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Gross Morphological and Osteometrical Evaluation of the Lumbosacral Vertebrae and Pelvic Bones of New Zea Land Rabbit (*OryctolagusCuniculus*) and their Clinical Applications

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ABSTRACT
This study examined gross morphology and osteometry of vertebrae [lumbar (L), sacrum (S) and coccygeal (C)] and pelvic bones of male New Zealand white rabbits *(Oryctolaguscuniculus)* and their clinical relevance. Ten healthy male rabbits weighing between 2.8-3.2kgwere used. The animals were sacrificed and their bones processed using standard methods. Gross morphological features of the vertebrae and pelvic bones were described and standard osteometric variables of lumbar, sacrum and coccygeal vertebrae and pelvic bone also determined. Results showed the lumbar vertebrae to be seven, with spinous and transverse processes that decreased in craniocaudal manner. The sacrum was fused, with the first three cranial sets bearing conspicuous spinous process oriented in different directions. The pelvic bone was rectangular consisting of ilium, ischium and paired pubic bones united by a symphyseal joint. The coccygeal bones numbered up to sixteen. Remarkable osteometric findings include: significantly decrease ($p<0.005$) vertebral body length in L_6 and L_7; increased transverse process of $L_{4,}$ L_5 and L_6;reduced spinous process length in L_{1-} L_3;decreased coccygeal vertebral body length in C_{13-} C_{16} and increased coccygeal vertebral body diameter in C_1. Osteometric variables on pelvic bone were not significantly different ($p<0.005$). The study demonstrates morphological and osteometric variables of the vertebrae; between last lumbar and first sacral (lumbosacral joint) and junction of *conusmedullaris*, and may form the basis for clinical, surgical and anatomical maneuvers in this animal, especially to access the subarachnoid space for cerebrospinal fluid or injecting local anesthesia and therapeutic substances into the epidural space.

Keywords: gross morphology, osteometry, lumbosacral, pelvic bone, *Oryctolaguscuniculus*

INTRODUCTION
Attention has been turned to New Zealand rabbit (White breed) which belongs to the family *Leporidae* and order *Lagomorpha*[1] for use as experimental animals in the laboratory of various biomedical researchers[2]. Also, it is a good source of meat protein and potential source of fur, hence the relevance of the osteomorphology of this animal as a basic tool in scientific researches[3, 4]is not out of place. Recently, this animal is being used as companion animal, and now the third most popular mammalian pet in the United Kingdom after dogs and cats [5, 6]. Although in Africa, particularly in Nigeria, it is bred mainly for meat however some people now rare rabbit as pet animals and this has led to a projectile increase in the level of veterinary care needed by owners of this animal[6, 7]. However, domestic rabbit is easy to keep because of its easy nature with respect to housing, feeding and rapid fecundity even with New Zealand[8].

The New Zealand falls within the range of medium to large breed of rabbit that appeared uniform in body proportion and stance[4]. Their length ranges from 25-50cm and body weight of 400-3000g for medium breed while large breed weighs 3500-5000g[1].

Although there are various investigations on the whole skeletal system of mammals including guinea pig and rat[9], the mink[10], hedgehog[11] and mole rat[12], little is known of the rabbit. On the skeletal systems of domestic rabbit, Brewer[13] have reported the skeleton to be relatively fragile and represents about 8% of the total body weight; Farag*et al.,*[8] studied the skull with special reference to the hyoid apparatus while Ajayi*et al.,*[4] studied the osteomorphometry of the bones of the thigh, crus and foot. Less emphasis has been placed on the osteology of the spinal column, especially the lumbosacral and coccygeal vertebrae which houses segments of the spinal cord, a component of the central nervous system and the pelvic bone which protects the reproductive organs of New Zealand rabbit. Therefore, this study seeks to investigate the possibilities.

MATERIALS AND METHODS

Animal care: The care and handling of all animals used for the research conformed to the rules and guidelines issued by the Faculty of Veterinary Medicine, University of Benin, Nigeria.

Animals and experimental model: The present study was conducted on the lumbar, coccygeal vertebrae and pelvic bones of ten healthy male adults *Oryctolaguscuniculus* weighing between 2.8-3.2kg. The animals were obtained from the local markets in Benin and transported to the Department of Veterinary Anatomy, University of Benin. They were housed in a clean cage, and fed with formulated pelleted feed with portable water for seven days in the Animal Research House Unit of the Department to acclimatize the animals. All animals used were then examined for absence of skeletal deformities before use. Afterwards, the rabbits were given a lethal dose of ketamine (100mg/kg) and xylazine (10mg/kg) combination[14]. They were then decapitated at the atlanto-occipital joint without cutting the bones. The rabbits were macerated with scalpel knife, removing as much flesh as possible so that the *conusmedullaris* was seen and observed at the lumbosacral region dorsal to the pelvic bone. The carcasses were then soaked in water overnight for further softening of the tissues attached to the bones. The used water was discarded while the carcasses were air dried then soaked in water again overnight. After 24 hours, the carcasses were transferred into water containing sodium hypochlorite and hydrogen peroxide to bleach the bones and soften the connective tissues attached to the bones. Bones were then rinse in clean water and air dried for gross anatomic and morphometric studies[15]. All measurement of the bones was carried out using digital Vernier caliper, protractors, set square and divider. The following parameters were evaluated on the lumbar, sacrum, coccygeal and pelvic bones:

LVBL (Length of body of lumber vertebrae): This is the distance between the cranial and caudal aspect of the body of the lumber vertebrae

LVTPL (length of transverse process of lumber vertebrae): This is measured from body to the tip of the transverse process.

LVSPL (Spinous process length of lumber vertebrae): This determined as distance between the origin of the spinous process to its tip.

ISD (Interacuate space depth): This is the height of the interacuate space measured from the highest point of the space to the bottom.

ISDT (Interacuate space diameter): This is the distance measured between the widest point of the space

SWD (Sacral Wing Diameter): The distance measured at the widest points on the bone

TSL (Total sacral length): The distance measured between the most cranial and most caudal aspects of the sacrum

IL (Iliac length): The whole length of the ilium measured between the two greatest points on the bone

OFL (Obturator foramen length): The length measured between the two widest points on the foramen

PCRL (Pubis cranial ramus length): The whole length of the cranial ramus of the pubis measured from the measured from the cranial aspect of the pubic symphysis to the point of articulation with the acetabulum

PCdRL (Pubis caudal ramus length): The whole length of the caudal ramus of the pubis measured from the cranial aspect of the pubic symphysis to the most caudal aspect of the bone

WLI (Whole length of ischium): The whole length of ischium measured from the center of acetabulum to the most caudal aspect of the bone

PWL (Pelvic whole length): The distance between the most cranial and most caudal aspects of the pelvic bone.

PD (Pelvic diameter): The distance measured between the widest points on the acetabula across the pelvic bone

PID (Pelvic inlet diameter): This is the widest distance measured between the brim of the ilium

PITD (Pelvic inlet transverse diameter): This is the widest distance measured at the region of the acetabulum on the inner aspect of the pelvis.

PSL (Pubis symphyseal length): The whole length of the pubis symphysis, measured from the cranial to the caudal aspect.

CVBL (Coccygeal vertebral body length): The longest distance measured on the bone

CVBD (Coccygeal vertebral body diameter): The widest distance measured on the body of the bone

Statistical analysis: Data obtained were expressed as mean ± SE. Student's t-test was used to evaluate significant difference between the osteometric variables of the paired structures on the pelvic bone. One-way ANOVA was used to determine significant differences in other morphometric parameters on the lumbar, sacrum and coccygeal vertebrae. Tukey Post-hoc test was used for multiple comparisons. The values of $p<0.05$ were considered significant.

RESULTS

Non-metric observations: The total number of the vertebral column of the rabbits studied from lumber to the coccygeal is 17. There were seven (**7**) lumbar, three (3) sacral and fifteen to sixteen (15-16) coccygeal bones represented as L_{1-7} S_{1-3} Co_{15-16}. The pelvic bone appears similar to other mammalian species.

Lumbar: The detailed lumbar appearance is shown in Figure 1. The lumbar vertebrae are 7 in numbers with the last lumbar vertebra (L_7) having a relatively short body compare to others. The transverse processes of all the lumbar vertebrae sweep cranio-ventrally at angle 50^0. The spinousprocess of the seventh lumbar vertebra (L_7) projects beyond the cranial articular process that extends from the lateral aspects of the vertebra arch, just dorsal to the transverse process, as it is observed for the sixth lumbar vertebra (L_6). For the fourth and fifth lumbar vertebrae ($L_4$and L_5), their spinous and cranial articular processes extend from the vertebral arch to reach the same level. From the first to the third lumbar vertebrae ($L_1$to L_3), the cranial articular processes projects beyond the spinous processes that has reduced progressively. Therefore the reduction in the spinous processes height is caudo-cranial. The cranial articular process also follows the same trend except that of the seventh with a smaller height compare to the sixth. The spinous processes of the lumbar vertebral column are oriented cranially at angle 50° to roof over the interacuate foramina.

Sacrum: The complete sacrum morphological appearance is captured in Figures 1-4. The sacrum is embedded in the wings of the ilium but their spinous processes project beyond the wings. The sacral vertebrae are not fused, leaving them with no medial and lateral sacral crest. The spinous process of the first sacral vertebra (**S_1**) is oriented at angle 90° while that of second (**S_2**) and third (**S_3**) are oriented at 60° and 40° respectively against the column (Fig. 4).

Coccygeal: The full coccygeal morphological details are revealed in Figure 1. The coccygeal vertebrae are typical of mammalian caudal vertebrae, numbering between 15-16, the transverse processes reducing as the coccyx transform into tapering columns of bones. The transverse process was completely lost from the 9th coccygeal bone. The last coccygeal bone was half the length of the 15th coccygeal bone. The spinous process was only obvious for the 1st-3rd coccyx.

Pelvic: The morphological details of pelvic bone are shown in Figures 1-5. The pelvic bone is rectangular in outline. The sacral promontory of the third sacral vertebra is directly dorsal to the pecten. The tuber coxa of the ilium is reduced to a small elevation of bone that is medially placed on the iliac wing. The tuber sacral and the iliac crest blend in with the tuber coxa to give the cranial aspect of the wing an oval appearance as it extends to shelter the last lumber vertebra. The body of the ilium is cylindrical but slightly indented medially so that the grove is continuous with the arcuate line. Cranial to it is the acetabulum which is considerably wide and deep beyond the lunar surface for the round ligament to be adequately lodged and connected to the fovea capitis of the femur. The two pubic bones are connected by symphyseal joint (not synotosis). The ischiatictuberosities are prismatic in shape with a deep ischiatic arch separating the two.

Gross Morphometric Parameters (Metric observations): Gross morphometric parameters evaluated in this study are presented in Tables 1-3.
Lumbar Vertebral Body Length (LVBL): The LVBL was significantly reduced ($p<0.05$) in the L_6 and L_7 compared to other lumbar vertebrae. Also, there was an insignificant increase ($p>0.05$) in LVBL of L_4 relative to others (Table 1).
Lumbar Vertebral Transverse Process Length (LVTPL): The LVTPL values were significantly increased ($p<0.05$) in the $L_{4,}$ L_5 and L_6 compared to other lumbar vertebrae. However, the values were markedly decreased in L_1 and L_2 vertebrae relative to others (Table 1).
Lumbar Vertebral Spinous Process Length (LVSPL): The LVSPL values were significantly reduced ($p<0.05$) inL_{1-} L_3 vertebrae compared to others. In addition, there was no significant difference ($p>0.05$) in the LVSPL values of L_{4-} L_7(Table 1).

Interacuate Space Depth (ISD): There was a significantly decreased ($p<0.05$) ISD from the interacuate space L_6-$L_{7\ to}$ S_3-C_1 compared to others (Table 2).
Interacuate Space Diameter (ISDT): The ISDT was significantly reduced ($p<0.05$) in the interacuate spaceT_{13}-L_1 relative to others (Table 2).
Sacral Wing Diameter (SWD): There was no significant difference ($p>0.050$) in the right and left sacral wing diameters (Table 3).
Total sacral length (TSL): The mean value of the total sacral length was 9.56±0.41 (Table 3).

Illiac length (IL), Obturator foramen length (OFL), Pubis cranial ramus length (PCRL), Pubis caudal ramus length (PCdRL) and Whole length of ischium (WLI): The above highlighted parameters were not significantly different ($p>0.050$) between the right and left constituent bones of the pelvis of New Zealand rabbit (Table 3).

Pelvic whole length (PWL) Pelvic diameter (PD), Pelvic inlet diameter (PID), Pelvic inlet transverse diameter (PITD) and Pubis symphyseal length (PSL) : The mean morphometric values of the parameters stated above are; 31.00±1.00 mm, 73.00±2.00 mm, 16.67±0.33 mm, 16.67±0.33 mm and 18.00±1.00 mm respectively (Table 3).
Coccygeal Vertebral Body Length (CVBL): There was no significant difference ($p>0.05$) in the CVBL of coccygeal vertebrae 1-12. The CVBL was significantly reduced ($p<0.05$) in coccygeal vertebrae 13-16 compared to others. **Coccygeal Vertebral Body Diameter (CVBD)**
The CVBD was significantly longer ($p<0.05$) in coccygeal vertebrae (CV) 1compared to others. The CVBD consistently displayed a non-significant trend ($p>0.05$) from coccygeal vertebrae 2-13 as well as from CV 14-16. The CVBD was significantly reduced in CV 14-16 when compared to others.

Table 1: Mean values of lumbar bone morphomertic parameters

LUMBAR VERTEBRA	LVBL (mm)	LVTPL (mm)	LVSPL (mm)
L_1	14.33 ± 0.66^a	8.66 ± 0.33^c	4.00 ± 0.90^b
L_2	14.67 ± 0.33^a	8.66 ± 0.31^c	4.00 ± 0.92^b
L_3	14.67 ± 0.33^a	14.33 ± 0.98^b	5.20 ± 0.20^b
L_4	15.33 ± 0.33^a	18.56 ± 0.74^a	8.22 ± 0.40^a
L_5	14.33 ± 0.33^a	18.56 ± 0.54^a	8.22 ± 0.20^a
L_6	13.00 ± 0.58^a	18.56 ± 0.74^a	8.22 ± 0.40^a
L_7	11.33 ± 0.33^b	14.50 ± 0.50^b	8.67 ± 0.33^a

Values with different alphabet superscripts in the row are significantly different
LVBL (Lumbar Vertebral Body Length), LVTPL (Lumbar Vertebral Transverse Process Length), LVSPL (Lumbar Vertebral Spinous Process Length)

Table 2: Mean values of interacuate space diameter and depth between the 13th thoracic (T_{13}) and the 1st lumbar bone (L_1) down to between the 3rd sacral (S_3) and the 1st coccygeal bone(C_1)

INTERACUATE SPACE (IS)	ISD (mm)	ISDT (mm)
T_{13} -L_1	6.33 ± 0.33^a	1.33 ± 0.33^d
L_1 -L_2	6.33 ± 0.33^a	3.00 ± 0.58^c
L_2–L_3	5.76 ± 0.32^a	3.00 ± 0.58^c
L_3-L_4	5.33 ± 0.33^a	3.67 ± 0.33^b
L_4-L_5	6.67 ± 1.20^a	4.33 ± 0.33^b
L_5-L_6	5.66 ± 0.33^a	5.33 ± 0.30^a
L_6-L_7	4.50 ± 0.51^b	6.00 ± 1.00^a
L_7-S_1	4.33 ± 0.30^b	5.67 ± 0.33^a
S_1-S_2	3.68 ± 0.33^b	3.33 ± 0.33^b
S_2-S_3	2.67 ± 0.10^c	2.67 ± 0.66^c
S_3-C_1	3.00 ± 0.58^b	2.67 ± 0.33^c

Values with different alphabet superscripts in the row are significantly different
ISD (Interacuate Space Depth), ISDT (Interacuate Space Diameter)

Table 3: The linear morphometric dimensions on the pelvic and sacral bones of New Zea Land rabbit (*Oryctolaguscuniculus*).

Bone	Morphometric Parameters (mm)	Right	Left	p-value
Pelvic bone	IL	39.33±0.66	39.00±1.00	0.1030
	OFL	14.67± 0.33	15.33±0.66	0.2400
	PCRL	17.67±0.33	17.00±0.57	0.1100
	PCdRL	17.33±0.33	17.33±0.33	0.4210
	WLI	31.00±1.00	31.00±0.60	0.2201
Sacral bone	SWD	16.00±1.52	15.00±1.55	0.5030
	Other Parameters			
Pelvic bone	PWL	31.00±1.00		
	PD	73.00±2.00		
	PEID	16.67±0.33		
	PITD	16.67±0.33		
	PSL	18.00±1.00		
Sacral bone	TSL	9.56±0.41		

IL - Iliac length, OFL, OFL - Obturator foramen length, PWL - Pelvic whole length, PD - Pelvic diameter, PID: Pelvic inlet diameter, PITD: Pelvic inlet transverse diameter, PSL - Pubis symphyseal length, PCRL - Pubis cranial ramus length, PCdRL- Pubis caudal ramus length, WLI - Whole length of ischium, SWD: Sacral wing diameter, TSL: Total sacral vertebrae length.

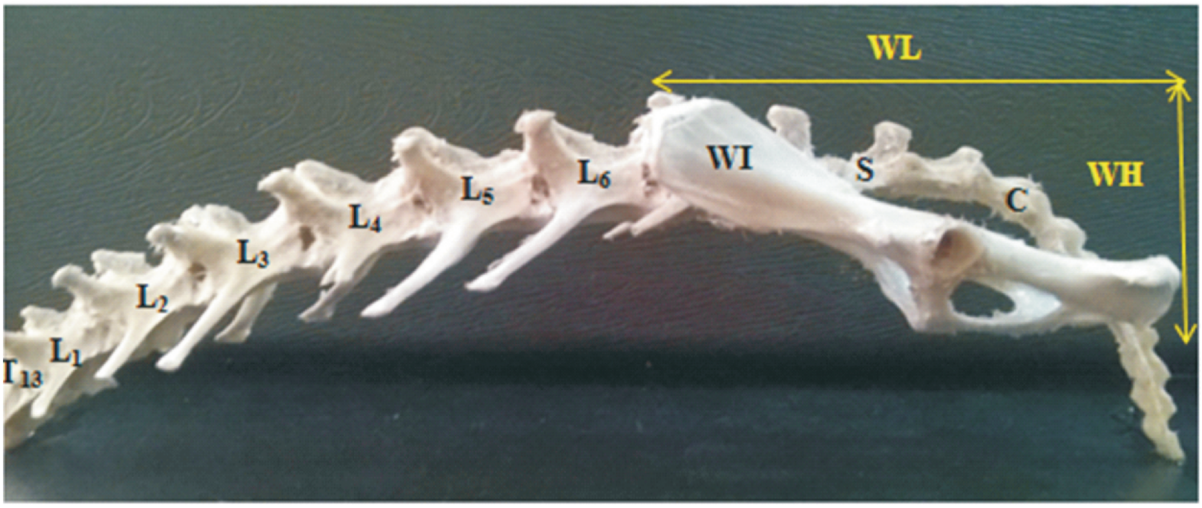

Figure 1: Lateral view of the caudal aspects of the vertebrae column of *Oryctolaguscuniculus* showing the last thoracic (T13), lumber (L_{1-} L_6), sacral (S) and coccygeal (C) vertebrae. Note that the seventh lumber vertebra is buried in the spatula-shaped wing (WI) of the ilium, and its spinous process projects slightly above the iliac crest. The maximum length (WL) and height (WH) of the pelvic bone.

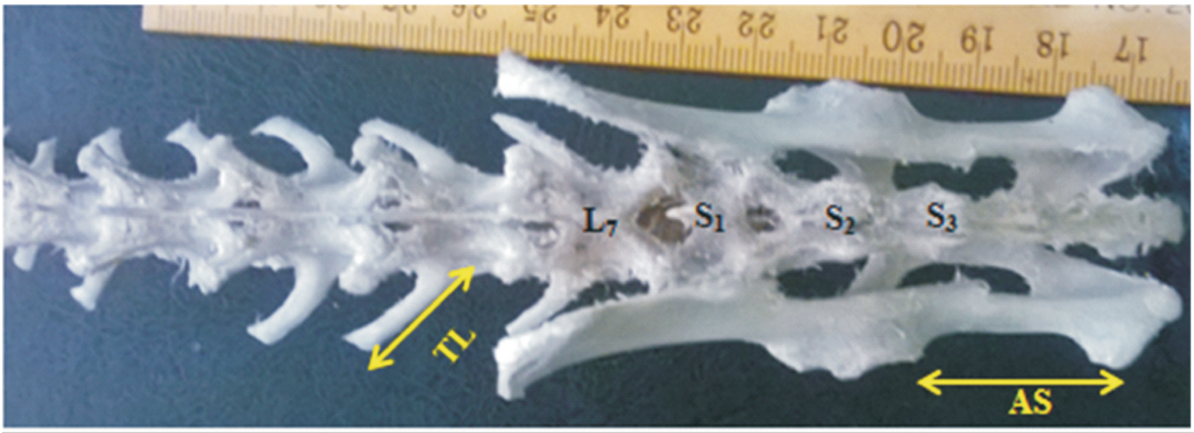

Figure 2: Dorsal view of the pelvic bone and caudal aspects of the vertebral column, showing the seventh lumber (L_7) and the first to third sacral ($S_{1-}S_3$) vertebrae spinous processes and the measured parameters; the transverse process length (TL) and the distance between the acetabulum and the ischial tubercle (AS)

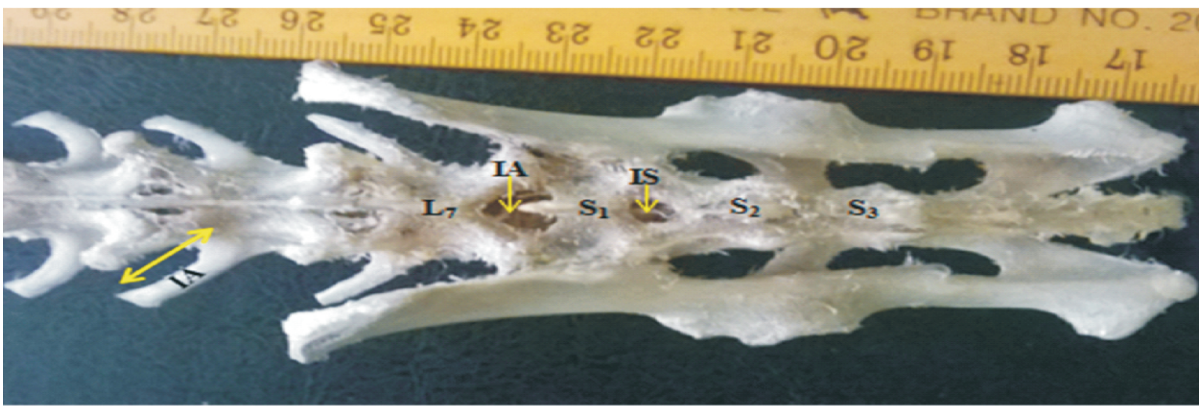

Figure 3: Dorsal view of the pelvic bone and caudal aspects of the vertebral column, showing theinteracuatespace (IA) between the seventh lumber (L_7) and the first sacral (S_1); and the space (IS) between first sacral (S_1) and second sacral (S_2) vertebrae. Note the relative wideness accessibility of IA and IS (yellow arrow) and without their spinous processes hanging over the spaces between them.

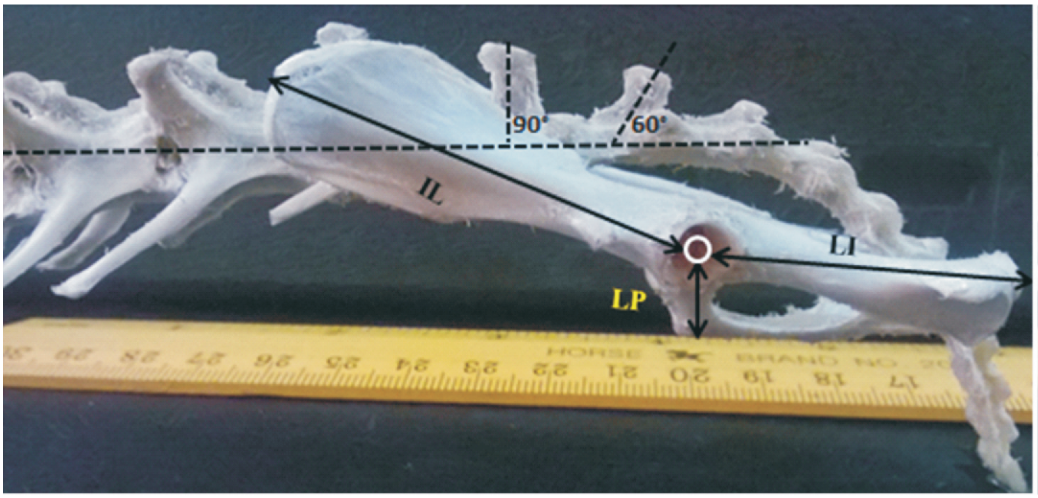

Figure 4: Lateral view of the pelvic bone and caudal aspects of the vertebral column, showing the measured parameters; the maximum length of the ilium (IL), ischium (LI), cranial ramus of the pubic bone (LP) and the angles the sacral spinous processes make with the long axis of the vertebral column.

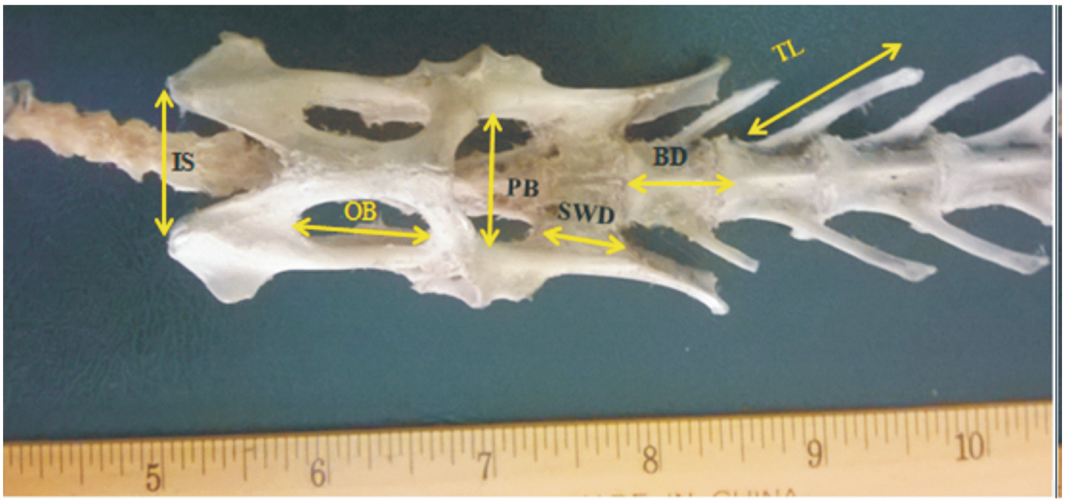

Figure 5: Ventral view of the pelvic bone and part of the lumber vertebral column, the sacral and coccygeal vertebrae with the various parameters measured;the diameter between the ischial tubercle (IS), the maximum diameter of the obturator foramen (OB), the maximum length of sacral wing (SWD), the transverse diameter of the pelvic inlet (PB), the maximum length of the vertebral body (BD) and the transverse length (TL).

DISCUSSION

The vertebral column has the reported to be of great importance in maintenance of posture, flexibility (especially at the caudal vertebra) and transmission of force during jumping, running or walking[16]. The results of this report showed the lumber, sacral and coccygeal vertebrae to be L_7, S_3 and $Co_{15\text{-}16}$ respectively. This is different from earlier reports of Oludeet *al.,*[17] in African giant rat (AGR); a rodent with L_6, S_5 and $Co_{31\text{-}36}$; Ozkan[12] in mole rat with L_6, S_4 and Co_5 and Lessertiseur and Saban [18] in *Spalacidae* with L_6, S_4, and Co_6. In a broader sense, Colvule and Bassert[19] gave L_7, S_3 and $C_{5\text{-}23}$ in cat; L_6, S_5 and $Co_{18\text{-}20}$ in cattle; L_7, S_3 and $Co_{20\text{-}23}$ in dogs; L_7, S_5 and $Co_{15\text{-}21}$ in horse. Our result of L_7 is consistent with most other species.

Concerning the lumber vertebra, this present study showed that the last lumber (L_7) has a relatively short body compare to $L_{1\text{-}6}$. Oludeet *al.,*[17] recently reported that the lumber vertebra of AGR appeared uniform in length. Also, Liebich and Konig[20] reported that the lumber vertebra of mammals have a more uniform shape of the bodies. However, the decrease diameter of the lumber vertebra bodies caudally in our report agrees with the report of Liebich and Konig [20]. Again, the present study showed that the transverse processes of the lumber vertebra of New Zealand rabbits sweep cranio-ventrally similar to reports of Dyce *et al*.,[21] but at angle 50^0. This is contrary to reports of Oludeet *al*.,[17] in AGR. In their report, the transverse processes of the lumber vertebrae are directed craniolaterally. Liebich and Konig[20], on the other hand, reported long, flattened and laterally projected transverse processes of the lumber vertebra in mammals.

Although Liebich and Konig[20], reported that the spinous process of lumber vertebra is usually about equal height and directed cranially, Oludeet al.,[17]reported increase in size of the spinous process caudally. They[17] also showed that the spinous processes become more erect in that order. Interestingly, the present study showed that the spinous processes of the lumber vertebra of New Zealand rabbits used are reduced in height caudo-cranially and cranially oriented at angle 50^0 to roof over the interarcuate foramina. This finding would probably support the reason why local injections in the area will be made with less difficulty.

The number of sacral vertebrae of New Zealand as reported in this findings is three (3) corroborating reports of 3-5 in mammals[20, 21] and recent report of Oludeet al.,[17]in AGR. Whereas earlier reports showed that these bones were fused together[17, 23, 24], we report that the sacral vertebrae are not fused; having no medial and lateral sacral crest. This will probably be the reason for arch shape posture of the rabbit sometimes. Our report showed that the first sacral vertebra is orientated at an angle of 90^0 while that of the second and third are orientated at 60^0 and 40^0 respectively against the vertebral column. This is unique about this animal. Moreover, since the interarcuate spaces in the lumber and sacral segments of their spinal column are small and the spinous processes project over the spaces making them very difficult for injection and collection of fluid; the lumbosacral junction and the junction between the first and second sacrals with 90^0 angle orientation and distance of 5mm and 3mm respectively and showing no vertebral spinous process obstruction are appropriate for injection and collection of fluid.

The baseline metric data generated from the lumbar, sacral and coccygeal vertebrae of New Zealand rabbits used in this work are unique and novel and will form basis for clinical, surgical and anatomical maneuvers.

CONCLUSION

As a basis for further related studies, it is suggested that CSF collection and drug administration should be carried out at the lumbosacral junction, between the seventh lumber vertebra and the first sacral vertebra because the spinal cord tapers to the *conusmedullaris* at that junction, and the relatively large diameter, with the spinous process of the first sacral vertebra oriented at angle 90^0 to the column. Also, for ease of administration of drug, this can be carried out between the first and second sacral vertebrae because their spinous processes give room for the procedure.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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