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3rd International Conference on Avian heRpetological and Exotic mammal medicine

PROCEEDINGS

ORGANIZED BY

European Association of Avian Veterinarians

Association of Exotic Mammal Veterinarians

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European College of Zoological Medicine

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MAPS

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28th March 2017

Scientific sessions herpetology

Scientific session HERPETOLOGY - **Reproduction**

ULTRASOUND SEX DETERMINATION IN SNAKES: CORRELATION BETWEEN HEMIPENES OR HEMICLITORIDS AND BODY MORPHOLOGICAL PARAMETERS

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ABSTRACT

In practice, snakes' sex is mostly determined using invasive techniques such as probing, popping or endoscopy. Ultrasonography has been used as a high sensitivity and non invasive technique in snake's sex determination since 2009, but few studies are still available and none of them ever considered the dimensions of copulatory organs or tried to correlate them to the morphometric parameters of snakes.

From August to October 2016, fifty snakes of the *Pythonidae*, *Boidae* and *Colubridae* families underwent the ultrasonographic examination of hemipenes and hemiclitoris in order to confirm their sex. Hemipenes and hemiclitoris (right and left) length, height and width were ultrasonographically measured in longitudinal and transverse scans of the cloacal region. At the same time, the body length, the tail length, the head length and width and the diameter of the cloacal segment of each snake was measured and recorded on a worksheet. The procedures never required anesthesia or sedation of the snakes, which were only manually restrained.

We confirm that ultrasonography is a sensitive, safe and harmless technique for snakes' sex determination. In addition, we found statistically significant correlations between hemipenes and morphometric parameters, but no correlation was found involving the hemiclitoris.

1. INTRODUCTION

As snakes do not show any fair sexual dimorphism (except for few species), the application of sex determination techniques is required.

Even though small physical variations exist between males and females, like size difference in big constrictors[1] or size of the cloacal spurs,[2] these are only statistic data, not applicable to an individual.

A very common technique between *Python regius* breeders is the evaluation of the length of the tail, which is longer in males. This method has no scientific fundament.[3]

Another well known technique is the use of an atraumatic probe, inserted the in the invaginated hemipenis or hemiclitoris. It's a simple repeatable and cheap method,[2] but the risk of injuring the copulatory organ and perforate the hemiclitoris and the tail,[4] or to vehicle serious infections with severe consequences is described.

On the opposite, the probe could be inserted partially, due to the presence of dense secretion and give wrong results.[2]

A widely used technique (especially for juvenile Colubridae) is the manual eversion of hemipenis. With finger pressure, the operator produces the prolapse of the organs, making them explorable. The disadvantage is the probability of traumatism and fractures of the spine and the ribs. It is also possible to fail seeing the hemipenis.[2]

The "saline injection", makes the hemipenis pro-

lapse, using the hydrostatic pressure of a sterile saline solution injected in the tail of the snake.
This gives a better vision of hemipenis and hemiclitoris. Unfortunately this method has also its troubles: injecting the liquid into the hemipenis could cause severe injuries.
Both eversion techniques are not applicable to large snakes, due to the opposition of the hemipenis retractor muscle.[2]
The use of a cystoscope instead of the probe gives also good results in clinical practice; however, they have the same invasiveness.
Endoscopy has been used to visualize the gonads, after a surgical preparation of a coelomatic access. It is a procedure with all the risks of a surgical anesthesia and with high cost due to the instrumentation.[2]
Ultrasonography has been considered a good method to observe the reproductive system of snakes as it helps to identify gonads modifications during the reproductive cycle.
In females the ovary could be detected dorsally or laterally to the kidney (due to the mesovary laxity) and the follicles can be easily seen in their maturation stages.[2]
Oviduct can also be seen in every phase of the reproductive cycle.
In males, testes can be identified cranially to the kidneys.[5]
The ultrasonographic appearance of hemipenis has been described in literature.[5,6]

2. MATERIALS AND METHODS

For this study, we used a portable ultrasound machine Esaote® MyLab Five, equipped with a linear multifrequency 8-12 MHz probe.
All the snakes examined were propriety of the reptilarium section of "Zoo Safari Park", Pombia (NO), of the wildlife park "Le Cornelle", Valbrembo (BG), of "Oasi di Sant'Alessio", Sant'Alessio (PV) and of the Natural Science Gallery and herpetological Centre "Reptiland", Riva del Garda (TN).

2.1 Observational study

This study comprehended snakes belonging only to *Boidae*, *Pythonidae* and *Colubridae* families. Animals with evident disease were excluded by this study.
Every specimen has been taken from its terrarium and physically restrained by a keeper and/or a proficient veterinary, in order to limit stress. No pharmacological restrain has been used.

2.2 Measurement

Keeping the animal in dorsal decubitus, an operator applied ultrasound gel on the analyzed body part and took three scans.
In the longitudinal scans of hemipenis/hemiclitoris, right and left, the echotexture of the copulatory organ was detected and digitally recorded. The maximum measurable length and height of each hemipenis/hemiclitoris were considered in these scans.
In the transverse section, scanning from the cloacal scale towards the tail, the maximum measurable height and width of each hemipenes/hemiclitoris were obtained.
Basing on the echographic appearance, the gender of the snake was determined.
After the ultrasonographic examination, the following morphometric measures were take using a tape measure and a digital gauge.

- Length of the snake (from the nose to the end of the tail)
- Distance between the cloaca and the tip of the tail
- Pelvis diameter at the cloaca
- Length of the head (from the caudal end of the square bones to the snout)
- Width of the head: (from one parietal bone to another).

All the data were filed in an Excel® table and later classified for family, genus and species.
For each of these groups, average and standard deviation of morphometric and echographic measurements were calculated. For each subject, average and standard deviation between the measurements of the right and the left hemipenes/hemiclitoris were considered.

2.3 Statistical analysis

The presence of a statistically significant correlation between the morphometric measures and the averages of the measures of the hemipenes/hemiclitoris was determined (MedCalc®). Only groups of at least 3 specimens were considered.
The statistical significance was considered for $p < 0,05$, with confidence interval at 95%, while the level of confidence was defined by these criteria: weak for $r < 0,3$; mild for $0,3 < r < 0,7$; strong for $r > 0,7$.

The t-test for independent values was performed to assess the existence of a significant difference ($p < 0,05$) between the morphometric values of the two genders.

3. RESULTS

The studied sample consisted in 55 snakes: 5 *Boidae*, 25 *Colubridae* e 25 *Pythonidae*.
Boidae comprehended 3 *Boa constrictor* (2 females and 1 male), 1 *Eunectes notaeus* (female) and 1 Acrantophis *dumerili* (male).
Colubridae comprehended 3 *Lampropeltis alterna* (2 males, 1 female), 3 *L. getula* (2 males, 1 female), 1 *L. pyromelana* (male), 7 *L. triangulum* (2 males, 5 females), *Pantherophis guttatus* (7 males, 1 female), 2 *Orthriophis taeniurus* (1 male e 1 female), *Pituophis melanoleucus* (male).
Pythonidae comprehend 1 *Liasis olivaceus* (female), 1 *Morelia spilota* (female), 1 *M. viridis* (male), 2 *Python curtus* (males), 7 *P. bivittatus* (4 males, 3 females), 12 *P. regius* (7 males, 5 females), 1 *P. reticulatus* (female).
The hemipenes (Fig. 1, A) showed an anechoic or hypoechoic structure, characterized by small echogenic foci and a hyperechoic and defined border. They were triangular shaped with the base near the cloaca (longitudinal scan) and a circular section (transversal scan). It was possible to find their opening near the cloaca.
Caudally it was possible to see the retractor mus-

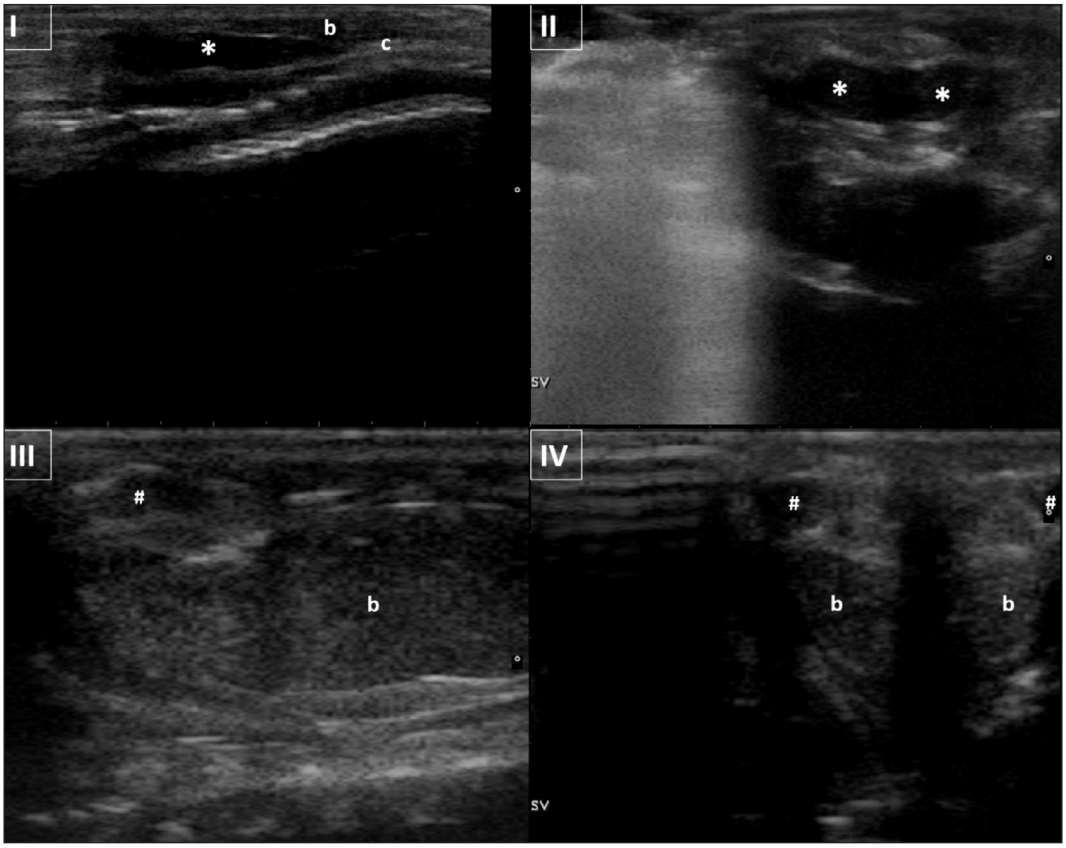

Figure 1 - I: longitudinal scan of a male *Acantrophis dumerili*, showing the triangular shaped markedly hypoechoic hemipenis, portion of the hypoechoic scent gland, and the hyperechoic retractor muscle; II: transverse scan of a male *Acantrophis dumerili*, showing the two hemipenes; III: longitudinal scan of a female *Python regius*, showing the irregularly shaped hypoechoic hemiclitoris and the heterogeneous scent gland; IV: transverse scan of a female *Python regius*, showing the two hypoechoic hemiclitoris and the scent glands. *: hemipenis, b: scent gland, c: hemipenis retractor muscle, #: hemiclitoris.

cle (Fig. 1, c), more echoic and homogeneous.
The scent gland, in males, was more echoic and homogeneous. It was dorsal to the hemipenis near the cloaca and becoming ventral near the tail.
Hemiclitoris were more difficult to find, always smaller than the hemipenes and sometimes asymmetrical. Their shape was irregularly ovoid, they looked mostly hypoechoic, with great variations, depending on their contents. They were always cranial to the scent gland that occupied the most of the tail, caudal to the cloaca.
In *Colubridae* family, the average length of the hemipenes was 2,32 ± 0,7 cm, while for the hemiclitoris was 0,67 ± 0,26 cm. The height, measured in longitudinal scan, was 0,41 ± 0,13 cm for the hemipenes, and was 0,18 ± 0,04 cm for the hemiclitoris. The height, measured in transversal scan was respectively 0,39 ± 0,13 cm and 0,18 ± 0,03 cm. The average width of the hemipenis was 0,45 ± 0,12 cm and for the hemiclitoris was 0,23 ± 0,05 cm.
The length of the snake, the diameter of the pelvis and the width of the head were mildly correlated to all the echographic measurements of the hemipenis.
In *Boidae* family, the average length of the hemipenes was 2 ± 0,16 cm, while the average length of the hemiclitoris was 0,81 ± 0,3 cm; the heights were 0,43 ± 0,05 cm for males and 0,18 ± 0,07 cm for females. In transverse scans were respectively 0,42 ± 0,03 cm and 0,18 ± 0,06 cm. The width of the hemipenis was 0,51 ± 0,01 cm and the width of the hemiclitoris was 0,25 ± 0,03 cm.
A strong correlation was found only between the length of the head of the females and the average height of the hemiclitoris (measured in transverse scan).
In the *Pythonidae* family, the average length of the hemipenes was 2,55 ± 1,25 cm, while the average length of the hemiclitoris was 1,03 ± 0,37 cm. The height measured in longitudinal scan was 0,51 ± 0,32 cm for the hemipenes and 0,19 ± 0,04 cm for the hemiclitoris. In transverse scan, the heights were respectively 0,51 ± 0,37 cm and 0,22 ± 0,04 cm; the width was 0,58 ± 0,32 cm for the hemipenis and 0,31 ± 0,05 cm for the hemiclitoris.
Females of this family showed a mild correlation between the diameter of the pelvis and the width of the hemiclitoris and between the length of the head and the width of the hemiclitoris.
Males showed a strong correlation between the length of the snake, the length of the tail, the diameter of the pelvis and the length of the head, with all the measures of the hemipenes.
The width of the head had a mild correlation with the height and the width of the hemipenis.
Considering the genera, statistical analysis was performed only for *Python spp.*, *Lampropeltis spp.* and *Pantherophis spp*.
Lampropeltis spp. showed a strong correlation between the diameter of the pelvis and the average width of the hemipenis. The length and the width of the head had a strong correlation with the height and the width of the hemipenis.
In *Pantherophis spp.* there was a strong correlation between length of the snake, the diameter of the pelvis and the length of the hemipenis.
Pyhton spp. males had strong correlations between length of the snake, length of the tail, diameter of the pelvis, length of the head and all the parameters of the hemipenes.
The width of the head shows medium level of correlations with all the parameters of the hemipenes.
In females a strong correlation was found between all the morphometric measures and the length of the hemiclitoris.
Concerning the species, we observed that *P. bivittatus* had correlations comparable with those of the *Python* genus, while *P. regius* didn't.

4. DISCUSSION

Use of ultrasound for gender determination in snakes proved to be a fast procedure and was well tolerated by the snakes: none of them showed signs of stress or pain, during the examination. Identification of the copulatory organs was easy and the post processing evaluation of the images avoided mistakes during the gender determination.
We were able to obtain diagnostic images for all subjects, starting from 1 cm of pelvic diameter.
In big snakes, we found artifacts that limited the quality of the images, because of the thickness of the scutae. Eventually in only 3 subjects we were not able to measure at least one parameter, but it was always possible to find the copulatory organs in at least one scan (longitudinal or transverse).
The effectiveness of the ultrasound in sex determination of snakes, in our population was witnessed by a sensibility and specificity of 100%, compared to the usual probe techniques.
We consider that the substantial lack of correla-

tions in females, could be a consequence of the small size of the hemiclitoris, which produced a great data dispersion.
Considering the families *Colubridae* and *Pythonidae*, we observed that the size of the hemipenis was proportional to the size of the snake.
In the *Colubridae* we found an inferior number of correlations compared with the *Pythonidae*. We speculate that it was caused by the greater heterogeneity of *Colubridae*.
Even though there were few correlations found in *Boidae*, the exiguous number of subjects examined, didn't allow to consider them reliable. These data should be confirmed on a wider number of animals.
The absence of a significant difference of morphological parameters between males and females confirm that visual sex determination is unreliable.
The great heterogeneity of our population resulted in interesting correlations mainly about the families. For the genera and for the species the fragmentation in different groups permitted to obtain correlations only for genera *Pyhton*, *Lampropeltis*, *Pantherophis* and the species *P. bivittatus*.
The strongest correlations were those observed between: hemipenes and the length of the snake and hemipenes and diameter of the pelvis, observed in all the families, in almost all of the genera and in *P. bivittatus*.
For these correlations, we also calculated the ratios:
The ratio between the length of the snake and the length of the hemipenis was 58,6±13,8 for *Colubridae*, 61,9±13,5 for *Pythonidae*, 59,8±11,7 for *Python* and 65,3±4 for *P. bivittatus*.
The ratio between the length of the snake and the height of the hemipenis (longitudinal scan) was 337,9±102 for *Colubridae*; 320,9±73,5 for *Pythonidae*, 304,8±43,8 for *Python spp.* and 296,7±19,2 for *P. bivittatus*.
The ratio between the length of the snake and the height of the hemipenis (transverse scan) was 353,5±114,9 for *Colubridae*; 328,3±66,3 for *Pythonidae*, 316,4±50,8 for *Python spp.* and 306,3±47,5 for *P. bivittatus.*
The ratio between the length of the snake and the width of the hemipenis was 294,5±73,2 for *Colubridae*; 277,6±50,2 for *Pythonidae*, 270,1±43,3 for *Python spp.* and 300,3±23,7 for *P. bivittatus.*
The ratio between the diameter of the pelvis and the length of the hemipenis was 0,6±0,1 for *Colubridae*, 1±0,3 for *Pythonidae*, 1±0,3 for *Python spp.* and 1±0,2 for *P. bivittatus*.
The ratio between the diameter of the pelvis and the height of the hemipenis (longitudinal scan) was 3,5±1 for *Colubridae*, 5,3±1,3 for *Pythonidae*, 5,3±1,3 for *Python spp.* and 4,3±0,4 for *P. bivittatus*.
The ratio between the diameter of the pelvis and the the height of the hemipenis (transverse scan) was 3,7±1,2 for *Colubridae*, 5,4±1 for *Pythonidae*, 5,5±1 for *Python spp.* and 4,5±0,7 for *P. bivittatus*.
The ratio between the diameter of the pelvis and the width of the hemipenis was 3±0,7 for *Colubridae*; 4,5±0,6 for *Pythonidae*, 4,6±0,6 for *Python spp.* and 4,4±0,5 for *P. bivittatus*.
In our experience ultrasonography confirmed to be an easy feasible, reliable, safe and harmless technique. It should be considered as an elective technique for sex determination in snakes. We think that our promising results could be considered for future studies aimed to establish reference values applicable to more genera and species.

The permission for publication has been obtained from all the authors on this manuscript.
The study was performed in accordance with the ethical standard dictated by the applicable law.

REFERENCES

1. O'Shea M. American Boas. In: O'Shea M, ed. Boas and pythons of the world. Princeton, NJ: Princeton University Press; 2011:38-63.
2. Denardo D. Reproductive biology. In: Mader DR, ed. Reptile medicine and surgery. 2nd ed. St. Louis, MO: Elsevier; 2006:376-390.
3. Millefanti M. La riproduzione. In: Millefanti M, ed. Boa e Pitoni. Milano, IT: De Vecchi; 2009:74-85.
4. Mattison C. Snakes and humans. In: Mattison C, ed. The new encyclopedia of snakes. Princeton, NJ: Princeton University Press; 2007:168-189.
5. Banzato T, Russo E, Finotti L, Milan MC, Gianesella M, Zotti A. Ultrasonographic anatomy of the coelomic organs of boid snakes (Boa constrictor imperator, Python regius, Python molurus molurus, and Python curtus). AJVR. 2012; 73:5.
6. Gnudi G, Volta A, Di Ianni F, Bonazzi M, Manfredi S, Bertoni G. Use of ultrasonography and contrast radiography for snake gender determination. Vet Radiol Ultrasound. 2009; 50:309-311.

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