Variations to somatic protein profiles of *Dicrocoelium dendriticum* in Iranian ruminants

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Abstract:

BACKGROUND: *Dicrocoelium dendriticum*, a liver trematode of herbivores, and man is very prevalent among ruminants of Iran, resulting in causing significant economic losses. Recently its phenotype polymorphism has been found in Iran, a phenomenon that may affect the protein profile of the parasite. **OBJECTIVES:** The aim of this study was to determine the somatic protein profiles of *D.dendriticum* in different ruminants in Iran. **METHODS:** Adult worms were collected from infected livers of sheep, goat, cattle, buffalo and camel. Somatic antigens were prepared separately by centrifugation of the homogenized adult worms and their patterns were determined using SDS-PAGE. **RESULTS:** Our findings revealed 18-20 protein fractions in somatic proteins of *D. dendriticum* of sheep and goat and 12, 11 and 10 in that of buffalo, cattle and camel respectively showing similar molecular weights of 14.4 to 116 kDa. Similar protein bands were seen in sheep and goats samples; but not for in other samples. **CONCLUSIONS:** The results showed polymorphism in the somatic protein patterns of *D.dendriticum* isolated from different hosts. A different number of protein bands with similar molecular weights of somatic antigens of *D.dendriticum* are described for the first time in the present study.

Introduction

Dicrocoelium dendriticum, a liver parasite, affects several host species, especially ruminants world wide. Several mollusks and ants can play a role as the first and second intermediate hosts. Dicrocoeliasis has been reported from different ruminants such as equine, hare, wild boar, wild sheep (Eslami et al., 1976; Eslami and Nadalian, 1987; Eslami et al., 2000; Eslami and Farsad-Hamdi, 1992) and human (Sohrabi, 1973) in Iran. Recently phenotype polymorphism for this parasite was shown in different herbivores of Iran (Taefie-Nasrabadi et al., 2008). It is important to recognize that trematodes have characteristics shared with some other invertebrates e.g. cestode which make it theoretically possible to have some "strains" adapted to a new host species. Therefore, a new strain (or race) of a trematode adapted to a new strain of intermediate or definitive host (s), and perhaps having distinct physiological characteristics, could arise (Smith and Halton, 1986). Meanwhile, high genetic variability was shown in adult *D.dendriticum* (Sandoval et al., 1999). The aim of present study was to determine the soumatic cell protein profiles of *D. dendriticum* in different ruminants in Iran.

Materials and Methods

Adult flukes were collected from infected livers

from sheep, goats, cattle and camels in Bassim as well as for buffalo in Bandar-Anzali abattoir. Flukes were washed several times with PBS, and homogenized with phosphate buffer saline (PBS) containing 1 mM phenyl methyl solphonyle fluoride (PMSF) in a glass tissue grinder. The homogenates were centrifuged at 12000 g for 30 min at 4°C. Protein concentration was determined according to Bradford (1976). The protein concentration of somatic products was ranging from 20-25 µg/µl. All prepared antigens were labeled and stored at -20 °C until used.

SDS-PAGE analysis: SDS-PAGE assays were performed according to Laemmli (1970). Protein fractions and somatic antigens of *D.dendriticum* were analyzed under reducing conditions using Mini Protean III cell apparatus (Bio-Rad) of 110 V constant voltages during 60 min with gel thickness 0.75 mm. SADS-PAGE carried out on 10% and 5% resolving and stacking gel respectively. The gels were stained with coomassie blue and photographed.

Results

The results of electrophoresis of somatic antigens of *D.dendriticum* of sheep, goat, buffalo, cattle and camel are shown in Figures 1-2. About twenty bands with 14.4 to 116 kDa molecular weight were determined in sheep and goats samples. Some fractions showing 16, 27, 33, 37, 44, 55, 58, 75 and 80 kDa were more visible.

D.dendriticum samples obtained from buffalo, cattle and camel resulted in producing 12, 11 and 10 fractions, respectively, having similar molecular weights to those of sheep and goat samples (14.4 to 116 kDa). Fractions showing 16, 36, 40 and 116 kDa were clear and common for all buffalo, cattle and camel samples. Also, fractions showing 70 kDa in buffalo sample and 23 and 27 kDa in cattle and camel sample were more clearly visible. Meanwhile the fractions showing 23 and 25 kDa were seen only in cattle and camel sample. In the two latter hosts the fraction gave above 35 kDa and were significantly less compressed than the other hosts.

Discussion

In the present study, we found that there is a possible emerging of a new "isolate", or called a "strain". According to Smith and Halton, 1986, due to hermaphroditism in trematodes, an unexpressed recessive mutant gene from one generation will appear in both male and female germ cells and at the same time in the next generation. Thus, a single mutation could theoretically give rise to homozygous double recessive and an 'instant' mutant could appear. In addition to these facts, because of multiplification of trematodes in the molluscan intermediate hosts by polyembryony, the mutant multiplies very rapidly (Rollinson et al., 1986). A study on the phenotype polymorphism of *D.dendriticum* of different herbivores from Iran showed some sort of polymorphism in different animals (Taefie-Nasrabadi et al., 2008). It was stated that even similar phenotype of a trematode, may reveal different pathogenesis, reproduction, immunity and chemotherapy (Rollinson et al., 1986). In this study, serotype polymorphism was shown in *D.dendriticum* of sheep and goat and with that of cattle, camel and buffalo using SDS-PAGE. Although all protein fractions share the same molecular weights (14.4 -116 kDa), differences were observed in the number of bands in sheep and goat (18-20) and camel, cattle, and buffalo (10,11,12) respectively. Our findings in cattle are in contrast with Wedrychowicz et al., 1995 and Revilla-Nuin et al., 2005, who found 8-9 polypeptides with 29 to 205 kDa in *D.dendriticum* surface proteins of cattle usind SDS-PAGE while 17 bands in SDS soluble or somatic proteins extracted with TBS. Meanwhile Revilla-Nuin et al., 2005, report 36 polypeptide bands in the somatic extract of sheep *D.dendriticum*. The variation in number and molecular weight of protein profiles of *D.dendriticum* of sheep and cattle reported by different workers could be due to geographical distribution of the parasite, different intermediate and final hosts belong to animal behaviors. Studying the genetic variability of *D.dendriticum* of sheep suggests that each sheep is infected by numerous genetically different parasites from one or more populations of infected ants (Manga-Gonzalez and Gonzalez-Lanza, 2005). Campo et al., 1998, characterization of adult *D.dendriticum* by isoelectric focusing in cattle, sheep and goat showed some differences in their enzyme types. According to Otranto et al., 2007, although no intra-specific morphometric differences were recorded for *D.dendriticum* from different hosts or localities, but

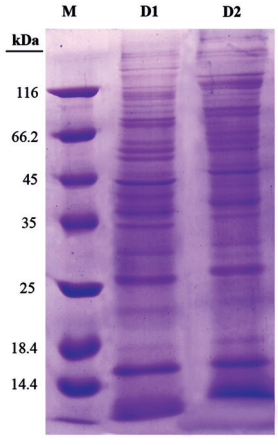

Figure 1. Protein profile of somatic antigens of *D.dendriticum*: goat sample (D1), Sheep sample (D2), Protein marker (M) (Fermentase=SM 0431).

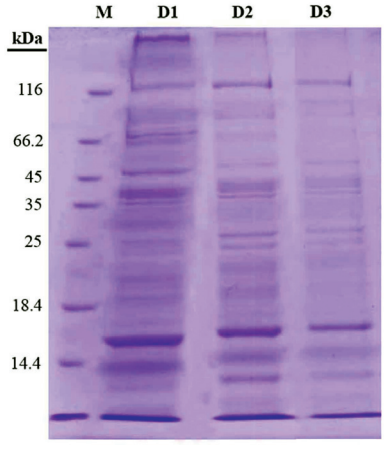

Figure 2. Protein profile of somatic antigens of *D.dendriticum*: Buffalo sample (D1), Cattle sample (D2), Camel sample (D3), Protein marker (M) (Fermentase=SM 0431).

there was a low level of intra-specific variation (i.e. 0.3%) found in the ITS-2 sequence of *D.dendriticum* irrespective of host species or locality. In contrast to Otranto et al., 2007 several reports exist on morpho-anatomic differences in *D.dendriticum* Phenotype, enzyme and genotype polymorphism that may be an indication for production of a new strain (Taefie-Nasrabadi et al., 2008; Smith and Halton, 1986; Sandoval et al., 1999).

According to Morozova et al., 2002, the high level of molecular polymorphism and heterogeneity in trematode adults of *D.dendriticum*, is probably caused by a great variety of microevolution factors affecting the population structure of parasites at different stages in different animal hosts. Of these factors, the most essential are various types of selection and the migration of host animals (Morozova et al., 2002). Although phenotype and serotype polymorphism shown in protein profiles of *D.dendriticum* from different hosts in Iran, further studies on the molecular or genomic levels, are needed to prove the probability of the arising of a new "isolate" or "strain".

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تنوع الگوی پروتئین‌های بدنی دیکروسلیوم دندریتیکم در نشخوارکنندگان ایران

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چکیده

زمینه مطالعه: دیکروسلیوم دندریتیکم یکی از ترماتودهای بافت‌کبدی علفخواران و انسان است که در نشخوارکنندگان ایران شایع بوده و ضرر و زیان اقتصادی شدیدی را همراه دارد. با بررسی‌های انجام گرفته پلی‌مورفیسم این ترماتود نشان داده شده است که می‌تواند بر الگوی پروتئینی انگل هم تأثیرگذار باشد. **هدف:** بررسی حاضر بمنظور تعیین الگوی پروتئین‌های بدنی دیکروسلیوم دندریتیکم در نشخوارکنندگان مختلف ایران انجام گرفت. **روش کار:** کرم‌های بالغ از کبد گوسفند، بز، گاو، گاومیش و شتر جمع‌آوری و آنتی‌ژن بدنی در هر مورد تهیه شد. این آنتی‌ژن‌ها براساس هموژنیزه کردن کرم بالغ و سپس سانتریفوژ تهیه و توسط SDS-PAGE آزمایش گردید. **نتایج:** بررسی الگوی الکتروفورتیک آنتی‌ژن‌های تهیه‌شده حاکی از وجود ۲۰-۱۸ باند پروتئینی در مورد دیکروسلیوم دندریتیکم گوسفند و بز و ۱۰، ۱۱ و ۱۲ باند پروتئینی به ترتیب در دیکروسلیوم دندریتیکم شتر، گاو و گاومیش بود. وزن مولکولی باندهای شناسایی‌شده در همه نمونه‌ها حدود ۱۴/۴kDa تا ۱۱۶ بود اگرچه باندهای پروتئینی در گوسفند و بز مشابه بودند ولی در سایر میزبان‌ها اختلاف وجود داشت. **نتیجه‌گیری نهایی:** در الگوی پروتئین‌های بدنی کرم بالغ دیکروسلیوم جداشده از میزبان‌های مختلف نوعی پلی‌مورفیسم دیده می‌شود. تعداد مختلف باندهای پروتئینی با وزن مولکولی مشابه از آنتی‌ژن‌های بدنی دیکروسلیوم دندریتیکم برای اولین بار از ایران گزارش می‌شود.

واژه‌های کلیدی: دیکروسلیوم دندریتیکم، آنتی‌ژن‌های بدنی، SDS-PAGE، نشخوارکنندگان، ایران.

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