Analysis on the association of biochemical-genetic and immunogenetic markers with the morbidity of goats belonging to the Carpathian breed

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Summary

The study makes an associative analysis of some genetic polymorphic structures with health status in goats of the Carpathian breed. During the experimental observations, a quarter of goat population was affected by various diseases; the metabolic disorders were most common, followed by infectious diseases and the by parasitosis; the hereditary maladies ranks last. Among the individual groupings, constituted in relation to their belonging to the genotypes of biochemical-genetic and immunogenetic systems, some differences were found concerning the resistance / receptivity to morbid phenomenon. Within the biochemical-genetic systems the HbAHbB haemoglobin heterozygotes, TfATfA transferrin homozygotes, AlbBAAlbB albumin and individuals with LK potassium phenotype are the most resistant to the action of pathogens, while the HbBHbB haemoglobin homozygotes, TfATfB transferrin heterozygotes, AlbAAlbA albumin homozygotes and individuals with HK potassium phenotype are most susceptible to various diseases. Immunogenetically, the healthy animals possess a richer dowry of blood group factors than the animals with different diseases due to contribution of erythrocyte factors Aa, Bf, Bi, Ca and Ma; only the blood factors Bb, Bc and Bd are slightly more common in ill animals than in the healthy ones. These associations can be used to achieve the genetic prophylaxis in farm animals.

Introduction

Modern human and veterinary medicine benefits more and more by biophysics, biochemistry, genetics and cellular biology data of the human and animal body to know and interpret the causes of different disorders which determine some pathological processes. Now the penetration rate of these issues in the medical theory and practice becomes more obvious, the pathology being based on the necessity to know the deterministic and biochemical substratum of different diseases, of their aetiology, in accordance with the cellular and biochemical processes that contribute to onset and manifestation of the pathological phenomena. Depth study of the biologic processes at molecular level thus allows a more complete approach and understanding of different diseases, in close relation with the biochemical individuality of the body. The concept of biochemical pathology, respectively molecular pathology, designates the ensemble of some qualitative and quantitative changes, especially at the level of some enzyme or protein molecules which involves the disturbance of some metabolic pathways or of their biologic function and which acts as the primary factor
in triggering and changing of some pathological phenomena. Some researches of molecular biology having implications in the animal pathology are scarcely in the beginning and the questions concerning these diseases are more numerous than the answers (Kramer, 2000; Menrad et al., 2002; Wojdak-Maksymiec, 2002; Güney et al., 2003; Smith & Sherman, 2009).

Some investigations of this kind tried by means of some biochemical, genetic and immune-serological methods to identify the so called “marker genes” belonging to some polymorphic systems of protein or mineral origin and to follow their evolution during the selection process of animals and also to establish whether these are correlated or not with some characters of normal physiology or physiopathology (Katusumato et al., 1998; Deza et al., 2000; Johnson et al., 2002; Mostaghni, 2004; Salako et al., 2007; Gurcan et al., 2011).

Some proteins (haemoglobin, transferrin, albumin, various isoenzymes) and mineral elements (potassium, sodium, copper, iron), some cellular structures with antigenic properties (blood group factors) with polymorph character or some molecular structures (AND variants), fundamentally involved in the intermediate metabolism or in the natural immunity installing, have an important role in ensuring a good health of the animals (Nguyen, 1990; Osterhoff, 1995; Katusumato et al., 1998; Deza et al., 2000; Johnson et al., 2002; Wojdak-Maksymiec, 2002; Güney et al., 2003; Mostaghni, 2004; Slalako et al., 2007; Gurcan et al., 2011).

The goat suffers with various diseases, which are caused by bacteria, viruses, parasites, metabolic factors and other non-infectious (genetic) agents (Dally et al., 1980; Buvanendran et al., 1981; Haba et al., 1991; Zartal-Zidani et al., 2002; Rychlik et al., 2005; Mendes-Ahid & Soto-Blanco, 2010). This is also the reason why we will try to approach the association between the main biochemical-genetic and immunogenetic systems and health in goats belonging to the Carpathian breed, as an important factor to increase their productivity by improving the health status of animals in accordance with the “animal welfare” objectives of the European agricultural policies.

Materials and methods

The biological material in order to achieve the associative analysis of the genetic markers with the goat health was composed of an animal sample of Carpathian breed representing a local population bred in the habitat conditions provided by The Research and Development Station for Sheep and Goat Breeding Popauti-Botosani, situated in the north-eastern Moldavia.

Laboratory analyses were carried out on blood samples taken by jugular vein puncture of animals: with heparin (for the determination of haemoglobin and potassium phenotypes), on sodium citrate (to determine the blood group factors) and without anticoagulant (for the determination of transferrin and albumin phenotypes).

The analysis of the biochemical-genetic systems in goats studied took into account the main protein and minerals with polymorphic character being highlighted electrophoretically and flamephotometrically:

Identification of the polymorphic protein phenotypes (haemoglobin, transferrin, albumin) was achieved by horizontal electrophoresis method, having as substrate the starch gel and used as electrolyte (according to the analyzed protein), buffer solutions consisting of Tris (hidroxymethyl) aminomethane, EDTA.Na2 and citric acid (for haemoglobin), boric acid and lithium hydroxide (for transferrin) or boric acid and sodium hydroxide (for albumin). Electrophoresis lasted four hours for haemoglobin and 6-8 hours for transferrin and albumin until the electrophoretic bands migrated to 4 cm respectively 11 cm from the starting line. The amperage was 40 mA and 50 mA, and the voltage was 300 V and 400 V for hemoglobin, respectively, for transferrin and albumin. Colouring of starch gels was performed with an alcoholic-acid solution of
amidoschwartz 10B (1%) for 15 minutes. For discoloration, the starch gels were placed in a bath with a solution of methanol, glacial acetic acid and distilled water (3/1/3) for several hours.

Identification of the potassium phenotypes was achieved by flame photometric method. Cationic concentrations of K+ from whole blood of animals were expressed in mEq/l. The detection of potassium phenotypes was based on the discontinuity range of the potassium ion distribution.

The laboratory analysis that provided the detection of erythrocyte factors was the characteristic one to antigen-antibody immunoserological reaction by the haemolytic test method. The basic reactive components have consisted of goat red blood cells from which the erythrocyte standard suspensions were prepared (as antigens), on the one hand, and monospecific reagent sera obtained by the isoinmunization technique (as antibodies), on the other hand. To finalize the antigen-antibody reaction, the rabbit complement adsorbed on goat erythrocytes was added to these components. For the method accuracy, in parallel, there have been made two serological surveys: for physiological serum and for complement. The immunoserological reactions occurred in the Takátsy microtitration device in thermostating conditions at 25-26°C and their reading was made at 1/2 hour, 21/2 hours and 5 hours. A scale of haemolysis degrees was estimated, denoted from 0 to 4; the reactions with lysis haematic degrees 2, 3 and 4 were considered positive.

The health status evolution of goat population was monitored for five years. At the experiment beginning, all animals were in perfect health condition. The main morbid entities which affected this small ruminant population were recorded being classified into four major categories of diseases: parasitical (kid coccidiosis, toxoplasmosis, echinococcosis, fasciolosis, cysticercosis, strongilosis, hydatosis, scabies, myiasis etc.), infectious (anthrax, anaerobiosis, gangrenosum mastitis, contagious agalactia, salmonellosis, etc.), metabolic (ruminal acidosis, ruminal alkalosis, pregnancy toxiemia, kid enzootic ataxia, kid miodistrofia etc) and genetic (congenital myotonia, functional sterility, impotence hereditary sterility, azoospermia, oligospermia, serious orhiepididimitis, necroseremia, genital infantilism, monorchidism, cryptorchidism etc.). There were calculated the frequencies of general morbidity on the whole population, but especially on each polymorphic protein or mineral structure.

Results and discussions

Veterinary medical bulletin in goats. During the experimental observations in the goat population, the general morbidity incidence was 24.34%. The metabolic disorders have met most frequently (9.93%). The infectious diseases were situated in second place (7.78%). Parasites have affected 5.21% of the goat population and hereditary maladies have happened in last place with a very low percentage (1.52%). The metabolic and infectious diseases are more present, they being manifested especially in young goat which, during this period, is more sensitive to the attack of infectious agents and to action of various physical or chemical agents which change more significantly the biochemical and metabolic parameters (fig. 1).

Fig.1. Incidences of morbid entities in goat population.
Reported to whole number of sick animals, the morbid entities in goats recorded the following shares: metabolic = 40.39%, infectious = 31.96%, parasitical = 21.41% and genetic 6.34% (fig. 2).

Association of haemoglobin system with health status of goats

In the Carpathian goats three haemoglobin phenotypes were identified in the electrophoretic field: two homotypes, HbA and HbB, and a heterotype, HbAB (Hrinca, 2010 a).

The association of haemoglobin genotypes with general health status of goats shows that the HbAHbB heterozygote genotype is more resistant to contracting the various diseases (16.38%) compared to the two homozygous genotypes. In the case of homozygotes the morbid disorders are less frequent within the type HbAHbA (19.14%) than in those of HbBhBb type (26.38%) (fig. 3).

There is not a clearer tiebreaker of disease frequencies in the three haemoglobin genotypes, but rather on the haemoglobin alleles; the incidence of disease in individuals with homozygous genotype for HbA allele is closer to that of the heterozygotes. Thus, one can speak of a genotype grouping in goats concerning the animal morbidity, on the one hand the homozygotes for the HbA allele (HbAHbA) and HbAHhB heterozygotes, and on the other hand the homozygotes for the HB allele (HbBHbB). This aspect shows that HbA allele confers to goats a selective advantage regarding the resistance to illnesses, both in homozygous status, but especially in heterozygous status. The HB allele, especially in homozygous status, increases the goat susceptibility of contracting various diseases. The differentiated predisposition of goats to diseases depending on the haemoglobin types could be explained by biophysical and physiological peculiarities (in the foreground being the O2 dissociation curve characteristics of oxyhaemoglobin molecule), but also by the specificities of the antigen structures on the surface of red blood cells of the two types of haemoglobin: HbA and HB (Dally et al., 1980; Buvanendran et al., 1981; Haba et al., 1991; Johnson et al., 2002; Zartal-Zidani et al., 2002; Mendes-Ahid & Soto-Blanco, 2010).

Association of transferrin system with health status of goats. In comparison to sheep, where the polymorphism at the determinant locus of serum transferrins is very pronounced, the goats have a moderate transferrin polymorphism of binary type. The two transferrin alleles determine the expression of three genotypes: two homozygous TfATfA and TfBTfB, and a heterozygous TfATfB (Hrinca, 2010 b). The lowest rate of illness happens to homozygous individuals for TfA allele.
(17.82%) and heterozygotes TfATfB are most affected by pathogenic agents (24.63%). The frequency of morbid disorders in the homozygous goats TfBTfB (21.58%) is intermediate between the incidences of various diseases that affect the heterozygotes, on the one hand, and the homozygotes for TfA allele, on the other hand (fig. 4).

Different sensitivity to various diseases of animals in relation to their transferrin genotype may be linked to the main role of transferrin (siderophilin), namely the iron binding capacity and transport function of iron ions in the body, properties which differ from one to another transferrin genotype. It can also be called into question the intermediate metabolism interference of hemoglobin and transferrin with major implications in the haemoglobin genesis process that probably presents specific aspects for each transferrin genotype (Katusumato et al., 1998; Kramer, 2000; Menrad et al., 2002; Mendes-Ahid & Soto-Blanco, 2010).

The AlbBAlbB homozygotes are most resistant to installing of different morbid entities (12.68%), while the other homozygotes, those for the AlbA allele, are most affected by various pathogens (27.93%). The disease incidence in the AlbAAlbB heterozygotes is moderate (19.56%) (fig. 5).

Association of albumin system with health status of goats. In the Carpathian goats three genetic variants were found at the determinant locus of serum albumin: AlbAAlbA homozygous genotype, AlbBAlbB homozygous genotype and AlbAAlbB heterozygous genotype (Hrinca, 2011).

The AlbBAlbB homozygotes are most resistant to installing of different morbid entities (12.68%), while the other homozygotes, those for the AlbA allele, are most affected by various pathogens (27.93%). The disease incidence in the AlbAAlbB heterozygotes is moderate (19.56%) (fig. 5).

Fig. 4. Health status of goats depending on the transferrin genotype.

Fig. 5. Health status of goats depending on the albumin genotype.

Albumin, like the most important blood serum protein fraction, in terms of quantity, carries out, inter alia, the important function of transport, being able to conjugate with metal ions, vitamins, hormones, fatty acids, anions, medicines etc. Perhaps these functions cause a differential receptiveness of animals to disease in relation to albumin genotypes (Katusumato et al., 1998; Wojdak-Maksymiec, 2002; Mendes-Ahid & Soto-Blanco, 2010).

Association of potassium system with health status of goats. By flame photometry, also in the caprine species, like in sheep, the two known potassium phenotypes were found: LK and the HK. In the Carpathian breed, the HK phenotype is one that is much more frequently than the LK phenotype, being prevalent in the potassium panel (Hrinca, 2012).

In the Carpathian breed, the frequency of illnesses was decreased in animals with LK phenotype (17.98%) than in the ones with HK phenotype (28.12%) (fig. 6). So the LK
phenotype gives to the Carpathian breed individuals a greater vitality compared to the HK phenotype.

At the level of five blood factors (Aa, Bf, Bi, Ca and Ma), the antigenic structures are more abundant in the healthy goats than in the diseased goats. In healthy animals, at the level of these loci, the number of positive reactions is much greater than that of the negative reactions, ranging from 54% for the Bf factor and to 92% for the Ma factor. The frequencies of the other three factors record values between 62% and 64%. In sick animals, although these factors have lower frequencies than in healthy animals, their values are quite significant. At the level of factors Bb, Bd and especially Bc there is a strong depression of dowry immunogenetic both in diseased animals as well as in healthy ones, being situated far below 50%. This time, however, the frequencies of blood group factors in these loci are lower in healthy animals (between 13% and 37%) than in diseased animals (between 18% and 43%). On average for all antigenic determinants, the immunogenetic dowry of the healthy goats is something richer (51.63%) than of the goats affected by various diseases (47.13%) (fig. 7).

In terms of phenotype, the healthy animals are associated with a richer immunogenetic dowry at the Aa, Bf, Bi, Ca and Ma factor level than in the diseased animals. In contrast, in the factors Bb, Bc and Bd, a better health status of the animals is associated with a certain weakness of antigenic structures than in the diseased animals. On a general level, one can say that the goat health is given by an adequate endowment with antigenic determinants. Certain differences between the
healthy and diseased animals also occur regarding the genotypic structure at the blood group factor loci. However, in both goat subpopulations, the recessive homozygotes are more frequent and the dominant homozygotes have low incidences. The heterozygotes have a good representation. However, in healthy animals, both the dominant homozygosity and the heterozygosity are better represented and the recessive homozygosity is poorer than in diseased animals (fig. 8). The differentiated association of erythrocyte factors with morbidity would be due to the antigenic structural particularities of each type of blood cell, as well as to the linkage of blood group factor loci with other polymorph loci, especially with those of haemoglobin and potassium (Nguyen, 1990; Haba et al., 1991; Osterhoff, 1995).

Fig. 8. Blood group zygosity status of goats depending on their health status.

The results of the present study suggest the existence of different associations of the morbidity overview in the Carpathian breed goats with some genetic structures presenting multiple molecular forms. Demonstration of these associations makes of biochemical-genetic and immunogenetic systems useful and efficient tools that can be integrated in the selection, improvement and breeding technologies of goats, both within Carpathian breed and also in entire caprine species. So the polymorphism of different genetic structures is a means of genetic prophylaxis achieving in goats. By selection the animals with genotypes and phenotypes of the biochemical-genetic and immunogenetic systems that are associated with disease resistance can be promoted for reproduction and the animals whose polymorphic structures are associated with various morbid entities will be removed from the reproduction circuit, taking another production destination. Therefore, the polymorphism of these structures must be maintained and even enhanced, fact that would justify their role as genetic markers for achieving a valuable and healthy gene pool for breeding of these animals. This study is only an overall analysis of the association of polymorphic genetic structures with morbid phenomenon in goats. To have an even greater plausibility, it should be extended towards achieving the association of each polymorphic genetic system with each nosological entity.

Conclusions

1. In goats belonging to the Carpathian breed there were observed associations between the structures with discontinuous variability of the biochemical-genetic and immunogenetic systems and the health status of animals.
2. Within the biochemical-genetic systems (polymorphic proteins and minerals) the lowest rate of diseases occurs in the HbAHbB haemoglobin heterozygotes, TfATfA transferrin homozygotes, AlbBAlbB albumin homozygotes and in individuals with LK potassium phenotype (mostly heterozygotes at the Ke locus).
3. The HbBHbB haemoglobin homozygotes, TfATfB transferrin heterozygotes, AlbAAlbA albumin homozygotes and individuals with HK potassium phenotype (recessive homozygotes at the Ke locus) are associated in the highest degree with various morbid entities in comparison with the other genotypic structures from the level of different biochemical-genetic loci.
4. In the majority of blood group factors (Aa, Bf, Bi, Ca and Ma), the hereditary dowry of antigenic determinants is richer in healthy animals than in the diseased ones; exceptions are the factors Bb, Bc and Bd at whose level the erythrocyte antigens are slightly more
numerous in animals suffering from certain diseases compared to the healthy ones.
5. The average of erythrocyte factors in all blood group systems shows a better immunogenetic endowment in goats unaffected by morbidity than those who contracted various diseases; this antigenic distribution determines a higher dominant homozygosity and heterozygosity and a lower recessive homozygosity in healthy individuals in comparison with the sick ones.
6. The exaggerated decrease of antigenic determinant dowry is associated with a lower organic resistance of the animal body.
7. The associative analysis of biochemical-genetic and immunogenetic systems with animal morbidity creates prerequisites using the genetic markers to improve the health status of the Carpathian breed goats.

References

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