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Authors: Jara, Luis Fernando, Sánchez, Jairo Mauricio, Alvarado, Hernán, and Nassar-Montoya, Fernando

Source: Journal of Wildlife Diseases, 41(2) : 431-434

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-41.2.431>

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Kurloff Cells in Peripheral Blood and Organs of Wild Capybaras

Luis Fernando Jara,¹ Jairo Mauricio Sánchez,^{1,4} Hernán Alvarado,² and Fernando Nassar-Montoya^{3,5}

¹ Convenio Universidad de la Salle-Fundación Jaime Duque, Bogotá, Colombia; ² Médico Patólogo, Bogotá, Colombia; ³ Convenio Universidad de la Salle-Fundación Jaime Duque, Parque Jaime Duque, Grupo de Investigación Medicina de la Conservación Universidad de La Salle-Fundación Araguatos, Carrera 15 No. 94-69, L. 4, Bogotá, Colombia; ⁴ Deceased; ⁵ Corresponding author (email: araguatos@etb.net.co)

ABSTRACT: Peripheral blood and tissue from twenty-two free-ranging, hunter-killed capybaras (*Hydrochaeris hydrochaeris*) collected between December 1996 and April 1997 in Casanare, Colombia (5°58'N and 71°33'W), were examined by light microscopy for Kurloff cells (KCs). Kurloff cells were observed in the blood of one pregnant adult female, and in organs from all the animals, including spleen (21 of 22 animals), liver (18 of 21), lungs (13 of 21), ovary (8 of 11), uterus (7 of 10), bone marrow (13 of 20), kidney (8 of 22), adrenal gland (6 of 20), and lymph node (4 of 14). The anatomic distribution of the KC in the wild capybaras was similar to that of the guinea pig.

Key words: Capybara, *Hydrochaeris*, Kurloff body, Kurloff cell.

The Kurloff cell (KC) is a mononuclear cell containing lymphocyte and monocyte properties (Eremin et al., 1980a). The role of the cell is not completely understood; however, in the guinea pig (*Cavia porcellus*), they have immune and natural killer activity (Eremin et al., 1980b; Debout et al., 1984) and anticancer properties (Debout et al., 1995; Pouliot et al., 1996; Debout et al., 1999).

Kurloff cells have been studied widely in the guinea pig since reported by Kurloff in 1888 (Revell, 1977); however, there are few reports of KCs in other rodent species. Kurloff-like cells were reported in peripheral blood of a female captive capybara (Messick and Willet, 1987), and Hawkey and Dennet (1989) showed a photomicrograph of a KC in a blood smear from this species. A study by Etzel in 1931 (cited by Revell, 1977) suggested the presence of KC-like structures in paca, capybara, agouti, and cavie.

Kurloff cells easily can be identified by light microscopy using Giemsa or periodic-acid Schiff (PAS) stains. The cells are ovoid, ranging from 10–25 μm in diame-

ter, and contain one large oval, elongated, or rounded cytoplasmic inclusion body, ranging from 1–11 μm in diameter. The inclusion body is homogeneous by light microscopy; however, artifacts caused during tissue processing can cause it to have granules, rodlike structures, and skeins. The inclusion body pushes the sickle-shaped nucleus towards the periphery of the cell, and its margin frequently has small vacuoles (Ledingham, 1940; Kortelainen and Korhonen, 1976a, b; Eremin et al., 1980a; Oulhaja, 1995).

Ultrastructurally, KCs have a smooth and rounded shape, a small number of microvilli projecting from the surface, and the usual cell organelles. Ultrastructural characteristics have been described in detail (Kortelainen and Korhonen, 1976b; Kittas et al., 1979; Landemore et al., 1986). Granule-filled vesicles of variable size and bundles of microfibrils are seen in the cytoplasm. The inclusion body, surrounded by a 4.5 nm membrane, can be completely electron-dense or slightly translucent, and has myelin figures peripherally.

Kurloff cells can be found in most organs of the guinea pig, yet counts are very low (Revell, 1977), and numbers vary with gender, age, and some physiological states. Counts are higher in females than in males, and increase during pregnancy and estrus. Treatment with estrogens increases KC counts in both males and females (Ledingham, 1940; Kittas et al., 1979; Sandberg and Hagelin, 1986). It also has been suggested that immunologic stimulation can raise KC counts (Revell, 1977).

The purpose of this study was to determine if KCs are present in wild capybaras and if there is a potential association be-

tween KC counts and physiological states or tissue lesions. Kurloff cells were identified by morphological characteristics using light microscopy (Kortelainen and Korhonen, 1976a). Other techniques to study KCs, such as electron microscopy and histochemistry, were not done because it was not practical under the field conditions of this study and because they do not conclusively identify KC.

Peripheral blood and tissues were collected from 22 free-ranging capybaras (*Hydrochaeris hydrochaeris*) between December 1996 and April 1997 to determine the presence of KCs. Animals had been killed by local inhabitants for food (legal hunting for subsistence, as allowed by Decreto 1608 of 1974 Colombian legislation) on two farms (El Triunfo and Penjamo) in Casanare, Colombia (5°58'N and 71°33'W). Capybaras are abundant in the region, and normally share pastures with livestock.

Blood was taken from the heart with a 20 ml syringe within 3 min after the animal was shot. Two blood smears were made immediately, and the remaining blood anticoagulated with ethylene diamine tetracetic acid (EDTA). Within 30 min of collection, a Wintrobe tube was filled with anticoagulated blood and centrifuged for 7 min at $760 \times G$. A smear was made of the resulting white blood cell layer to enhance the probability of visualization of KCs. Blood and white blood cell smears were fixed and stained with Giemsa stain.

Number of KCs per 300 white blood cells was counted in the whole blood and white blood cell layer smears by light microscopy at $400\times$. Kurloff cells were identified by morphological characteristics, which included cell form and the presence, staining properties, and shape of an inclusion body in the cytoplasm. Only cells with KC morphology as described by Kortelainen and Korhonen (1976b) were counted.

Pieces of spleen, mesenteric lymph node, bone marrow, ileum, liver, kidney, lungs, adrenal, ovaries, and uterus, ap-

proximately 1 cm^3 , were fixed in buffered 10% formalin. Tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) and PAS for examination by light microscopy. Only tissues with perfect integrity were examined for KCs; thus total number of samples examined for each organ varied.

Slides were examined at $400\times$ by light microscopy and KCs in the entire tissue section were counted. Because KC counts were very low (normally lower than 30 KCs per slide) in liver, lung, kidney, adrenal, ovary, uterus, and blood marrow, total counts in the entire tissue (approximately 1 cm^2) were recorded, except in spleen. Total KC counts in each slide were ranked on an ordinal scale to diminish counting bias due to the variance on the tissue size, in order to carry out non-parametric analyses to evaluate distribution of the KCs among the organs (Siegel, 1990). Ranking was as follows: 0 KCs per slide = 1; 1–5 KCs per slide = 2; 6–15 KCs per slide = 3; 16–30 KCs per slide = 4; and >30 KCs per slide = 5. A Mann-Whitney *U*-test was done to evaluate KCs counting rank differences in liver, lung, kidney, adrenal, ovary, uterus, and blood marrow and between pregnant and nonpregnant females. In spleen, KCs were counted in ten $400\times$ fields for each slide, because KCs were abundant in this organ. A MANOVA (multivariate analysis of variance) test was done to evaluate KC counting differences in spleen, among populations (farms), gender, and age.

Eight adult males, 10 adult females, two juvenile males, and two juvenile females were sampled. Kurloff cells were observed in peripheral blood in a smear of white blood cells from one pregnant female. The total count was 32 KCs per 300 white blood cells. Kurloff cells had a mean length of $9.94 \mu\text{m}$ (SD 1.77, range 6–14 μm , $n=50$) and a mean width of $9.3 \mu\text{m}$ (SD 1.57; range 6–12 μm , $n=50$). They contained an oval, reticular, vacuolated, and eosinophilic cytoplasmic inclusion body with a mean length of $4.98 \mu\text{m}$ (SD

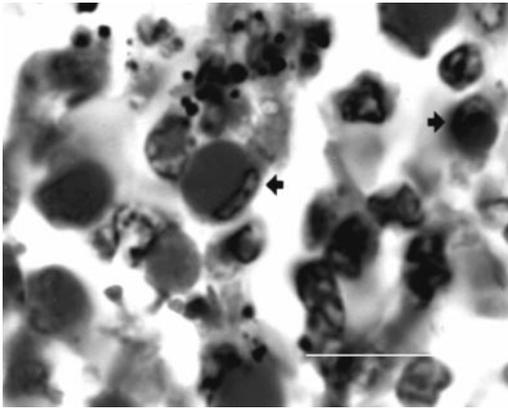


FIGURE 1. Kurloff cells (arrows) in a section of the spleen from a wild capybara. PAS stain. Bar = 10 μm .

1.65 μm , range 2–9 μm , $n=50$) and a mean width of 9.3 μm (SD 1.57 μm ; range 6 μm –12 μm ; $n=50$). Organs from all 22 capybaras had KCs that were oval or rounded with an oval or rounded magenta cytoplasmic inclusion body when stained with PAS. Kurloff cells were found in the spleen (21 of 22 animals) where they were abundant in the red pulp but rare in splenic white pulp; liver (18 of 21); alveolar walls and capillaries of lungs (13 of 21); center and periphery of the corpora lutea of ovaries (8 of 11); endometrial capillaries of uterus (7 of 10); disseminated along the hematopoietic zone of bone marrow (13 of 20); in peritubular capillaries of kidneys (8 of 22); adrenal medulla (6 of 20); and the follicular zones and into the post-capillary veins of lymph nodes (4 of 14) (Fig. 1).

Highest KC counts were recorded from spleen with a mean of 28.6 (range 0–101) KCs in ten 400 \times fields; using MANOVA, there were no differences in splenic KC counts among farms ($F=0.00127$, $P=0.972>0.05$), gender ($F=0.47943$, $P=0.50>0.05$), and age ($F=2.1466$, $P=0.164>0.05$). Kurloff cell counting ranks were higher in pregnant/lactating females (six animals) than in nonpregnant females (six animals) using Mann-Whitney U -test: in liver ($U=3.5$, $P=0.013<0.05$), lung ($U=0$, $P=0.002<0.05$), kidney ($U=5.5$, $P=0.032<0.05$), adrenal ($U=1.5$,

$P=0.019<0.05$), and ovary ($U=1$, $P=0.004<0.05$). Males had low counts, similar to those of non-pregnant females.

There was no apparent relationship between KC localization in organs and microscopic changes. Kurloff cells were not seen in or around congestion in kidney (2 of 22 animals), lung (7 of 22), liver (1 of 22), or spleen (6 of 22), fat degeneration in liver (2 of 22), endarteritis in lung (1 of 22) and kidney (6 of 22), and a lung abscess (1 of 22).

This study demonstrates the presence of KCs in peripheral blood and organs of wild capybaras; characteristics and distribution of capybara KCs were similar to the guinea pig (Kortelainen and Korhonen, 1976a; Revell, 1977; Kittas et al., 1979; Lettaief et al., 1993). In addition, pregnancy/lactation was related to presence and increased tissue counts of KCs, which might explain why KCs were observed in peripheral blood from one animal only, a pregnant female. Age, gender (non-pregnant females), and farm population did not influence counts. There was no association between KC distribution and tissue lesions; however, this is based on very few changes if tissue congestion is discounted.

The presence of KCs in peripheral blood of only one pregnant female might explain why KCs are not normally reported in capybaras, and emphasizes the importance of histological studies when evaluating the presence of KCs. Including KC identification and counting in post-mortem protocols for capybaras will increase our understanding of the cell. The spleen is a good target organ for screening purposes. Further research to compare KC morphological and histochemical characteristics between capybaras and guinea pigs, and to understand the function of KCs in captive and wild populations of capybaras is needed. The natural killer activity of KCs as reported for guinea pig (Debout et al., 1984, 1995; Pouliot et al., 1996; Debout et al., 1999) and potential immunologic and anticancer activities also need to be investigated. Further studies

and screening for KCs should be extended to other South American rodents, including agouti, paca, and cavie (Revell, 1977).

We thank El Triunfo and Penjamo; L. A. Vega, V. Cotrino, and M. Quiroga for helping us with laboratory analyses; C. Marinkelle (CIMPAT, Universidad de los Andes, Bogotá) for identifying the parasite *Cruorifilaria tubero cauda*; Convenio Universidad de La Salle-Fundación Jaime Duque for supporting the study; and A. Gómez and V. Pereira for reading this manuscript.

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Received for publication 9 October 2002.