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Acute phase protein response in Alpine ibex with sarcoptic mange

Md. Mizanur Rahman [a,b], Cristina Lecchi [a], Cristina Fraquelli [a], Paola Sartorelli [a,c], Fabrizio Ceciliani [a,c,*]

[a] Department of Animal Pathology, Hygiene and Veterinary Public Health, Via Celoria 10, 20133 Milano, Italy
[b] Department of Medicine & Surgery, Chittagong Veterinary and Animal Sciences University, Zakir Hossain Road, Chittangong 4202, Bangladesh
[c] Interdepartmental Center for Studies on Mammary Gland (CISMA), Università di Milano, Italy

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ABSTRACT

The acute phase proteins (APP) are a group of serum proteins that change their concentration in animals following external or internal challenges, such as infection, inflammation or stress. The concentrations of four APPs, including serum amyloid A (SAA), haptoglobin (Hp), α_1-acid glycoprotein (AGP) and ceruloplasmin (Cp) were determined in serum collected from healthy Alpine ibexes (*Capra ibex*) and ibexes with *Sarcoptes scabiei* mange. Primary structures of all four APPs were determined by cDNA sequencing. The concentrations of all four APPs were higher in serum of animals with clinical signs of sarcoptic mange when compared to healthy animals. Two of the APPs, including SAA and AGP, acted as major APPs, since their serum concentrations were increased more than 10-folds when compared to healthy animals ($P < 0.001$). The other two APPs, including Hp and Cp, acted as minor acute phase proteins, as their concentrations were increased from two to five folds ($P < 0.001$).

These findings provide a remarkable potential as diagnostic markers for the early detection of sarcoptic mange in free ranging animals.

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1. Introduction

Sarcoptic mange is a severe contagious disease worldwide affecting humans and other mammalians caused by the burrowing mite *Sarcoptes scabiei* (Arlian, 1989; Burgess, 1994; Walton et al., 2004). Scabies epizootics have been reported in almost all European wild ruminants, including chamois (both subspecies *Rupicapra pyrenaica* and *Rupicapra rupicapra*) (León-Vizcaíno, 1990; Rossi et al., 2007), ibex (both subspecies *Capra pyrenaica* and *Capra ibex*) (León-Vizcaíno et al., 1999; Schaschl, 2003; Ondersheka et al., 1968; Rossi et al., 1995), Barbary sheep (*Ammotragus lervia*) (González-Candela et al., 2004), red deer (*Cervus elaphus*) (Oleaga et al., 2008a), roe deer (*Capreolus capreolus*) (Oleaga et al., 2008b) and different other wild animals around the world (Pence and Ueckermann, 2002).

Sarcoptic mange first induces a typical hypercheratosis and local inflammation, followed by a hyperplasia in the stratum granulosum. Eventually, affected areas feature severe alopecia and scab formation (Arenas et al., 2002; Arlian et al., 1990). Prolonged infestations with scabies, or infestations in already debilitated animals, have effects on organs (Burgess, 1994) and even body weight and size (Serrano et al., 2007). The blood oxidant/antioxidant balance is likely to be modified as well, as it was found in sarcoptic dogs (Camkerten et al., 2009). Affected animals often succumb to the infestation (Pence and Ueckermann, 2002). Immune response against mange infestation is not completely understood. Scabies extracts modulate immune response of the host by down-regulating the keratinocyte expression of IL-1 – receptor antagonist and by increasing the expression level of other cytokines, including IL-6, granulocyte-colony stimulating factor

* Corresponding author at: Department of Animal Pathology, Hygiene and Veterinary Public Health, Via Celoria 10, 20133 Milano, Italy. Tel.: +39 02 50318100; fax: +39 02 50318095.
E-mail address: fabrizio.ceciliani@unimi.it (F. Ceciliani).

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(G-CSF) and IL-10 (Arlian et al., 2003, 2006). Moreover, cutaneous lesions caused by sarcoptic mange are likely to serve as a route for secondary invaders, such as bacteria for example. A systemic inflammatory status due to mange infestation probably occurs, but it has not been demonstrated, at least so far. This acute phase reaction is supposed to be caused by the invasion of bacteria from cutaneous lesions or by the immunomodulatory activity of mange products on pro-inflammatory cytokines, including IL-6 and IL-1β which, in turn, up-regulate the expression of acute phase proteins (Suffredini et al., 1999).

The acute phase proteins (APPs) are a group of structurally unrelated serum proteins that change in concentration in animals subjected to external or internal challenges, such as infection, inflammation, surgical trauma or stress. The circulating concentration of the APP is species-specific and related to the severity of the disorder and the extent of tissue damage in the affected animal. Since APP's serum amount may increase (positive APPs) or decrease (negative APPs), it may also provide diagnostic and prognostic information, thus representing a powerful means of monitoring animal health (Petersen et al., 2004).

To the best of knowledge of the authors, the few reports on haematological and biochemical intervals in ibex (Sartorelli et al., 1991; Pérez et al., 1999, 2003; Degiorgis et al., 2000; Lastras et al., 2000) did not include APPs. Therefore, the dynamic of acute phase response in ibex is still unknown.

The present study was carried out to gain insight into the acute phase reaction during *S. scabiei* infestation in ibex. In detail, our experiments aimed, in a first instance, to determine the primary structures of the APPs: SAA, Hp and AGP. These proteins were selected because they are the most common APPs in ruminants (Petersen et al., 2004), but also because a very recent report demonstrated that these three proteins increased after pro-inflammatory challenge in goats (González et al., 2008). Ceruloplasmin concentration was also studied, since several reports demonstrated that this protein is an indicator of infection in ruminants (Murata et al., 2004), and its amount can be measured in wild ruminants as well (Barboza and Blake, 2001). This information was considered the requisite to reach the second objective, that was to compare the serum APP concentrations between healthy ibexes and those affected by sarcoptic mange. Results are presented hereby.

2. Materials and methods

2.1. Identification of animals and diagnosis of mange

The study was carried out on a population of wild Alpine ibex that was located in Dolomite Alps, Northern Italy (Universal Transverse Mercator coordinates: 32T 712826 mE 5150746 mN) during a major outbreak of *S. scabiei* epidemic that occurred between years 2004 and 2005. Blood samples were collected from 43 males (mean age: 5.7 years $\pm$ 2.45 s.d.) and 10 females (mean age: 6.9 years $\pm$ 4.53 s.d.) from years 2003 to 2005. Age of animals was determined by horn segment counts (Habermehl, 1985).

APP concentration was assayed from 15 clinically healthy ibexes (Group 1) (4 females and 11 males). These animals were also sarcoptes mange negative, as determined by ELISA search for specific antibodies (Rambozzi et al., 2004). A second group (Group 2: 38 animals – 6 females and 32 males) included animals with evident clinical signs of sarcoptic mange, as determined by skin lesions associated with detection of the causal agent *S. scabiei* from cutaneous biopsies.

Blood samples were obtained by jugular veinpuncture of ibexes captured for management/therapy purposes during the summer season (from July to September). Blood containing vials were refrigerated at 4 °C, left to clot and then centrifuged at $2500 \times g$ for 15 min. Serum was separated and stored at −20 °C until analysed for Cp, SAA, AGP and Hp.

2.2. Experimental protocol

In a first series of experiments the determination of the primary structure of the four APP included in this study, i.e. SAA, Hp, AGP and Cp, was carried out. Following this first, preliminary phase, the immunological methods available for the measurement of APP in cattle were tested for cross-reactivity in Alpine ibex by means of Western blotting using, as primary antibodies, those included in the commercial kit for APP determination.

Once the method is validated, the concentration of APP in serum obtained from Alpine ibex affected by spontaneous mange infestation was measured.

2.3. Determination of primary structure of ceruloplasmin, haptoglobin, α_1-acid glycoprotein and serum amyloid A

Total RNA was extracted from euthanized ibex liver using the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. The reverse transcription (RT) reaction was carried out on 1 mg RNA using iSCRIPT cDNA SYNTESIS Kit (Bio-Rad, Segrate, Italy). The cDNA was used as the template for the PCR (Eppendorf Mastercycler1) (Eppendorf, Milan, Italy).

PCR reactions were performed in 10 μl final volume under the following condition: 1× buffer Eppendorf, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1 mM of each primer and 0.5 unit of Taq Polymerase (Eppendorf, Milan, Italy).

The primers used to amplify the coding sequences of ibex AGP, SAA, Hp and Cp are listed in Supplementary Material, Table 1, together with their thermal profiles.

PCR products were applied on a 1.5% agarose gel electrophoresis and the segments of predicted molecular weight obtained were gel-purified using the QIAquick gel extraction kit (Qiagen, Milan, Italy) and then were quantified by NanoDrop® ND-1000. The fragments were sequenced directly with ABI technology using an automated DNA sequencer (ABI PRISM 310 Genetic Analyzer). The predicted amino acid sequence was obtained using the ExPASy proteomic server (www.expasy.ch). Interproscan and prosite (www.ebi.ac.uk) analysis was carried out to detect post-translational modifications of the four proteins.

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