MORPHOLOGICAL STUDIES OF VOMERONASAL ORGAN IN THE WILD JUVENILE RED- FLANKED DUIKER *Cephalophus rufilatus* (GRAY 1864)

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ABSTRACT

The vomeronasal organ (VNO) of the juvenile Red-flanked duiker (Cephalophus rufilatus) weighing between 0.8-1.4 kg was studied by gross dissection and light microscopy. The organ was found to be present at the base of the nasal septum completely housed by the vomeronasal cartilage, but the various soft tissue components of the organ were not remarkably present as in most adult mammals. The average palatal length of the organ was 1.9 cm, while the transverse diameter of the lumen of the duct measured 0.5 cm. The average thickness of the vomeronasal sensory epithelium on the medial wall was 50.05 µm, while that of the 'non sensory' respiratory epithelium on the lateral wall was 40.05 µm. Our findings suggest that the VNO of the juvenile duiker is rudimentary at this stage and may not be able to support vomeronasal functions. Further development of the various components is required to achieve its functional capacity.

Keywords: Red-flanked duiker, *Cephalophus rufilatus,* Vomeronasal organ, Anatomy, Histology

INTRODUCTION

In several mammalian species, olfactory sensory perception is mediated by two anatomically and functionally distinct organs, the main olfactory and vomeronasal organ(VNO) (Dulac and Axel, 1998).The VNO is a highly variable structures located bilaterally in the mucosa of the base of the nasal septum (Bhatnagar and Smith, 2003). It is typically encased within a cartilaginous or bony capsule (Salazar *et al.,* 1995). The VNO plays important role in chemosensory-mediated phenomenal effects on endocrine regulation, social and sexual behaviour (Halpern and Martinez-Marcos, 2003). It contains the peripheral chemoreceptors necessary for detection of pheromones (Wysocki *et al.,* 1991).

The VNO is also thought to function as an organ to determine the flavour of food in the mouth by olfaction (Dursun, 1994). Some researchers (Kumar *et al.*, 1981; Soler and Suburo, 1998; Doving and Trotier, 1998) suggested that the VNO may be related to "frehmen behaviour" (lip curl) displayed especially by felids and ungulates. The forest duikers *Cephalophus* spp are small little-known group of 15 species of African bovids which is ~~wi~~dely distributed throughout sub-Saharan Africa (Ansel, 1971). Forest duikers are considered primitive antelopes, which diverged early in bovid evolution and thus thought to have retained numerous primitive characteristics ((Estes, 1974; Kranz and Lumpkin, 1982). Moreover the group is relatively homogenous. All *Cephalophus spp* are small (4 – 64 kg) with build, gait and short slanted horns seem well adapted to movement through thick vegetation of the forest habitats. All duikers are browsers but individual species may be exclusively frugivorous, or frugivorous and herbivorous (Gautier-Hion *et al.,* 1980). They are true ruminants. Red-flanked duiker (*Cephalophus rufilatus*) is rare specie of forest duikers found throughout Central and Western Africa. They are among the few duiker species found outside equatorial rain forests (Estes, 991). Red-flanked duikers have the largest preorbita (maxillary) glands of all duiker species (Kingdon, 1984). These glands exude a scent used in territorial marking. Duikers *Cephalophus spp* are important source of food and income throughout forest regions of Central and West Africa. Local inhabitants kill duikers for food by capturing them in live traps or with nets. In West Africa, it is one of the most common meats sold in both rural and urban markets.

There is scant literature on the morphological and behavioral differences between wild and domesticated African bovids. In addition, some questions regarding the breed-related differences in the topographical location in a given species-large and small ruminants, goat, swine, sheep, cats as well as the boundaries of the location

of the sensory and non sensory epithelium of the vomeronasal organ in animals remain unclear. To date, there has been no conservation projects aimed at protecting populations of this species in the wild (Fischer and Linsenmair, 2001). Knowledge on the ecology and reproductive behaviour is still very limited (Newing, 2001). Moreover, information on the morphology of the vomeronasal organ in the Red-duiker (*C. rufilatus)* is apparently lacking. This study is therefore designed to shed light on the morphological aspect of the VNO in this species which hitherto is lacking.

MATERIALS AND METHODS

Six wild juvenile *C. rufilatus* (4 males and 2 females) weighing between 0.8 kg-1.4 kg purchased at intervals from farmers engaged in kill-trapping in the forests around Ogurugu/Opanda, in Nsukka area of South Eastern, Nigeria, were used for this study. Following decapitation, the heads were washed with normal saline and their vomeronasal organs were dissected out along with the nasal septum and hard palate. This was examined grossly using a dissecting microscope. The palatine and vomer bones were trimmed and the VNO dissected out from the tissues by sawing with small hand saw. These blocks of tissue containing the VNO was fixed in 10% neutral buffered formalin, decalcified using formic acid-sodium citrate solution according to Bhatnagar and Kallen (1974) and Smith et al., (1997). The tissues were dehydrated in graded series of ethanol, cleared in xylene and embedded in paraffin wax. The blocks were sectioned in transverse plane at 6 µm thickness using a rotary microtome. Every tenth section was mounted on glass slides and stained with Haematoxylin and Eosin. The middle sections of the organ were studied with a Hund Wetzlar 600H light microscope with Moticam 1000 digital camera attachment and images captured into a computer. Ocular micrometer, calibrated with a stage micrometer was used to measure the thickness of the sensory and nonsensory epithelium.

RESULTS

Gross Anatomy: The VNO was observed to be paired and oval shaped and located on the ventral part of the nasal cavity, closely associated with the vomer, maxillary and incisive bones (Figures 1, 2 and 3). The rostral and middle segments of the organ were observed to be completely encapsulated by the vomeronasal cartilage, while the caudal extremity of the organ was partially surrounded by a coma-shaped vomeronasal cartilage. The VNO extended from the incisive ducts of the oral cavity to the 1st and sometimes the 2nd premolar tooth. The duct of the VNO was found to be oblong-shaped, though the size and internal contour of the duct varied along its longitudinal axis. The mean palatal length of the VNO was 1.9 cm, while the mean lumen diameter of the duct was 0.5 cm.

Histological Features: The vomeronasal ducts of the middle segment of the VNO were bounded medially and laterally by cartilaginous walls. The medial and lateral walls were observed to be concave and convex respectively. The epithelium lining the medial wall was thicker (50.05 µm) than the lateral wall (40.05 µm).The mean diameter of the lumen of the vomeronasal duct was 530.05 µm and 260.05 µm for the long and short axis of the duct respectively. The medial wall of the vomeronasal duct was lined with olfactory epithelium (vomeronasal sensory epithelium) (Figure 4)**.** This epithelium is comprised of three cell types, namely supporting cells, olfactory cells and basal cells. The supporting cells were elliptical in shape and apico-peripheral in location. The olfactory cells were observed to be bipolar neurons with microvillous apical surface projections. The basal cells were round in shape and located close to the poorly differentiated basement membrane. The lamina propria of the medial wall was very prominent and contained few nerve bundles. Sparse vascularisation and few glandular ducts were observed near the boundary with the lateral wall. The vomeronasal nonsensory epithelium on the lateral wall was lined with ciliated pseudostratified columnar cells with goblet cells and basal cells. Blood vessels and lamina propria were sparsely distributed in the lamina propria (Figure 5).

DISCUSSION

This study describes the existence of a VNO in the juvenile *C. rufilatus*, a model of the primitive bovid ancestors*.* Our results in the juvenile duiker showed that the VNO is a rudimentary structure compared to that of juvenile blind mole rats (Zuri *et al.,* 1998), other rodents (Garrosa *et al.,* 1986; Sangari *et al.,* 2002), neonatal and adult goat (Besoluk *et al.,* 2001; Igbokwe, 2006). There were no apparent morphological and morphometric differences between the male and female animals studied. In the *C. rufilatus* studied, the average palatal length of the VNO was 1.4 cm, while the average transverse diameter of the vomeronasal duct was 0.5 cm and the VNO terminated caudally between the first and second developing premolar teeth. In adult goats, the VNO terminated at the level of the third premolar

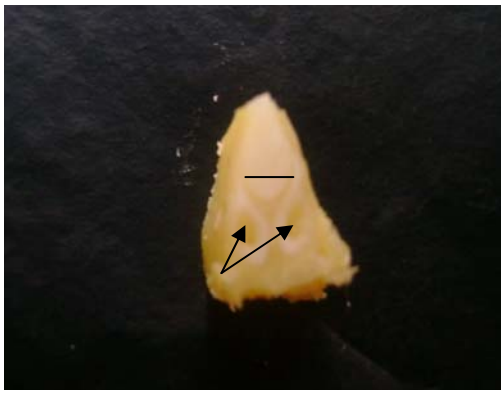

Figure 1: Gross dissections, showing the paired VNO (arrows) and its lumen and nasal septum (bar). H & E ×100

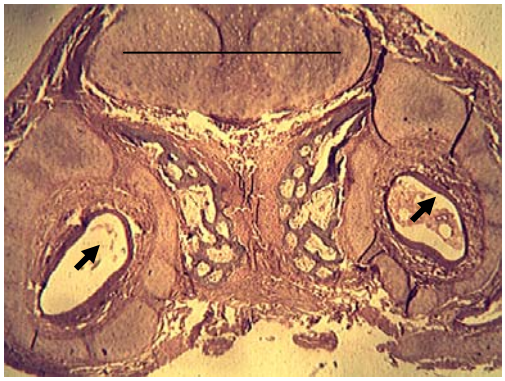

Figure 2: Histological section of the middle segment showing the paired VNO, and surrounding skeletal bones, nasal septum (bar). H & E ×100

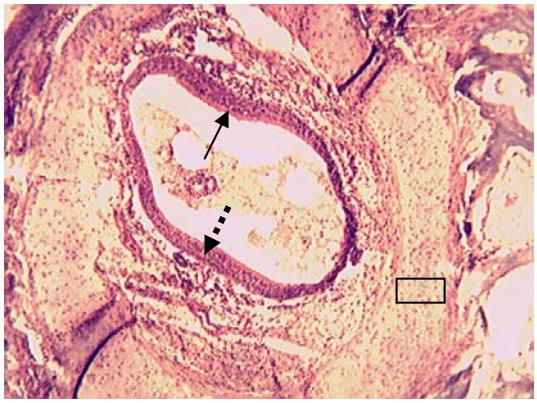

Figure 3: VNO housed by hyaline cartilage (box), vomeronasal sensory epithelium on medial wall (unbroken arrow), 'non sensory' respiratory epithelium on lateral wall (broken arrow): No marked differences in thickness of both epithelia H & E×200

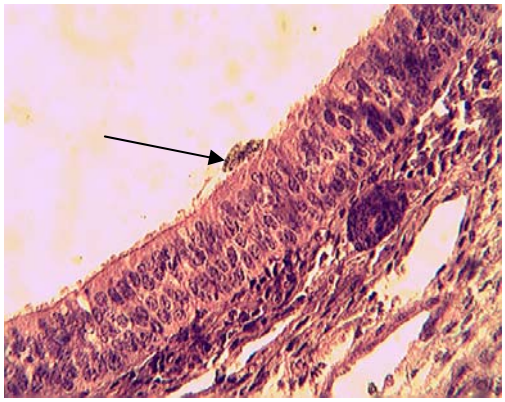

Figure 4: vomeronasal sensory epithelium (pseudostratified columnar) comprising microvillary surfaces(dark arrow), surporting cells (a), bipolar sensory neurons (b), basal cells(c), nerve bundle(n), lamina propria (L). H & E × 400

(Besoluk *et al.,* 2001). The vertical diameter in goats in its central part is 5 mm (Takigami *et al.,* 2000). In adult sheep, the caudal part ends at the first premolar or second premolars (Kratzing, 1971). The vertical diameter of the VNO lumen has a width of 1 cm in its central part (May, 1964). In swine, the VNO length is up to 4 cm; it ends blindly and reaches the second premolar level (Salazar *et al.,* 1997). In cattle, the caudal part of the VNO ends at the level of the first and second premolar (Kumar et al., 1981). The lumen of the ventral part of VNO is not covered with cartilage that constitutes the organ (Kostov, 2007). Jacobs et al., (1981) recorded that the ventral part of the vomeronasal duct in cattle was not covered with the vomeronasal cartilage. However, we observed that the rostral and middle part of the VNO in the duiker was completely enveloped by the vomeronasal cartilage except at its caudal part. Besoluk et al. (2001) reported that only the dorsal part was lacking the vomeronasal cartilage in Angora goats (*Capra hircus*), while, Adams and Wiekamp (1984) reported lack of vomeronasal cartilage in the dorsolateral part of the vomeronasal organ in dog.

There is a wide agreement that the vomeronasal duct is lined by 2 types of epithelium, respiratory and sensory in several mammalian species (Wysocki and Meredith, 1987; Salazar *et al.,* 1995; Doving and Trotier, 1998; Evans, 2003)). This has been demonstrated by several methods (Banister and Dodson, 1992). The vomeronasal organ of the red-flanked duiker is no exception to this rule and our results confirm these findings. We measured the thickness of the vomeronasal sensory epithelium of the medial wall to be 50.05 µm, that of the 'non sensory' respiratory epithelium on the lateral was 40.05 µm. Vaccarezza *et al.* (1981) reported that the vomeronasal sensory epithelium and 'non sensory' epithelium were 140 µm and 30 µm respectively in rats, being a macrosomatic animal. In the dog, Adams and Wiekamp (1984) found that the sensory and 'non sensory' epithelium were 55 – 121 µm and 34 – 72 µm thick respectively.

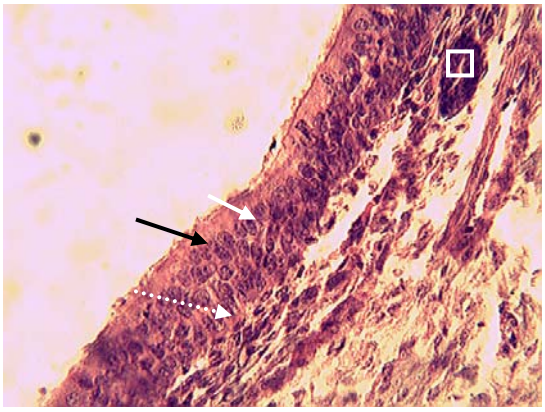

Figure 5: Non sensory respiratory epithelium comprising surporting cells (dark arrow), ciliated columnar cells (white arrow), basal cells (white arrow), glandular ducts (box), H & E× 400

The vomeronasal sensory epithelium of the medial wall is thin, approximately the thickness of the VNO of a 13 day rat embryo (Yoshida *et al.*, 1993) and that of adult male ferret (Weiler *et al.,* 1999). Only about 1-2 rows of sensory bipolar neurons were seen. This was also observed in the rudimentary VNO of ferret. We did not observe intruding capillaries in the sensory epithelium as described for other mammals (Yoshida *et al.,* 1993; Sangari *et al.,* 2002). Intruding capillaries usually reflect a high metabolic activity (Vaccarezza *et al.,* 1981).Their absence suggests low metabolism and is described in species with a thin epithelium in which the VNO seems not to play important role in behaviour. In species with functionally important VNO, capillaries intrude prenatally when VNO has reached certain developmental stage (Breiphol *et al.,* 1981). Taniguchi *et al.* (1992) stated that there are no venous sinuses in the VNO. These have been observed in neonatal goats (Igbokwe, 2006) and by Salazar *et al.* (1997) in adult pigs, cows and horses. Our findings in the Red-flanked duiker did not reveal groups of vessels which can be called venous sinuses. Numerous glandular ducts open at the junction of the lateral and medial walls in the rat (Vaccarezza et al., 1981) and goat (Igbokwe, 2006).This feature was not observed in the juvenile duiker. Duikers are known to exhibit 'flehmen' behaviour (Estes, 1991). Soluble substances may enter the VNO via the incisive duct through either the nasal vestibule or the oral cavity.

C .rufilatus reaches sexual maturity at 9 – 15 months (Kingdon, 1984). It is probable that the full development and functionality of the VNO takes place several months after birth. The VNO may play more important role in territorial marking than reproductive functions. *C. rufilatus* posses the largest preorbital gland of all duiker species used in scent marking between conspecifics.

In conclusion, we hope that this study revealing macroscopically and microscopically the structure and function of the vomeronasal organ in the juvenile Red-flanked duiker (*C. rufilatus*) will shed light on future studies on the reproductive biology and ecology of this endangered specie suitable as microlivestock.

REFERENCES

ADAMS, D. R. and WIEKAMP, M. D. (1984). The canine vomeronasal organ. *Journal of Anatomy,* 138: 771 – 787.

ANSEL, W. F. H. (1971). Order Artiodactyla. Pages 1 – 84. *In:* MEESTER, J. and SETZER, H. W. (Editors). *The Mammals of Africa. An Identification Manual,* Part 15, Smithsonian Institute Press, Washington D.C.

BANNISTER, L. H. and DODSON, H. C. (1992). Endocytic pathways in the olfactory and vomeronasal epithelia of the mouse: Ultrastructure and uptake of tracers. *Microscopic Research Technique,* 23: 128 – 141.

BESOLUK, K., EKEN, E. and BOYDAK, M. (2001). The vomeronasal organ in Angora goats (*Capra hircus*). *Veterinarski ARHIV,* 71(10): 11 – 18.

BHATNAGAR, K. P and KALLEN, F. C. (1974). Morphology of the nasal cavities and associated structures in *Artibeus jamaicensis* and *Myotis lucifugus*. *American Journal of Anatomy,* 139: 167 – 190.

BHATNAGAR, K. P. and SMITH, T. D. (2003)**.** The human vomeronasal organ: An Interpretation of its discovery by Ruysch, Jacobson or Kolliker with English translation of Kolliker (1877). *Anatomical Record,* 270 B: 4 – 15.

BREIPHOL, W., BHATNAGAR, K., BLANK, M. and MENDOZA, A. (1981). Intra-epithelial blood vessels in the vomeronasal neuroepithelium of the rat. A light and electron microscopic study. *Cell Tissue Research,* 215: 465 – 473.

DURSUN, N. (1994). *Veterinary Anatomy 11.* Medisan Publishing House, Ankara.

DOVING, B. K. and TROTIER, D. (1998). Structure and function of the vomeronasal organ (Review). *Journal of Experimental Biology,* 201: 2913 – 2925.

DULAC, C and AXEL, R. (1998). Expression of candidate pheromone receptor genes in vomeronasal neurons. *Chemical Senses,* 23: 467 – 475.

ESTES, R. D. (1974). Social organization of the African bovidae. Pages 1 – 511. *In:* GEIST,

V. and WALTHER, F. (Editors). *The behaviour of ungulates and its relationship to management.* Volume 1. International Union of Conservation, National Publication Service.

ESTES, R. D. (1991). *The behaviour guide to African mammals (including hoofed mammals, carnivores and primates).* University of California Press, California.

EVANS, C. (2003). *Vomeronasal chemoreception in vertebrates: a study of the second nose.* Glasgow Caledonian University Press, United Kingdom.

FISCHER, F. and LINSENMAIR, K. E. (2001). Decreases in ungulate population densities: Examples from the Comoe National Park, Ivory Coast. *Biological Conservation,* 101(2): 131 – 135.

GARROSA, M., COCA, S. and MORA, O. (1986). Histological development of the vomeronasal complex in the prenatal and postnatal rat. *Acta Otolaryngology,* 102: 291 – 301.

GAUTIER-HION, A., EMMONS, L. H. and DUBOST, G. (1980). A comparison of diets of three groups of primary consumers of Gabon (primates, squirrels and ruminants). *Oceologia,* 45**:** 182 – 189.

HALPERN, M. and MARTINEZ-MARCOS, A. (2003). Structure and function of the vomeronasal system: an update *Progress in Neurobiology,* 70: 245 – 318.

IGBOKWE, C. O. (2006). *Morphological studies on the development of vomeronasal organ in the Red Sokoto goat (Capra hircus).* M.Sc Dissertation, University of Nigeria, Nsukka.

JACOBS, V. L., SIS, R. F., CHENOWETH, P. J., KLEMM, W. R. and SHERRY, C. J. (1981)**.** Structure of the bovine vomeronasal complex and its relationship to the palate and tongue manipulation. *Acta Anatomica,* 110**:** 48 – 58.

KOSTOV, D. (2007). Vomeronasal organ in domestic animals. *Bulgarian Journal of Veterinary Medicine,* 10: 53 - 57.

KINGDON, J. (1984)**.** *East African mammals. An atlas of evolution in Africa.* University of Chicago Press, Chicago.

KRANZ, K. R. and LUMPKIN, S. (1982). Notes on the yellow backed duiker *Cephalophus* s*ylvicultor* in captivity with comments on its natural history. *International Zoo Year Book*, 22: 232 – 240.

KRATZING, J. (1971). The structure of the vomeronasal organ in sheep. *Journal of Anatomy*, 108: 247 – 260.

KUMAR, S., DHINRA, L. D. and SINGH, Y. J. (1981). Anatomy of vomeronasal organ of buffalo (*Bubalis bubalis*). *Journal of Anatomical Society of India,* 30: 63 – 66.

MAY, N. D. S. (1964). The anatomy of the sheep. Queensland University Press, Brisbane.

NEWING, H. (2001). Bushmeat hunting and management implications on duiker ecology and interspecific competition. *Biodiversity and Conservation,* 10(1): 99 – 108.

SALAZAR, I., QUINTEIRO, P. and CIFUENTES, J. (1995). Comparative anatomy of the vomeronasal cartilage in mammals- mink, cat, dog, pig, cow and horse. *Annals of Anatomy,* 177: 475 – 481.

SALAZAR, I., SANCHEZ-QUINTEIRO, P. and CIFUENTES, J. M. (1997). The soft tissue components of the vomeronasal organ in pigs, cows and horses. *Anatomy Histology and Embryologia,* 26: 179 – 186.

SANGARI, S. K., SENGUPTA, P., PRADHAN, S and KHATRI, K. (2002). Intraepithelial capillaries in the neuroepithelium of the vomeronasal organ in adult guinea pig. *Journal of Anatomical Society of India,* 51(1): 50 – 52.

SMITH, T. O., SIEGEL, M, I., MOONEY, M. O., BURDI.A A. R., BURROWS. A. M and TODHUNTER, J. S. (1997). Prenatal growth of the human vomeronasal organ. *Anatomical Record,* 248: 447 – 455.

SOLER, M. V. C. and SUBURO, A. M. (1998). Innervation of blood vessels in vomeronasal complex of rat. *Brain Research,* 811: 47 – 56.

TAKIGAMI, S., MORI, Y. and ICHIKAWA, M. (2000). Projection pattern of vomeronasal neurons to the accessory olfactory bulb in goats. *Chemical Senses,* 25: 387 – 393.

TANIGUCHI, K., MATSUSAKI, Y., OGAWA, K. and SAITO, T. (1992). Fine structure of the vomeronasal organ in the common marmoset (*Callithrix jaacchus*). *Folia Primatologia,* 59: 176 – 189.

VACCAREZZA, O. L., SEPICH, L. N. and TRAMEZZANI, J. H.(1981). The vomeronasal organ of the rat. *Journal of Anatomy*, 132: 165 – 167.

WEILER, E., APFELBACH, R. and FARBMAN, A. I. (1999). The vomeronasal organ of the male ferret. *Chemical Senses,* 24:127 – 135.

WYSOCKI, C. J and MEREDITH, M. (1987). The vomeronasal system. Pages 125 – 150. *In:* FINGER, T. and SILVER, W. (Editors). *The neurobiology of taste and smell,* Wiley. New York.

WYSOCKI, C. J., KRUCZEK, N., WYSOCKI, L. M. and LEPRI, L. J. (1991). Activation of reproduction in multiparous and primiparous voles blocked is by vomeronasal organ removal. *Biology of Reproduction,* 45: 611 – 616.

YOSHIDA, J., KIMURA, J., TSUKISE, A. and OKANO, M. (1993). Developmental study on the vomeronasal organ in the rat fetus. *Journal of Reproduction and Development,* 39: 47 – 54.

ZURI, I., FISHELSON, L. and TERKEL, J. (1998). Morphology of the nasal cavity and vomeronasal organ in juvenile and adult blind mole rats (*Spalax ehrenbergi*). *Anatomical Record,* 251: 460 – 471.