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# Reversible switch from hemoglobin A to C in sheep and recovery from anemia following experimental infection with *Anaplasma ovis*

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**ABSTRACT -** Anemia causes a change in the type of circulating hemoglobin (Hb) in sheep carrying the βA-globin haplotype, where the Hb A is replaced with Hb C, unlike Hb B. The effect of the substitution of Hb A with Hb C on the recovery from anemia was investigated by comparing the hematological picture of sheep, following experimental infection with *Anaplasma ovis*. The blood values were obtained from 3 AB and 3 BB Hb sheep after the development of the disease where anemia is a pathognomonic symptom. The expression of the silent gene encoding for Hb C was detected by isoelectric focusing and quantified by high performance liquid chromatography. Both Hb AB genotype and Hb C occurrence were involved in the lower recovery from anemia in the trial.

*Key words:* Hb polymorphism, Silent gene expression, Response to anemia, Sheep anaplasmosis.

**Introduction -** Goats and some sheep under conditions of erythropoietic stress (anemia) or hypoxia, synthesize a juvenile hemoglobin (Hb) type, Hb C, where β-globin is encoded by the silent gene *HBBC*. Anemia causes a change in the type of circulating hemoglobin only in sheep carrying βA-globin haplotype, where Hb A is replaced with Hb C. Pioneered by the work of van Vliet and Huismam (1964), the Hb C in Caprinae species has been thouroughly studied and particularly the mechanism of the reversible switching has triggered focused research in the 70's (Litt and Kabat, 1972; Nienhuis and Anderson, 1972; Nienhuis and Bunn, 1974; Nienhuis *et al.*, 1980). Few pieces of information are available on the effect of the substitution of Hb A with Hb C. Owing to the high oxygen affinity of Hb C (Huisman and Kitchens, 1968), the reversible switch from Hb A to Hb C may be considered a way to cope with the reduced amount of oxygen available at higher altitudes and therefore suggest a positive effect on the fitness of mountain Caprini. However, in the case of erythropoietic stress, Hb C might negatively affect the peripheral oxygen delivery and then, it might worsen the clinical picture of sheep breeds native of areas with endemic hemotropic pathogens. This work aims at expanding the knowledge on this issue analysing *in vivo* the response to anemia in sheep.

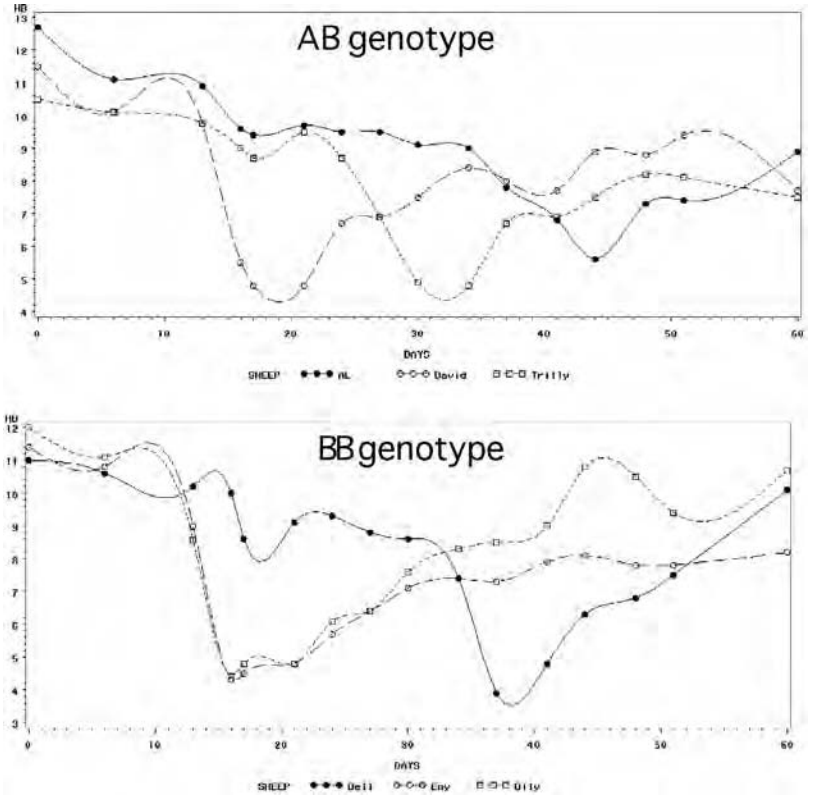
**Material and methods -** Six Suffolk lambs, less than six months of age, were housed at the Medical Clinics of the Department of Animal Health and Well-being of the University of Bari. In particular, the lambs, selected based on different β-globin genotypes and equally divided between heterozygous AB and homozygous BB, were used as a model for analysing the response to the disease obtained by an experimental infection with *Anaplasma (A.) ovis*. *A. ovis* was isolated from a splenectomized sheep al-

lowed to be naturally infected pasturing in tick areas. At the peak of parasitaemia in the sheep, a blood sample was obtained from the jugular vein and parasitaemia was estimated by the buffy coat method. About 200 ml of blood were then obtained from the donor sheep and each lamb in the two "genotype" groups was inoculated intraperitoneally with 25 ml of infected blood. Clinical evaluation was done on a daily basis and rectal temperatures were recorded every morning for 12 weeks post infection. Blood and serum samples were collected twice a week during the observation period. Hematological variables were evaluated using a hematology analyzer. The expression of *HBBC* gene was detected by isoelectric focusing and quantified by high performance liquid chromatography. An analysis of variance was carried out in the 6 analysed animals by GLM procedure (SAS, 1990), considering the interaction with the hemoglobin type (2 levels: AB and BB) of the linear and quadratic regression of each hematological variable on the number of days from the infection. Moreover, the Hb C and hematocrit (PCV) trends were analysed in 2 animals where Hb C was switched on.

**Results and conclusions** - Figure 1 shows, as an example, the different trend after infection (days=0) of Hb content for the AB and BB lambs respectively. A part for individual differences observed in reaching the lowest Hb values, the recovery was always faster in BB sheep, as also indicated by the significantly higher quadratic regression coefficient of Hb for BB *vs.* AB genotype (0.0045 *vs.* 0.0027, P<0.03).

It is also important to point out the different behavior of the heterozygous subject AL compared

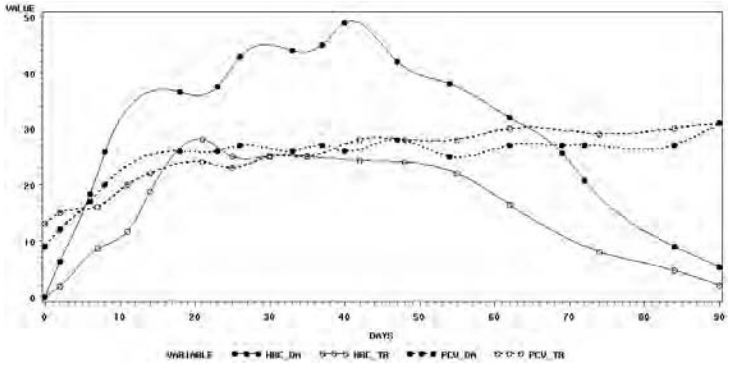
Figure 1. Trend of Hemoglobin (Hb, g/dl) as a function of the number of days from the infection (DAYS) for the hemoglobin genotypes AB and BB.



with the other two heterozygous animals (Figure 1). The less severe hematological picture of AL was related to the lower anoxemic stress of this subject, also confirmed by the lack of the Hb A to C switch in the same animal. In fact, only 2 AB animals developed Hb C, which was higher in one of them, whereas PCV showed a similar trend in both animals (Figure 2).

In conclusion, both the Hb AB genotype and the occurrence of Hb C were involved in the lower recovery from anemia in the trial performed. Since southern Italian sheep breeds are mostly BB, these findings support previous results highlighting that the ability of the above breeds to thrive in endemic TBD areas (Pieragostini and Petazzi, 1999) is somehow related to the Hb genetic system (Pieragostini *et al.*, 2003, 2005).

Figure 2. Trend of Hemoglobin C (HBC) and hematocrit (PCV) content (VALUE) as a function of the number of days from the start of the disease (DAYS) for the 2 subjects David (DA) and Trilly (TR).




*This work is part of a study concerning the improvement of sheep breeding by the use of genetical markers of resilience to tick borne disease (TBD) caused by* hemotropic pathogens, such as Anaplasma spp, Babesia spp, and Theileria spp. In turn, the *study* above *is included in a project, aiming to the improvement of animal breeding by the use of molecular genetics (SELMOL) and sponsored by the Italian Ministry for Agriculture, Food and Forestry Policy (MIPAAF).*

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