

CLOISONNÉ KIDNEY IN MOUFLON SHEEP (*OVIS ORIENTALIS MUSIMON*)

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ABSTRACT.— This study describes a case of cloisonné kidney associated with infection by *Babesia ovis* in a wild mouflon (*Ovis orientalis musimon*) found dead in year in eastern Sierra Morena (Andalusia, Spain). The major macroscopic lesions were a) icteric coloration in ocular and oral mucosa, and b) dark metal coloration on the kidneys' external surface. Intense dark coloration alternating with intense greyish strips was observed on renal cortex sections. Blood samples were collected for haematological and parasitological analyses, as well as samples from various organs for histopathological examination. Infection by *B. ovis* was confirmed by using the Polymerase Chain Reaction test (PCR). Histopathological examination revealed tubulonephrosis associated with thickening of the basal membrane of the convoluted portions of the renal tubules (both proximal and distal), which took on an intense brownish pigmentation. These renal lesions have been described as "cloisonné" kidney. Cloisonné kidneys show uncommon coloring of the renal tubular system, associated with ferritin and hemosiderin deposits in these structures resulting from a chronic hemolytic process (hemoparasites, toxins, etc.). In our case, hemolytic phenomena are associated with infection by *B. ovis*. The seroprevalence of this hemoparasite in wild mouflon populations in other regions of Spain (Catalonia) is up to 15%.

RÉSUMÉ.— Nous étudions un cas de rein cloisonné associé à l'infection par *Babesia ovis* sur un mouflon sauvage (*Ovis orientalis musimon*) qui a été trouvé la même année dans la partie orientale de la Sierra Morena (Andalousie, Espagne). Les principales lésions macroscopiques étaient: a) coloration icterique sur les muqueuses oculaire et orale, et b) coloration métallique foncée sur la partie extérieure du rein. Cette coloration foncée intense alternait avec des bandes grisâtres dans les sections du cortex rénal. Nous avons pris des échantillons de sang pour les analyses hémathologique et parasitologique, ainsi que d'autres échantillons de divers organes pour l'analyse histopathologique. L'infection par *B. ovis* était confirmée par

le test de la réaction en chaîne de Polymérase (PCR). L'analyse histopathologique a révélée une tubulonephrose associée avec l'épaississement de la membrane basale des portions en boucle des tubules rénaux (aussi bien proximaux que distaux), lesquels prenaient une intense pigmentation brunâtre. Ces lésions rénales ont été décrites comme rein "cloisonné". Le rein cloisonné montre une coloration extraordinaire du système rénal tubulaire, associé avec un dépôt de ferritine et hémosidérine sur ces structures à la suite d'un processus hémolytique chronique (hémoparasites, toxines, etc.). Dans notre cas, les phénomènes hémolytiques étaient associés à l'infection par *B. ovis*. La séroprévalence de cet hémoparasite dans les populations sauvages du mouflon d'autres régions d'Espagne (Catalogne) s'élève jusqu'à 15%.

RESUMEN.— En el presente estudio se describe un caso de riñón lacado asociado a una infección por *Babesia ovis* en un muflón salvaje (*Ovis orientalis musimon*) encontrado muerto en Sierra Morena (Andalucía, España). Las lesiones macroscópicas más aparentes fueron: a) coloración icterica en la mucosa oral y ocular y b) coloración metálica oscura en la superficie externa de los riñones. Una intensa coloración oscura alternando con unas bandas grisáceas era visible en las secciones del córtex renal. Se tomaron muestras de sangre para los análisis parasitológicos y hematológicos, además de muestras de varios órganos para su examen histopatológico. Se confirmó la infección por *B. ovis* gracias a la Reacción en Cadena de la Polimerasa (PCR). El análisis histopatológico reveló tubulonefrosis asociada a un engrosamiento de la membrana basal del sistema contorneado de los túbulos renales (tanto proximal como distal), la cual adquiere una intensa pigmentación marronácea. Estas lesiones renales han sido descritas como riñón "cloisonné" o riñón lacado. Los riñones lacados muestran un color poco común en el sistema tubular renal, asociado a depósitos de ferritina y hemosiderina como resultado de un proceso crónico hemolítico (hemoparásitos, toxinas, etc.). En este caso, el fenómeno hemolítico está asociado a una infección por *B. ovis*. La seroprevalencia de este hemoparásito en el muflón salvaje de otras regiones de España (Cataluña) puede alcanzar un 15%.

1. Introduction

The mouflon (*Ovis orientalis musimon*) is distributed in Spain where there exist wild populations in Sierra de Cuenca, Montes de Toledo, Extremadura, Sierra de Cazorla (Jaén), and Sierra Morena Oriental (over 3,500 individuals), as well as in many private big game estates all over Spain. Not much is known about the main pathologies affecting this species in Spain because little research has been conducted on this subject (MARCO-SÁNCHEZ *et al.*, 1996; GÓMEZ-BAUTISTA *et al.*, 1996; FERRER *et al.*, 1998; LASTRAS *et al.*, 2000; MORENO *et al.*, 1999).

The pigmented thickening of the proximal convoluted tubular (PCT) basement membrane have been described in goats (ZAHAWI, 1957; THOMPSON *et al.*, 1961; LIGHT, 1965; KHAROLE, 1967; GROSSMAN & ALTMAN, 1969; ALTMAN *et al.*, 1970; METIN, 1980), horses, (MARCATO & SIMOI, 1982) and

sheep (METIN, 1980; ORYAN *et al.*, 1993; HATIPOGLU & ERER, 2001). The lesion has been termed cloisonné kidney by Light, because of the resemblance of the thickened membranes to enamel work separates by wires. Cloisonné kidney is considered to be a non clinical entity, and the etiology of the condition is unknown (LIGHT, 1965; KHAROLE, 1967; GROSSMAN & ALTMAN, 1969; ALTMAN *et al.*, 1970; METIN, 1980; HATIPOGLU & ERER, 2001).

This work describes microscopic appearance of the lesions on cloisonné kidney in an mouflon sheep (*Ovis orientalis musimon*) and his possible relation with a *B. ovis* infection.

2. Material and methods

A wild mouflon was found dead in year in eastern Sierra Morena (Andalucía, Spain). Blood samples were collected for parasitological study as well as samples from kidneys for microscopically study. These were fixed in 10% folmol solution. Samples for histopathological study were routinely processed and embedded in paraffin wax. Four micrometre thick sections were stained with haematoxilyn & eosin, peryodic acid Schiff reagent (P. A. S.) and Gomori's method for iron.

Polimerase chain reaction (PCR) was used for hemoparasites, *Babesia/Theileria* spp., detection. DNA was isolated from blood with the aid of the Blood Spin kit purchased from MO-BIO (Solana Beach, California, USA). Primers used for DNA amplification/sequencing are BT1-F, BT1-R, BTH-1F, BTH-1R, BT2-F and BT2-R. Diagnosis of *Babesia/Theileria* was performed with the seminested PCR assay (CRIADO-FORNELIO *et al.*, 2003).

3. Results

The major macroscopic lesions were moderate icteric coloration in ocular and oral mucosa and dark metal coloration on the kidney's external surface. Intense dark coloration alternating with intense greyish strips was observed on renal cortex sections (Figure 1).

Histopathological examination of haematoxilyn & eosin stained sections revealed thickening of the basement membrane of proximal and distal convoluted tubules due to dark brown pigmentation (Figure 2). The lesions were mostly witnessed in the cortex extending to cortico-medullary junction. No changes were observed in basement membrane of medullar tubules and Bowman capsules. No proliferative connective tissue was observed in associ-

ation with the thickened basement membrane of the tubules. The affected basement membranes showed specific positive reaction for iron and gave PAS positive reaction (Figure 3).

Infection by *Babesia ovis* was confirmed by PCR test in blood specimens.

4. Discussion

This work represents the first report of cloisonné kidney in a mouflon (*Ovis orientalis musimon*) and in addition proposes an etiopathogenic mechanism in his development.

Pigmented thickening of the PCT was described the first time in goats, named as "symmetrical cortical siderosis" in 1957 (ZAHAWI, 1957). LIGHT (1965) termed this lesion as "cloisonné kidney" in goats in 1960, because of the resemblance of the tickened membranes to enamel work separates by wires. This condition has been extensively reported in goats, but we have scarce descriptions for sheep's kidneys (METIN, 1980; ORYAN *et al.*, 1993; HATIPOGLU & ERER, 2001). The incidence of this pathology in sheep's is lower (aproximately 0.04%, HATIPOGLU & ERER, 2001) than in other reports on goats (up to 14% according to LIGHT, 1960). However, our report is still insufficient to be conclusive about the degree of prevalence of this condition in mouflon populations in south-eastern Spain.

Similarly to ORYAN *et al.* (1993), we found microscopical changes in both, proximal and distal convoluted tubules, although the most severe lesions were observed in PCT (HATIPOGLU & ERER, 2001). As in HATIPOGLU & ERER, 2001; THOMPSON *et al.*, (1961) and LIGHT, (1965), pigmented basement membrane gave PAS positive reaction, in contrast to KHAROLE (1967). Likewise, in contrast to the descriptions by HATIPOGLU & ERER, 2001; LIGHT & METIN (1980), no appreciable increase of collagen fibers and no celular infiltration were observed on the peritubular interstitium.

Although the etiology of this condition is unknown, some researchers have suggested a number of possible reasons for this pigmentation. ZAHAWI (1957) claimed that iron presence in PCT cells was irreversible and may be the cause of the hypertrophy of the basement membrane. This author claimed that protein- and cobalt-low, but iron-rich diets may be responsible for this condition.

According to other authors (ALTMAN *et al.*, 1970), high serum ion-binding saturation, small-sized goat erythrocyte, low enzyme activity in membranes, and increased osmotic fragility may be a more likely cause of intravascular haemolysis through any number of inciting causes (poisonous plants, copper-rich soils, etc.).

In our particular case, parasitisation by *B. ovis* was confirmed. This may well have caused chronic erythrolysis, which in turn would provoke ferritin and hemosiderin deposition in the basement membranes. However, since we studied a free-ranging mouflon, other causes cannot be ruled out, such as the influence of toxic plants, common in the area mentioned, or an iron-rich diet, or copper-rich soils.

On the basis of these reflections, we may conclude that it is necessary to undertake further studies in order to discover the pathogenesis of cloisonné kidney in sheep, whether domestic or wild.

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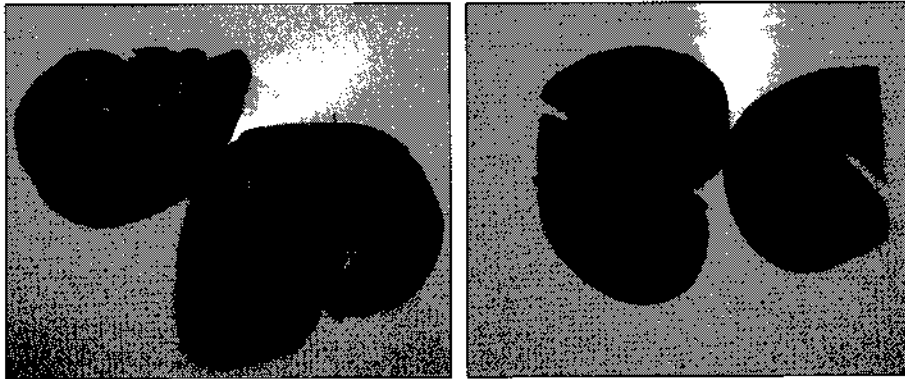


Figure 1 (A, B). Gross appearance of renal surface. Black color, narrow, radiating streaks on the cut surface of the cortex.

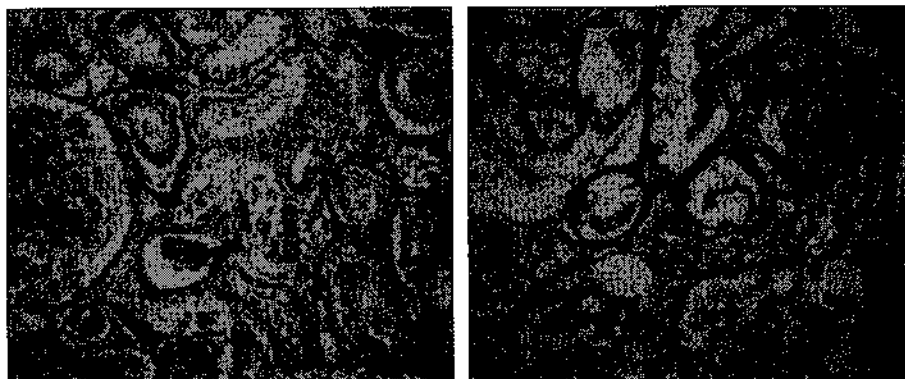


Figure 2 (A, B). Microscopic lesions. Dark brown thickening of basement membranes of convoluted tubules. Hematoxilin & eosin. (x 400).



Figure 3 (A, B). Microscopic lesions. Pink brown pigmentation in the basement membranes of convoluted tubules. P. A. S. (x 400).