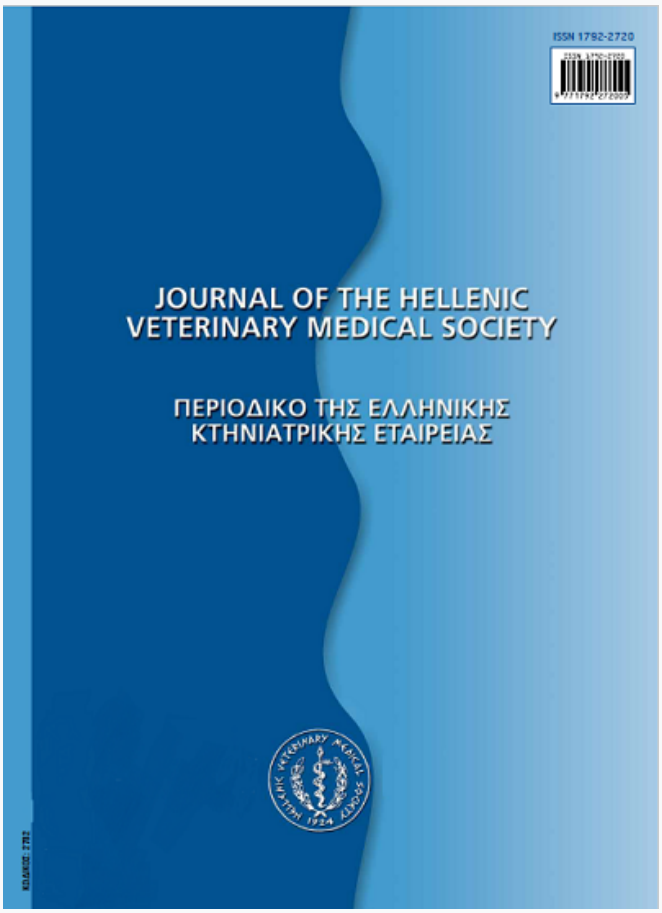

Characterization of immunogenic protein fractions of Sheep Cysticercosis in Cairo, Egypt

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Research article
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Characterization of immunogenic protein fractions of Sheep Cysticercosis in Cairo, Egypt

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ABSTRACT. Cysticercosis is a parasitic infection that causes severe economic and public health problems. The overall incidence of infection in sheep slaughtered in Cairo, Egypt during meat inspection was 31.22% and precisely 19.72% for *Cysticercus tenuicollis* and 11.50% for *C. ovis*. Sera collected from infected animals used to evaluate the diagnostic efficacy of the extracted antigens using ELISA. The sensitivity of the test was 100% for both *C. tenuicollis* and *C. ovis*, while the specificity was 80.26% and 87.03%, respectively. Using EITB the fractions of 36 KDa and 23 KDa appears to be specific for diagnosis of *C. tenuicollis*; while, 77 KDa and 73 KDa were specific for diagnosis of *C. ovis.* Moreover, several protein fractions which were detected in all antigens extracted didn't react with its target sera but at the same time did react specifically versus sera from animals infected with another cysticerci. Those fractions are considered as common immunogenic fractions between different cysticerci, so strictly identified specific fractions must to be used for diagnosis of Cysticerci infection to avoid cross reactions with other cysticerci antibodies.

Keywords: Cysticerci - ELISA- EITB- Sheep, Egypt.

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INTRODUCTION

Cysticercosis is a parasitic disease caused by metacestodes of *Taenia* species infecting human and carnivores all over the world, producing severe economic and public health problems related to its zoonotic nature (Ahmadi and Badi, 2011). The disease has the special economic significance of the veterinary level as it causes severe losses due to the condemnation of infected meat and organs (Goswami *et al*. 2013).

Until now, no routine technique is adopted for diagnosis of infection by cystisercosis in animals, animals other than postmortem meat inspection (PM) Abdel-Radi, (2014). A variety of serological techniques were applied for diagnosis of metacestod infection in different animals (Oliveira et al. 2010; Paulan et al. 2013 & Mousa et al. 2014); however, the sensitivity and specificity of these techniques were highly affected by the type of the tested sera as well as a degree of purity of the antigen especially with the complex nature of metacestodes antigen, which exhibits variable degrees of cross reactivity (Varela-Diaz et al. 1977). A combination of more than one serological test, using of the sera of known infection history and purified antigens was recommended to achieve a proper diagnosis of cysticercosis (Sultan et al. 2012).

The present study aimed to determine the incidence of infection by different Cysticerci in slaughtered sheep in Cairo governorate, Egypt via PM inspection. Sera collected from infected animals will be used to determine the diagnostic values of different Cysticerci antigen using ELISA. Moreover, these antigens were fractionated by SDS-PAGE and then the sensitivity and specificity of the obtained fractions were investigated aiming to spot some light on the level of cross reactions between ant-bodies of different infection versus the fractioned antigens using EITB technique.

MATERIALS AND METHODS

Inspected animals and collected samples*:*

Aiming to determine the incidence of infection by *C. tenuicollis* and *C. ovis* in Cairo governorate., a total of 426 sheep slaughtered at Cairo abattoirs (El-Basatin) during the period from January to December 2012 were exposed to postmortem (PM) inspection according to Abdel-Radi, (2014) The muscles and visceral organs, particularly the lung, liver, kidney, heart and spleen of each animal, were examined carefully for the presence of the previous cysts. All the detected *Cysticerci* were collected in separate identified suitable cellophane bags and transported to the laboratory in an ice box for further examination and antigen preparation. Rectal fecal and blood samples were collected one week before slaughtering and at the time of slaughtering from each inspected animal. After PM inspection cysticerci infected carcasses were identified.. Fecal samples were examined for diagnosis of different internal parasitic stages. Using concentration flotation (by salt solution) and concentration sedimentation of water as described by Pritchard and Kruse (1982) Thin Giemsa stained blood smears were prepared and examined for detection of different blood parasites according to Garcia, (2007). Sera of animals proved to be free from enteric and blood parasites were selected and used for serological study.

Antigen preparation

Two cysticerci antigens (Ag) were extracted from *C. tenuicollis* and *C.ovis* cysts. Viable non calcified cysts with cystic fluid and clear inviginated scolex free from surrounding tissue, Love and Hutchinson, (2003) were selected for antigen preparation. *C. tenuicollis* antigens were prepared from the whole cysts, according to Goswami *et al.* (2013) While, *C. ovis* crude Ag was prepared, according to (Melcher, 1943). The protein content of each antigen was measured according to Lowry et al. (1951), The antigens were allocated in 1ml vial and stored at -20 oC until use.

Hyper-immune sera (HIS)

Rabbit hyper-immune sera (RHIS) were produced versus the 2 tested antigens according to (Fagbemi *et al.* 1995). Two white New Zealand rabbits (2 months old) per each antigen, were bled for negative control sera then injected with 1.2 mg protein for each antigen, mixed with an equal volume of mineral oil subcutaneously. After 3 weeks, 3 consecutive injections of 0.4 mg protein antigen in equal volume of oil were given intra-muscularly at biweekly intervals. Rabbits were bled from the ear vein for serum collection 10-14 days after the last injection. The collected sera were stored at –20°C until used.

Serodiagnostic studies

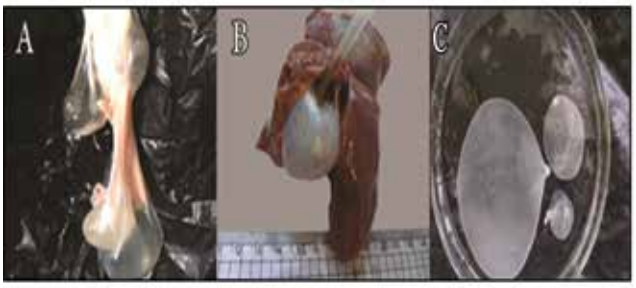
Indirect- enzyme linked immunosorbent assay (ELISA):

The test was performed as described by Iacona *et al.* (1980)**.** The plate was coated with each antigen (*C.tenuicollis* and *C.ovis*) separately in (4 ug / ml in coating buffer) which adjusted after checkerboard titration. After overnight incubation at 4oC, the plate was washed, then blocked with 0.1% bovine serum albumin fraction V (Sigma), 100ul/well. Serum samples were used at 1:50 dilution. RHI sera was used as reference positive control. Horseradish peroxidase conjugated (HRPC) rabbit anti-sheep IgG, {Heavy and light chains (H & L)} (Sigma) as well as HRPC goat anti-rabbit IgG (whole molecule) (Sigma) was used in 1:2000 dilution. Ortho-phenylenediamidine-OPD was added at a concentration of 340 ug/ ml substrate buffer. Absorbency was read by 490nm using full automated Titerteckmultiskan ELISA reader. The cut off points were set as 2SD above the mean of control negative samples. After optimization of the test by checker board titration, the test was used to determine the value of different Cysticerci antigens in capturing the specific and cross reacted anti-bodies in naturally infected sheep sera aiming to spot some light on the specificity and sensitivity of them in the diagnosis of infection in field collected serum sample.

Electrophoretic fractionation of antigens and western blot analysis:

For identification of immunogenic proteins, polypeptide profiles of cystic fluid antigen and whole cyst lysate antigen preparations of each *C. tenuicollis* and *C. ovis* were fractionated using SDS-PAGE (Genei Bangalore, India) containing 10% gel under protein denaturing condition according to (Laemmli, 1970). Standard molecular weight marker protein (Lonza, USA) was run simultaneously. Two separate gels for each sample ran in same conditions using the same antigen samples prepared from each Cysticerci. After completion of running of the SDS-PAGE, one gel along with molecular weight markers was shifted to stain with Coomassie Brilliant Blue. The remaining one was washed with transfer buffer and Cysticerci proteins resolved by SDS-PAGE were electrophoretically transferred (Genei, Bangalore) from the gel to a nitrocellulose membrane (NC) as per the standard method according to (Towbin *et al.* 1979) and probed by western blotting using laboratory prepared RHIS in comparison with sera of naturally infected animals and a negative control one. *C. tenuicollis* and *C. ovis* protein fractions that reacted versus reference positive sera and at the same time did not react versus the negative control were considered as specific protein fractions.

Figure 1. (1): *Cysticercus tenuicollis* of sheep. (A) In omentum (B) In liver. (C) Obtained C. *tenuicollis* with different size.



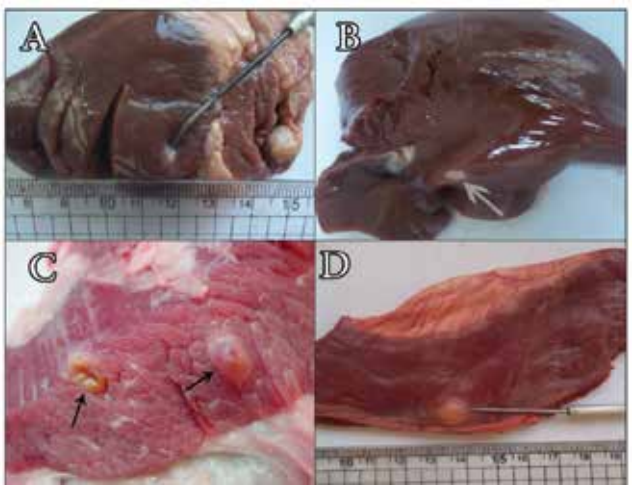
RESULTS

Incidence of Cystcerci infection in the inspected sheep.

The total incidence of infection with the different cystcerci was 133 (31.22%) from 426 inspected slaughter sheep during one year at Cairo abattoir. It was found that 84 (19.72%) for *C. tenuicollis* and 49 (11.50%) for *C. ovis* in the different infected organs (Fig. 1-2).

The diagnostic value of different antigens was evaluated using ELISA versus control and natural infected sera. Optimization of ELISA using checker board titration revealed that 2µg antigen/well, with

Figure 2. *Cysticercus ovis of sheep.*
A) In heart B) In liver C) In muscles D) In diaphragm.



1:100 serum dilution and 1:2000 conjugate, appear as the best condition for application of the assay under the condition of the present study.

The examined number of collected sera (n = 62) for *C. tenuicollis* & (n =30) for *C. ovis* from naturally infected sheep at PM inspection (cyst/animal). These sera reexamined by using ELISA which, aiming to determine the level of sensitivity and specificity of each of the two antigens in capturing of its specific antibodies in the sera of natural infected and control animals. The data revealed absolute sensitivity for each of the tested antigens (100%) in capturing its target antibodies in sera of infected animals. Serum samples gave a positive reading when tested at 1:100 serum dilution, Table (1).

For estimating the degree of specificity, each type of antigen was exposed to sera of animals infected by the other types of Cysticerci as well as its original HIS obtained from rabbits vaccinated by the same antigen. The data in table (1) revealed the level of cross reactions as sera containing anti-*C. tenuicollis* Ab cross reaction with *C. ovis* and this fact is decreasing the specificity of the assay of 87.09%. Also, sera containing anti-*C. ovis* Ab crossed reacted with the *C. tenuicollis* showing a decrease in specificity of up to 73.33%.

From the previous results, anti-*C. tenuicolis* Ab in infected sera was considered the less cross reacted Ab with tested antigen, followed by that of *C. ovis*. At the same time no cross reactions could be detected in treatment of any of the two antigens versus control RHIS or negative sera of sheep.

Specific and cross reacted Cysticerci protein fractions:

In order to further investigate the level of cross reactions between the different tested Cysticerci antigens SDS-PAGE was applied and the specificity and sensitivity of each fraction was determined after transferring them to NC membrane and treating of them versus specific and nonspecific antibodies in sera of file collected naturally infected sheep as demonstrated in Table (2) & Fig. (3).

The data revealed that 4 protein bands corresponding to molecular weights (MW) of (97, 63, 36 & 23kDa) were reacting specifically after treatment of *C. tenuicollis* fractioned antigen versus *C. tenuicollis* infected sheep sera. At the same time two protein bands (at 78KDa &17KDa) were reacting specifically after treatment of corresponding NC strip versus the sera of *C. ovis* infected animals.

By the same approach, NC strips caring *C. ovis* fractioned antigens, showing 4 bands at MW of (97KDa, 77KDa, 73KDa & 65kDa) which reacted specifically versus sera of sheep infected with *C. ovis.* At the same time, the fractions at the level of (48KDa & 26KDa) reacted specifically when treated a corresponding NC strip by the sera of sheep infected with *C. tenuicollis*. At the same time 2 other bands

Table 1. Sensitivity and specificity of the two antigens in capturing the specific and cross reacted anti-bodies in naturally infected sheep sera

Testing the sensitivity & Specificity		Sera selected from animals infected at PM inspection by (at 1:100 serum dilution)	
	Antigen ELISA Plate	*C.tenuicollis (n= 62)*	*C.ovis (n=30)*
Sensitivity test	*C.tenuicollis*	62 (100%)	-
	C.ovis	-	30 (100%)
Specific RHIS for each Ag		+	+
Specificity test	*C.tenuicollis*		8 (73.33%)
	C.ovis	8 (87.09%)	-
Negative rabbit or animal sera		-	--

-(n) = The number of testing serum samples collected from naturally infected sheep.

on the level of (135 KDa & 52KDa) were detected at treatment of similar NC strip by *C. ovis* non-infected sheep sera, Table (2) & Fig. (3).

DISCUSSION

Incidence of infection by different Cysticerci in slaughtered animal still has special importance as it gives a clear picture about the role of slaughtered animals as a source of infection by different *Taenia* species infecting dogs and human for special area. This in fact points out the importance of application of proper meat inspection and condemnation of the infected parts as well as prevents the arrival of stray dogs to animal farms.

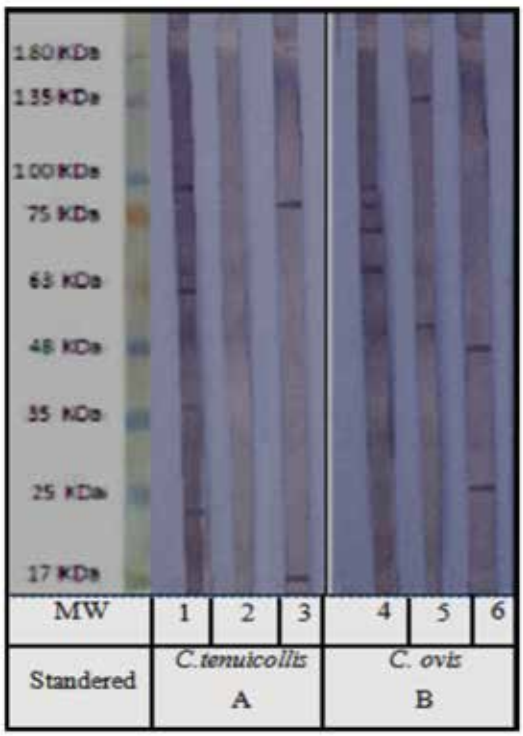
During the present study, incidence of *C. tenuicollis* was 19.72% in examining sheep. The same result was previously recorded by El-Massry (1988**)** The authors recorded an incidence reached to 19.5% in the same localities of our study. While, the incidence was slightly lower than that recorded in Ethiopia by (Endale *et al*. 2013) as it reached 22% On the other hand, El-Dakhly *et al.* (2012) recorded a very low percentage (1.3%) in middle Egypt. Moreover, the recorded incidence in our study was in the range previously mentioned by Abdel-Maogood, 2005 & Sissay *et al*. 2008 as they determine a percentage of 5.66% in Egypt & 26.0% in eastern Ethiopia, respectively.

In the author's opinion and agreement with El-Massry (1988**)** incidence of cysticercosis recorded among the sheep in the present study may be related to the high infection rate of dogs with the *Taenia* worm in the study sites. This can supported by some habit of the people in Egypt, as they usually slaughter sheep at home and throw offal's in garbage which usually eaten easy by the present stray dogs.

In the author's opinion, the difference in the incidence rate recorded in the present study in comparison with the other similar one may be related to different grazing systems, eating habits, accuracy of PM inspection protocol, the hygienic and control measures applied in each area.

The second part of the present study was directed to use sera collected from infected animals in evaluating the diagnostic values of different Cysticerci antigen from the aspect of their ability to capture of its specific antibodies (Ab) using ELISA. Each of testing antigens (*C. tenuicollis* and *C. ovis*) revealed absolute sensitivity (100%) in capturing of its target Ab in the tested sera obtained from animals infected by each cyst alone. This result came in agreement with similar previous studies on diagnosis of *C. tenuicollis* and *C. ovis* (Abdel-Maogood, 2005 and Radwan, 2008). This result disagreed with that recorded by Sultan et al. (2012) who recorded sensitivity rate for ELISA in diagnosis of *C. tenuicollis,* which reaches up 90%. These variations might be related to the degree of purification of the used antigen as well as the standardization of the assay and the reference control sera.

Figure 3. Specific and non-specific protein fractions of different cysticerci antigen on NC treated versus positive and negative sera.
A) *C. tenuicollis*
1-NC strip is treated with *C. tenuicollis* +ve serum.
2-NC strip is treated with *C. tenuicollis* -ve serum.
3-NC strip is treated with *C. ovis.* + ve serum
B) *C. ovis*
4- NC strip is treated with +ve serum of *C. ovis.*
5- NC strip is treated with -ve serum of *C. ovis.*
6- NC strip is treated with +ve serum of *C. tenuicollis.*



Concerning the specificity of each antigen in capturing of antibodies (Abs) present in the sera of animals infected by the other two types of Cysticerci, the obtained results clear different degrees of decrease in specifying as sera containing anti-*C. tenuicollis* Ab was cross react with *C. ovis* Ag is decreasing the specificity of the test to 87.09%. Moreover, sera containing anti-*C. ovis* Ab crossed reacted with the antigen of *C. tenuicollis* showing decrease in specificity reached to 73.33%. This result is supported by (Varela-Diaz et al., 1977) who mentioned that metacestodes have a complex nature of antigen and exhibits cross reactions among themselves. Also, Kandil et al., (2004) found that a high level of non-specific cross reactions to occur when crude somatic extracts or cyst fluid preparations of *Taeniid* cestodes are used for the immunodiagnosis of larval cestodes infections. But this result disagrees with Abdel-Maogood, (2005), who recorded that the specificity of *C. tenuicollis* and C. *ovis* was 50% and 54.38%. (Sultan et al. 2012) who found that the specificity of non-purified crude antigens derived from the whole cyst of *C. tenuicollis* was 60%, so the purification of antigens is required.

From the previous results, anti-*C. tenuicollis* Ab in infected sera considered the less cross reacted Ab with other tested antigens; this may be related to the habitat. The nature of these larvae as they usually present hanged in the peritoneal cavity non-embedded in tissue as the condition of *C. ovis* metacestode.

The last part of this study was directed to investigate the presence of common or cross reacted protein fractions in each of the evaluated antigen responsible for the demonstrated degree of cross reactions between them. For this reason the antigens were fractionated by SDS-PAGE, then the sensitivity and specificity of the obtained fractions were investigated using EITB technique. The data revealed that the fractions at the MW level of 36KDa & 23KDawere found to be specific for diagnosis of *C. tenuicollis*. While that at 77 &73 KDa were specific for diagnosis of *C. ovis*. This result was in agreement with (Goswami *et al.* 2011) who recorded that 36.2 &23 kDa protein fractions are recognized as immunodominant polypeptides and could be explored for serodiagnosis of *C. tenuicollis* infection. On the other hand, this result disagrees with that reported by Abdel-Maogood (2005) who found that the specific epitope for diagnosis of *C. ovis* infection in sheep was 26.494 KDa, While 74.224, 66.725, 55.567 & 21.224 KDa were the specific for diagnosis *C. tenuicollis* in sheep

Concerning the level of cross reactions between different antigenic fractions, the data revealed that two protein bands corresponding to MW of 78 &17 KDa were present in the fractioned *C. tenuicollis* Ag and react specifically versus sera of *C. ovis* infected animals. These data mean the presence of some common fractions between *C. tenuicollis* antigens and anti-bodies of other Cysticerci, rather than

Table 2. Protein bands detected on NC strips containing different fractioned antigens after treatment by positive and negative sera

Tested sera	Protein bands detected on NC strips containing different fractioned antigens after treatment by its positive sera and other sera MW (KDa)		
	C. tenuicollis	*C. ovis*	Non specific bands
C.tenuicollis +ve	97,63,36 & 23	48 & 26	63
C.ovis +ve	78 & 17	97 ,77, 73 & 65	
C.tenuicollis –ve	-	-	
C.ovis –ve	-	135&52	

+ve= positive -ve =negative
Non Specific Bands =commen bands between *C. tenuicollis* and *C. ovis*

presence of unapparent infection by *C. tenuicollis* in the examined sera**.** In the same time, the fractions at the level of (48 & 26 KDa) were reacting specifically when treat NC strips carrying *C. ovis* fractioned Ags by the sera of sheep infected with *C. tenuicollis* These protein fractions considered to be common immunogenic fractions between these Cysticerci as it captures the Ab of other cyst and in the same time did not react versus its own Ab. The specific fractions obtained in the present study were in agreement with that obtained by Abdel-Maogood (2005) who found that *C. tenuicollis* antigen gave bands ranging between 88.298-16.16 kDa and *C. ovis* Ag gave bands ranging between 94.806-15.894 kDa, Moreover, Goswami *et al.* (2013) who recorded that the band at 63 kDa MW considered to be specific for diagnosis of *C.tenuicollis* infection. This was disagreeing with Kandil *et al.* (2003).

The concerning presence of cross reacted fractions between different Cysticerci Ag, the obtained data were in agreement with Lawson, (1994) who recorded that *C. tenuicollis* and *C. ovis* shares some antigenic properties that could result in partial cross-protection in the intermediate host under natural conditions. While, Kandil *et al.* (2004) reported that the common band between *C.tenuicollis* and *C. ovis* antigens were at the level of 45 kDa. In the authors opinion, the difference in the size of the specific fractions described by other author's may be related to some genetic variations in the parasites between different localities, age of the parasite as well as to the condition of the test and the method of antigens preparation and purification as described by Kandil *et al.* (2003).

CONCLUSION

The present results revealed that the sensitivity of *C.tenuicollis* and *C. ovis* was 100% for each one. While, the specificity was 80.26% and 87.03%, respectively by using EILSA. The EITB analysis cleared that 36 and 23 KDa protein bands were found to be specific bands for diagnosis of *C.tenuicollis,* While 77 and 73 KDa protein bands were found to be specific bands for diagnosis of *C.ovis*.

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