



# Study on giraffe (*Giraffa camelopardalis*) urinary proteome

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## Background

Due to their unique physiology and susceptibility to handling and anesthesia, the investigation of health status in giraffe is difficult (Dagg, 2014). Thus, every diagnostic based on non-invasive practices will help to obtain clinical information. Under this point of view, urine can be considered an excellent biological sample, because it can be obtained non-invasively and repeatedly without contact with the animals (Kersey et al, 2014). In addition, urine is considered a good source of biomarkers, which can give information on kidney function and general health status.

## Objectives

The aim of this research project were to: 1) perform a complete urinalysis of urine samples collected from captive giraffes hosted in different Italian facilities and 2) separate the urine proteome by SDS gel electrophoresis.

## Materials and methods

Fourteen urine samples were collected from 13 specimens with different age, sex and subspecies living in two Italian zoos: Parco Zoo Falconara Marittima (Ancona) and Parco Zoo Safari Ravenna (Ravenna) (**Fig. 1**).

Urine samples were collected from the ground with a syringe, according to Glatston & Smith, 1980 who used the same technique for the okapi.

Urine samples were subjected to a routine urinalysis as follows: physical examination; chemical examination by semi-quantitative dipstick test; centrifugation at 1500g for 10 min; microscopic examination of urine sediment; protein quantification and urine protein-to-creatinine ratio (UPC) determination. To separate the urine proteome sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on urine supernatants.



Fig. 1 Giraffe housed in Safari Park Ravenna

## Results

**Routine Urinalysis:** the giraffe's urine had an alkaline pH between 8 and 9. Five samples showed a positivity (1+) to protein and the microscopic examination of the sediment had not shown significant results.

**Urine total protein concentration (UTP) and creatinine:** mean values were  $20.14 \pm 11.42$  mg/dL and  $159.95 \pm 77.10$  mg/dL respectively.

**UPC:** a mean value of  $0.12 \pm 0.04$  was determined.

**SDS-PAGE of urine proteome:**  $6 \pm 2$  protein bands were present in all the examined samples. The most represented protein bands had an apparent molecular weight (MW) of 91 kDa, 83 kDa, 69 - 70 kDa, 49 kDa, 28 kDa, 16 and lower than 14 kDa (**Fig. 2, Fig. 3**).

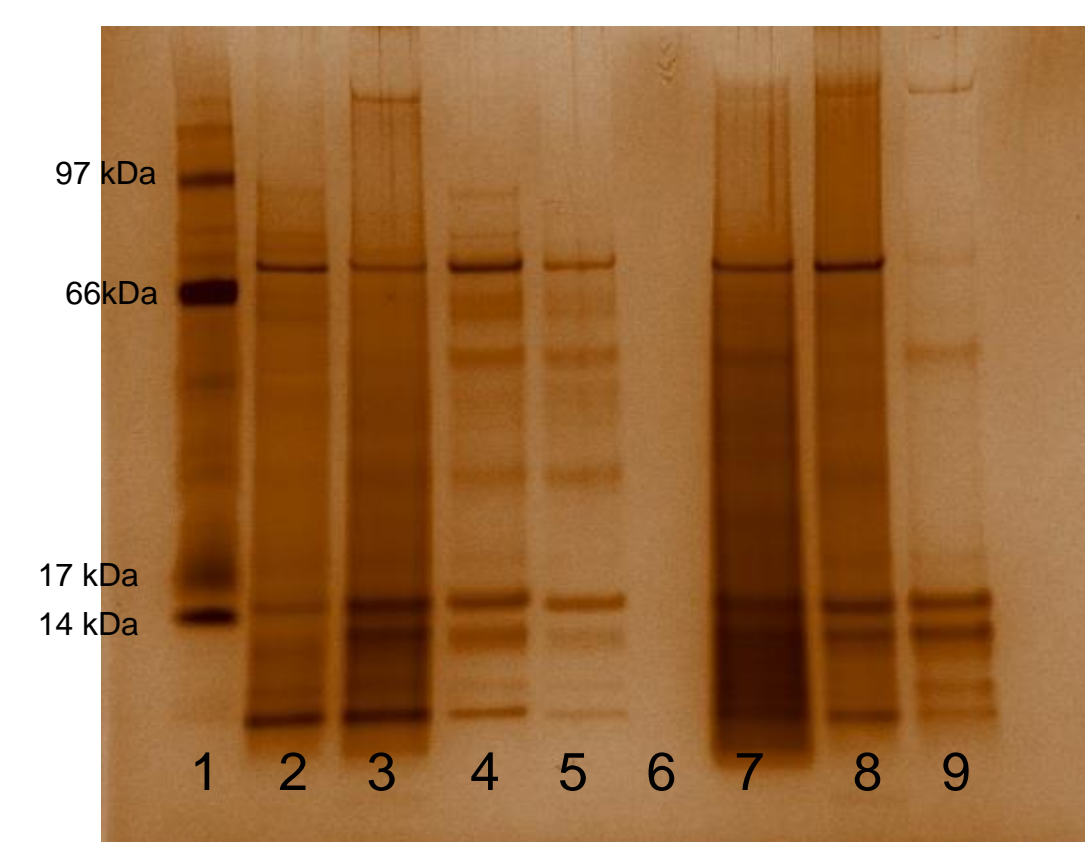


Fig. 2 Electrophoresis gel (SDS-PAGE).  
Lane 1: marker Low Range Markers: sc-2360  
Lane 2 - 3 - 7: urine samples from the giraffe housed in Ancona. Lane 4 - 5 - 8 - 9: urine samples from the giraffe housed in Ravenna.  
The numbers to the left of the figure indicate the molecular weights of the marker.

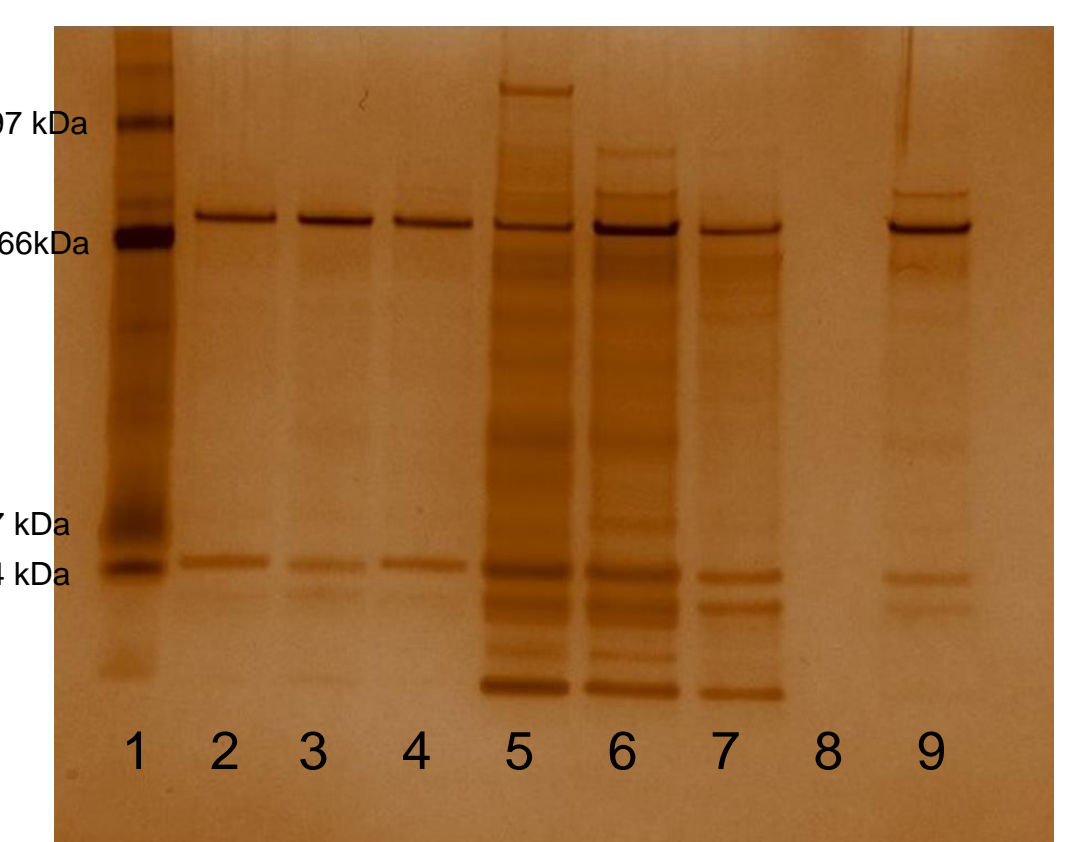


Fig. 3 Electrophoresis gel (SDS-PAGE).  
Lane 1: marker Low Range Markers: sc-2360  
Lane 2 - 3 - 4 - 5 - 6 - 7 - 8 - 9: urine samples from the giraffe housed in Ravenna.  
The numbers to the left of the figure indicate the molecular weights of the marker.

## Discussion

Concerning urine physical and chemical examination and sediment analysis, the giraffe's urine presented an alkaline pH, similar to okapi, bovine and goat. Due to the alkaline pH, the protein quantification with the dipstick test might produce false positives; as a consequence, we suggest to determine urinary total proteins by the Pyrogallol red-molybdate assay. Regarding the UPC, no data are reported in the literature for giraffe. Comparing our data to reference values reported for domestic animals, specimens should be considered non-proteinurics ( $UPC < 0.2$ ) (Lees et al, 2005).

SDS-PAGE of urine proteome showed a characteristic protein profile of giraffe urine, with a most evident band at 69-70 kDa, which could be identified as albumin, on the basis of molecular weight. Other faint protein bands are present at 91kDa, putative uromodulin, 83 kDa, putative transferrin. Other protein bands are present at low molecular weights, namely at 28 kDa and 16 kDa.

## Conclusion

Our data, though obtained on a limited number of specimens, can be considered the first attempt to obtain data on urine parameters in healthy giraffe and SDS-PAGE resulted in a useful diagnostic tool that could help clinicians in qualitative evaluation of proteinuria and for monitoring of renal function in giraffe.

## Bibliography

- Dagg A. I., Physiology, In: Giraffe, biology, behaviour and conservation, edited by Dagg A. I., Cambridge University Press, 2014, New York, USA, 118-134.  
Glatston A. R. - Smith S., Analysis of the urine of the okapi (*Okapia johnstoni*), Acta Zoologica et Pathologica Antverpiensia, 1980, 75, 49-58.  
Kersey, D. C. - Dehnhard M., The use of noninvasive and minimally invasive methods in endocrinology for threatened mammalian species conservation, General and Comparative Endocrinology, 2014, 203, 296-306.  
Lees G. E. - Brown S. A. - Elliott J. - Grauer G. F. - Vaden S. L., Assessment and management of proteinuria in dogs and cats, 2004 ACVIM Forum Consensus Statement (Small Animal), Journal of Veterinary Internal Medicine, 2005, 19, 377-385.