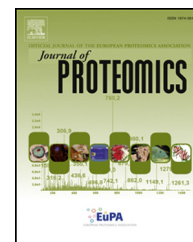




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Review

In between — Proteomics of dog biological fluids



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ABSTRACT

Dogs are relevant to biomedical research in connection both to veterinary medicine for their role as pets and to basic investigations for their use as animal models in pathology, pharmacology and toxicology studies. Proteomic analysis of biological fluids is less advanced for dogs than for other animal species but a wealth of information has already been gathered, which we summarize in this review. As a remarkable feature, we also assemble here for due reference a number of 2-DE serum/plasma or urine patterns in health and disease; some of them correspond to unpublished data from the University of Veterinary Medicine Vienna.

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Abbreviations: 1d, first dimension; 2d, second dimension; APP, acute phase proteins; APR, acute phase reactant; BALF, bronchoalveolar lavage fluid; CSF, cerebrospinal fluid; PAA, polyacrylamide; PSP, prostate specific protein.

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1. Introduction

In the last few years, with the help of a mixed crowd of colleagues we have reviewed literature data about the proteomics of the biological fluids in a few animal species. One manuscript was devoted to farm animals — tissue proteomics was also accounted for in there [1]; two dealt with laboratory animals, mice [2] and rats [3], the latter a follow-up to an earlier summary of mainly in-house work [4].

This new manuscript is shifting interest on companion animals. We focus on dogs — mainly because more literature data are available than for any other pet (>300000 entries in PubMed (<http://www.ncbi.nlm.nih.gov/pubmed/>)). In a sociological perspective, in the US — for which reliable census data are available — dog ownership involves 39% of households, to a total of 78 million owned (<http://vetmedicine.about.com>). Connected with more detailed workout of the patients, veterinary medicine for dog treatment results in higher cost rates than for other pets but also yields a higher number of case reports and of clinical databases. In a basic science perspective, dog was and still is often used as animal model in pathology, pharmacology and toxicology studies (>20000 entries in PubMed). Relevant to this point are mainly the biological peculiarities of the species but also practical considerations (handling, diversity of breeds).

A detailed knowledge of the protein composition of biological fluids in a given species under a number of conditions is relevant to both veterinary routine and scientific advancement. For the former, changes in analyte concentrations provide evidence of disease; for the latter, understanding all the biological roles of the affected components helps explain complex physio-pathological mechanisms. We pointed out both of these aspects in all of our previous writing about biological fluid proteomics. We like to stress here one more time that physiologists and pathologists are still learning about the functions of even the major and long-characterized plasma/serum proteins. This especially applies to the acute phase proteins, which we will deal with in detail in the following.

2. Dog plasma/serum (at baseline)

The genome of the dog has been completely sequenced [5] (<http://www.broadinstitute.org/mammals/dog>) and ca.

thousand reviewed entries may currently be found for *Canis familiaris* in UniProt (<http://www.uniprot.org>). Among the latter, however, only twelve cover complete sequences of major proteins secreted in plasma/serum: albumin, α -fetoprotein, β -2-glycoprotein 1, apolipoprotein A-I, C-I, C-II and E, clusterin, haptoglobin, coagulation factor IX, von Willebrand factor, plasminogen, and one is a major protein secreted in urine: uromodulin. All of these proteins are common across animal species and the information reported in the UniProt entries devoted to dog items seldom deals with exclusive properties. This is the case only for albumin, whose role as dog allergen is quoted, and for von Willebrand factor, which is connected with a bleeding disease in the Scottish Terrier breed. Conversely, no major dog-specific protein has been identified/reported in plasma/serum. Our own experimental data confirm that none of the prominent spots in a 2-DE map of dog serum corresponds to a protein unique to this genus. In conclusion, the list of fully characterized proteins is very short (not even such relevant components as transferrin or α_1 -antitrypsin being included) and annotations are scanty: qualitative peculiarities may have gone undetected as yet, or may have been inadequately reported/reviewed.

Back in 1975, early work on dog serum proteome already involved the 2-DE approach: some major serum components (glycoproteins as a group) were identified by histochemical and autoradiographic techniques [6] and changes possibly due to sex or age (six months to 10 years) were evaluated (mean concentration, SD, CV) [7]. These investigations focused on Beagles as the breed most frequently used as experimental animal. Intensive dog breeding over centuries resulted in a multitude of different dog breeds; the Fédération Cynologique Internationale, an international federation of kennel clubs, lists more than three hundred breeds (<http://www.fci.be/nomenclature.aspx>). Routine electrophoresis on agarose gels has reported different reference ranges, depending on breed, for overall serum protein concentration as well as protein distribution (zone electrophoretic separation in albumin, α - to γ -globulin zones) in healthy animals [8].

A 2-DE map of dog serum proteins run under reducing and denaturing conditions (and with a NL 4–10 IPG for the 1d isoelectric focusing step) is shown in Fig. 1 (our unpublished results and [9]); the sample is from a healthy male Beagle. Spot identifications are marked, deriving either from immunoblots

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