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A Case of Abnormal Pregnancy in *Vipera ammodytes* (L., 1758) (Reptilia: Viperidae) from Bulgaria

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Abstract: In the second half of August 2016, a female nose-horned viper (Vipera ammodytes) was captured and brought in the laboratory. The viper seemed gravid and one month after its capture showed the typical sign of parturition (the previously thickened posterior part becomes much thinner with abundant skin folds). However, no neonates were found, but only a yolky mucous uniform mass, a product of the slow degradation of the eggs or embryos. The snake was kept for few more days but no additional expulsion of reproductive material occurred. This viper lost almost 50% of its pre-parturition body weight and its post-parturition body condition index was very low. One plausible explanation for the pregnancy failure could be the female's low nutrient reserves in the onset of vitellogenesis, although alternative explanations such as parasites or internal disease could not be excluded. Egg- or embryo-resorption during gestation seems unlikely for this specimen.

Keywords: snakes, nose-horned viper, gestation, pregnancy failure, resorption.

Introduction

Most viviparous squamates are primarily lecithotrophic, obtaining most of their nutrients indirectly from the mother in yolk deposited into follicles during vitellogenesis (Stewart & Thompson 2000, Blackburn 2005, Blackburn & Sidor 2014, Van Dyke et al. 2014). This yolk deposition relies on the use of body reserves (capital breeding) and in squamates from the temperate zones is often associated with low reproductive frequency (Hahn & Tinkle 1965, Baron 1992, Bonnet et al. 1998). During embryogenesis, each developing fetus converts its own yolk protein into body tissues (Van Dyke & Beaupre 2011). In such species, life history theory predicts that a threshold level of energy reserves is necessary for a reproductive cycle to begin. (Schaffer 1974, Bull & Shine 1979). Such threshold has been proven in some viper species (Bonnet et al. 1994, Bonnet & Naulleau 1995, Naulleau & Bonnet 1996, Diller & Wallace 2002, Baron et al. 2012). Females with body condition under that threshold either do not initiate reproduction, or have a very low reproductive success (Bonnet et al. 1994). Reproductive failure is predicted to be costly because it jeopardizes resources accumulated over a long period of time that were invested in reproductive activities and embryo development (Lorioux et al. 2016). Egg- and embryo-resorption has been suspected to exist as a compensatory mechanism for minimizing nutrient losses during failed attempts at reproduction (Blackburn 1998, Blackburn et al. 1998, Blackburn et al. 2003, Bonnet et al. 2008, Lorioux et al. 2016). However detailed studies showed no evidence of such resorption (Blackburn et al. 1998, Blackburn et al. 2003).

Here we present a case of abnormal pregnancy in a nose-horned viper (*Vipera ammodytes*). The probable reasons for the pregnancy failure are discussed.

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The nose-horned viper is a viviparous lecithotrophic species. Mating is in May-June (Beshkov 1977) and parturition is usually in the end of August and the first half of September (Luiselli & Zuffi 2002), although in very hot summers it may happen earlier (Beshkov 1977, Strugariu et al. 2011). Females usually have a biennial reproductive cycle (Beshkov 1977, Luiselli & Zuffi 2002) but in some areas they may reproduce each year (Dushkov 1978). In Bulgaria, *V. ammodytes* is found throughout the country and is represented by two subspecies: *V. a. ammodytes* (inhabiting northwestern Bulgaria and northern part of the Krayshte Region) and *V. ammodytes montandoni* Boulenger, 1904 (inhabiting the rest of the country) (Stojanov et al. 2011).

Materials and Methods

The presented observation and the accompanying morphometric data are result from a long-term study (2013-2017) on the ecology of the nose-horned viper (*V. ammodytes*) in Bulgaria (the remaining results are irrelevant to the topic under consideration and will be the subject of another publication). The Gravid females (8 specimens, determined by palpation, from 3 localities in the Western part of the country) were measured and weighted, and were brought to the laboratory (Natural Museum of Natural History in Sofia) until parturition. They were kept in plastic boxes (31/22/16 cm) with sawdust for substrate. A cable heating source was provided on the one end of the box and a shelter in the other. Water was provided *ad libitum*. The snakes were not fed since pregnant females rarely eat during this stage of gestation (Bea et al. 1992). We checked them every day (sometimes several times a day) to obtain if parturition occurred. After parturition the females were weighted again. Neonates (total of 46 specimens) were measured and weighted.

The body condition of the females after parturition was evaluated with the body condition index (BCI) developed by Leloup (1976) and used by Bonnet et al. (1994) and Bonnet & Naulleau (1995). The BCI is calculated with the following formula: **BCI=M/TM**, where **M** is the actual body mass (g) of the studied animal and **TM** is the theoretical body mass (g) of the same animal. **TM** was calculated with the formula $\mathbf{TM = (L / l)^3 * m}$, where **L** is the snout to vent length (SVL, cm) of the studied animal, **l** is mean SVL of neonates of the species studied and **m** is mean body mass (g) of neonates of the species studied. The mean length and body mass of the newborns were calculated using the measurements obtained from the above mentioned 46 specimens.

Results

The data of the measurements and the calculated indexes for the 8 gravid vipers, brought to the laboratory are presented in Table 1. In 7 of them a normal parturition was observed and the neonates (2-10) were vital and without visible external defects (in 7 occasions) or stillborn (in 2 occasions). In one of the gravid vipers (captured on 18.08.2016 near the village of Karlukovo and classified as pregnant with some uncertainty, because the thickening was softer than usual in vipers at this stage of gestation) was registered a process, which has not yet been reported for *V. ammodytes* (at least in our knowledge).

On the afternoon of 18.09.2016 the female showed the typical sign of parturition: the previously thickened posterior part becomes much thinner with abundant skin folds. After checking the box no neonates (live or stillborn) were found, but only a yolky mucous uniform mass which dried up in about 30 minutes (Fig. 1). We palpated the female to check for any reproductive material left in the uterus, but we did not detect any. We also palpated the stomach of the viper to check if it had swallowed undeveloped eggs or neonates after the parturition. No items were found. The snake was kept for few more days but no additional expulsion of reproductive material occurred. The viper‘s BCI after parturition was 0.65 Only two vipers had lower BCI after parturition (see Table 1). The mean BCI after parturition of all vipers was 0.71 (range: 0.55−0.97, SD=0.13, n=8). Even though no viable reproductive material (developed neonates) was produced this female lost almost 50% of her pre-parturition body weight. Higher weight loss was registered only in the females with the highest clutch size (50.24% and 52.44%, in both cases with birth of 10 neonates) (see Table 1).

Discussion

Pregnancy failure in squamates is not unusual and experimental studies show that in most cases it is caused by unsuitable thermal regimes, stress, diseases or parasites (Fox 1948, Beuchat 1988, Lourdais et al. 2004, O’Donnell & Arnold 2005, Hare & Cree 2010, Lorioux et al. 2013, Michel et al. 2013). However, in most cases pregnancy failure is in the form of stillborn neonates (Fox 1948, Lourdais et al. 2004, Lorioux et al. 2013, Michel et al. 2013). A similar case of abnormal pregnancy failure is reported for *Vipera aspis* (L., 1758) by Michel et al. (2013). The authors stated that 4 of the females expelled undeveloped items often impossible to count precisely (uniform yolk mass with poorly developed embryos embedded within), and other 5 females, although positively palpated several weeks before (at the onset of the experiment and then again one month later during early gestation), did not produce any "reproductive item" and further palpation failed to detect remaining item into their oviducts. However, these females were kept in constant cool ambient temperatures (23°C) from the mid vitellogenesis to the parturition period. During mid-gestation (July), they observed skin disease on those vipers. The authors were not certain for the exact cause of the pregnancy failure (disease versus cool temperature, or a combination of both factors). In our study the pregnant viper was brought to the laboratory in the late stage of gestation, when the embryos are already formed (Beshkov 1977, Bonnet et al. 2001) and it was provided with a heating source, so cold temperature should not be considered as plausible reason for the observed pregnancy failure. Furthermore, other pregnant vipers were kept under the same conditions and they all produced viable offspring.

Table 1. Localities, morphometric data and neonates number for the studied gravid females of *V. ammodytes*. M1 and M2 refers to the body weight of the vipers in the moment of capture and immediately after parturition. WL% refers to the weight loss after parturition, or (M1-M2)/M1*100. NN refers to the clutch size, i.e. number of neonates. SVL and BCI are referred in the text.

Locality	SVL	M1	M2	WL%	NN	BCI
Balsha (N42.856° E23.252°)	55.8	172.9	92.96	46.24	7	0.71
Balsha (N42.856° E23.252°)	49.4	139.4	86.9	37.66	5	0.97
Balsha (N42.856° E23.252°)	56.8	199.6	101.2	47.49	6	0.74
Balsha (N42.856° E23.252°)	57	166.1	103.7	37.57	6	0.75
Karlukovo (N43.179° E24.061°)	55.5	147.8	70.3	52.44	10	0.55
Karlukovo (N43.179° E24.061°)	44.5	58.8	40.2	31.63	2	0.61
Karlukovo (N43.179° E24.061°)	51.6	133.24	66.67	49.96	-	0.65
Kresna's gorge (N41.769° E23.151°)	53.6	164.17	81.69	50.24	10	0.71

Fig. 1. The reproductive product of the female V. ammodytes with the abnormal pregnancy, expulsed on 18.09.2016 (a yolky mucous uniform mass, photographed before it dried up).

No traces of skin disease were evident, so this explanation is also unlikely. Since no signs of embryos were detected, the most probable explanations for the observed abnormal pregnancy are: 1) the female carried only unfertilized eggs, which underwent degeneration or 2) the embryos died in the early stage of gestation and also underwent degeneration. It is possible that the pregnancy failure was due to low nutrient reserves of the female in the onset of vitellogenesis. Vitellogenesis involves a very intensive mobilization of maternal reserves, fat bodies, liver, vertebral bone, and muscles (Bonnet et al. 1994, Bonnet & Naulleau 1995) and a threshold levels of energy reserves, necessary for a reproductive cycle to begin are found to exist in vipers (Bonnet et al. 1994, Bonnet & Naulleau 1995, Naulleau & Bonnet 1996, Diller & Wallace 2002, Baron et al. 2012). In the study of Bonnet et al. (1994) only 3 female *V. aspis* with body condition under this threshold (BCI<0.70) became vitellogenic but they had very low reproductive success: almost all the litters were comprised by eggs or stillborns, only one healthy young being produced. According to Tinkle (1962), follicles containing yolk, but obviously smaller and sometimes malformed probably would become atretic or, if ovulated and fertilized, would not develop normally. However since we did not dissect the female viper an alternative explanations such as parasites or internal disease could not be excluded.

Another important question is whether or not egg or embryo-resorption was the reason for the observed abnormal pregnancy. Many biologists have inferred that pregnant lizards and snakes can terminate reproduction by resorbing eggs and embryos from their oviducts (see Blackburn 1998 and references therein). According to Blackburn et al. (2003) the inferences are based chiefly on three lines of evidence: 1) the presence of degenerating and infertile eggs in maternal oviducts; 2) the apparent disappearance of embryos from the reproductive tract of captive, pregnant females, without evidence of parturition; 3) histological observations suggestive of uterine resorption. The same authors stated that the first two lines of evidence were very weak, because abortive eggs could be retained indefinitely or could be extruded from the reproductive tract, where they might go unnoticed or be ingested by the mother (Blackburn et al. 2003). Their observations showed no signs of resorption and indicated that the oviductal eggs and embryos underwent substantial postmortem degeneration, necrosis, and homogenization and passed down the oviducts to be extruded through the cloaca. Bonnet et al. (2008) described a case of disappearance of 2 eggs in the last stage of gestation in *V. aspis*. They stated that one of the plausible explanations of this was resorption of the eggs, although they did not exclude other explanations such as the slow degradation of the eggs with gradual expulsion of very small amounts of tissue or fluids through the cloaca during the last stage of gestation; consummation of the unviable eggs from the mother after the parturition; leakage from the oviduct, expulsion into the peritoneal cavity; resorption by the oviducts. Lorioux et al. (2016) stated that female *V. aspis* that failed at pregnancy in their study regained muscular performance (traction force) likely by reabsorbing the yolk content in their reproductive tract. According to our results egg- or embryo-resorption during gestation seems unlikely for *V. ammodytes*. As mentioned earlier, after parturition the female with abnormal pregnancy was very emaciated and it BCI was 0.65. It also lost almost 50% of its body weight after parturition. According to Madsen & Shine (1993) the female *Vipera berus* (L., 1758) that was most emaciated after parturition was the one that had produced unusually large litters relative to maternal body size. Bonnet et al. (1994) and Bonnet & Naulleau (1995) stated that the mean BCI of the females *V. aspis* after parturition is 0.52. The same authors found that at the beginning of vitellogenesis, wild population of *V. aspis* involves three classes of individuals: potentially reproductive females (high BCI=0.70), intermediate non-reproductive females (0.52<BCI<0.70), and thin females (low BCI, with a BCI<0.52) and that females with a BCI of less than 0.52 should have a low survival in comparison to other categories. According to our data the mean BCI after parturition in *V. ammodytes* is 0.71. The estimated BCI of the viper with abnormal pregnancy is lower than the mean value, which shows that this viper was in bad body condition and that it should have a low survival probability. If egg- or embryo-resorption appears, it should be expected that the snake would be in better condition and that the mass loss should not be that high. Our results also show that the "product" of the failed pregnancy underwent degeneration and homogenization and was extruded through the cloaca as suggested by Blackburn et al. (1998) and Blackburn et al. (2003). Having in mind that this homogenized mass was not easily detectible in the box and dried up quickly it can be said that it easily can be overlooked, especially among the post-parturitient products (fluids and membranes) of live neonates, which could be the explanation of the case in Bonnet et al. (2008).

Conclusions

It seems that pregnancy failures occur not only in snakes held in captivity, but also in free-living individuals. The reasons for such miscarriages are unclear. Although resorption of the abortive material from the mother's organism seems like the perfect compensatory mechanism for reduction of the negative effect in such pregnancies, our study does not confirm the existence of such mechanism in *Vipera ammodytes*. Further studies are needed for the better understanding of the reproductive biology of the Old-world's vipers.

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References

Baron J-P. 1992. Rergime et cycles alimentaires de la vipe`re Your Sincerely, Your Sincerely, Your Sincerely, Your Sincerely, Your Sincerely, Your Sincerely, d'Orsini (*Vipera ursinii* Bonaparte 1835) au Mont Ventoux, France. Revue d'Ecologie (Terre Vie) 47 (3): 287–311.

Baron J-P., Le Galiard J-F., Ferrière R. & Tully T. 2012. Intermittent breeding and the dynamics of resource allocation to reproduction, growth and survival. Functional Ecology 27 (1): 173–183.

Bea A., Braia F., Baron J-P. & Saint Girons H. 1992. Regimes et cycles alimentaires des viperes Europeennes (Reptilia: Viperidae). Année Biologique 31 (1): 25–44.

Beshkov V. 1977. A study of biology and ecology of the snakes in Maleshevska Planina Mts. (Southwestern Bulgaria). III. Food and reproduction of the Nose-horned viper (*Vipera ammodytes meridionalis* Boulenger). Ekologiya (Ecology) 4: 3–12 (in Bulgarian).

Beuchat C. A. 1988. Temperature effects during gestation in a viviparous lizard. Journal of Thermal Biology 13 (3): 135–142.

Blackburn D. G., Kleis-San Francisco S. & Callard I. P. 1998. Histology of abortive egg sites in the uterus of a viviparous, placentotrophic lizard, the skink *Chalcides chalcides*. Journal of Morphology 235 (2): 97–108.

Blackburn D. G. 1998. Resorption of oviductal eggs and embryos in squamate reptiles. Herpetological Journal 8 (2): 65–71.

Blackburn D. G. 2005. Amniote perspectives on the evolutionary origins of viviparity and placentation. In: Grier H. & Uribe M. C. (Eds): Viviparous fishes. Homestead, Florida: New Life Publications, pp. 301–322.

Blackburn D. G. 2006. Squamate reptiles as model organisms for the evolution of viviparity. Herpetological Monographs 20 (1): 131–146.

Blackburn D. G. & Sidor C. A. 2014. Evolution of viviparous reproduction in Paleozoic and Mesozoic reptiles. The International Journal of Developmental Biology 58 (10-12): 935–948.

Blackburn D. G., Weaber K. K., Stewart J. R. & Thompson M. B. 2003. Do Pregnant Lizards Resorb or Abort Inviable Eggs and Embryos? Morphological Evidence From an Australian skink, *Pseudemoia pagenstecheri*. Journal of Morphology 256 (2): 219–234.

Bonnet X. & Naulleau G. 1995. Estimation of body reserves in living snakes using a body condition index (BCI). In: Llorente G. A, Montori A., Santos X. & Casreteso M. A. (Eds) Scienta Herpetologica. Barcelona: Asociacion Herpetologica Espanola, pp. 237–240.

Bonnet X., Akoka S., Shine R. & Pourcelot L. 2008. Disappearance of eggs during gestation in a viviparous snake (*Vipera aspis*) detected using non-invasive techniques. Acta Herpetologica 3 (2): 129–137.

Bonnet X., Bradshaw D. & Shine R. 1998. Capital versus income breeding: an ectothermic perspective. Oikos 83 (2): 333–342.

Bonnet X., Lourdais O., Shine R. & Naulleau G. 2002. Reproduction in a typical capital breeder: costs, currencies and complications in the aspic viper. *Ecology* 83 (8): 2124-2135.

Bonnet X., Naulleau G. & Mauget R. 1994. The influence of body condition on 17-ß Estradiol levels in relation to Vitellogenesis in female *Vipera aspis* (Reptilia, Viperidae). General and Comparative Endocrinology 93 (3): 424–437.

Bonnet X., Naulleau G., Shine R. & Lourdais O. 2001. Short-term versus long-term effects of food intake on reproductive output in a viviparous snake, *Vipera aspis*. Oikos 92 (2): 297–308.

Bull J. J. & Shine R. 1979. Iteroparous animals that skip opportunities for reproduction. The American Naturalist 114 (2): 296–303.

Diller L.V. & Wallace R.L. 2002 Growth, reproduction, and survival in a population of Crotalus viridis oreganus in North Central Idaho. Herpetological Monographs 16 (1): 26–45.

Dushkov D. 1978. A study of the food and reproduction of the Nose-horned viper (*Vipera ammodytes meridionalis* Boulenger) in the Thracian Plane.- Master's thesis, Faculty of Chemistry and Biology, PU "P. Hilendarski", 32 pp (in Bulgarian).

Fox W. 1948. Effect of temperature on development of scutellation in the Garter snake, *Thamnophis elegans atratus*. Copeia 1948 (4): 252–262.

Hahn W. E. & Tinkle D. W. 1965 Fat body cycling and experimental evidence for its adaptive significance to ovarian follicle development in the lizard Uta stansburiana. Journal of Experimental Zoology A 158 (1): 79–85.

Hare K. M. & Cree A. 2010. Incidence, causes and consequences of pregnancy failure in viviparous lizards: implications for research and conservation settings. Reproduction, Fertility and Development 22 (5): 761–770.

Leloup P. 1976. Méthode simple pour calculer approximativement et comparativement les matières de réserve des serpents vivans. Acta Zoologica et Pathologica Antvepiensia 64: 91–93.-

Lorioux S., AngeLier F. & Lourdais O. 2016. Are glucocorticoids good indicators of pregnancy constraints in a capital breeder?. General and Comparative Endocrinology 232: 125–133.

Lorioux S., Vaugoyeau M., DeNardo D. F., Clobert J., Guillon M. & Lourdais O. 2013. Stage dependence of phenotypical and phenological maternal effects: Insight into Squamate reptile reproductive strategies. The American Naturalist 182 (2): 223–233

Lourdais O., Shine R., Bonnet X., Guillon M. & Naulleau G. 2004. Climate affects embryonic development in a viviparous snake, *Vipera aspis*. Oikos 104 (3): 551–560.

Luiselli L. & Zuffi M. 2002. Female Life History Traits of the Aspic Viper (*Vipera aspis*) and Sand Viper (*Vipera ammodytes*) from the Mediterranean Region. In: Schuett G. W., Höggren M., Douglas M. E. & Greene H. W. (Eds.): Biology of the Vipers, Utah, Sandy: Eagle Mountain Publishing, pp. 279–284.

Madsen T. & Shine R. 1993. Costs of reproduction in a population of European adders. Oecologia 94 (4): 488–495.

Michel C. L., Pastore J-H. & Bonnet X. 2013. Impact of cool versus warm temperatures on gestation in the aspic viper (*Vipera aspis*). Comparative Biochemistry and Physiology, Part A 165 (3): 338–342.

Naulleau G. & Bonnet X. 1996. Body condition threshold for breeding in a viviparous snake. Oecologia 107 (3): 301–306.

O'Donnell R. P. & Arnold S. J. 2005. Evidence for selection on thermoregulation: Effects of temperature on embryo mortality in the Garter snake *Thamnophis elegans*. Copeia 2005 (4): 929–933.

Schaffer W. M. 1974. Optimal reproductive effort in fluctuating environment. The American Naturalist 108 (964): 783–790.

Stewart J. R. & Thompson M. B. 2000. Evolution of placentation among squamate reptiles: recent research and future directions. Comparative Biochemistry and Physiology A 127 (4): 411–431.

Stojanov A., Tzankov N. & Naumov B. 2011. Die Amphibien und Reptilien Bulgariens. Frankfurt am Main: Chimaira. 588 p.

Strugariu A., Zamfirescu S. R., Gherghel I. & Sahlean T. C. 2011. Unusual early parturition in temperate region viviparous snakes during the atypically hot summer of 2007. Herpetologica Romanica 5, 43–49.

Tinkle D. W. 1962. Reproductive potential and cycles in female *Crotalus atrox* from northwestern Texas. Copeia 1962 (2): 306–313.

Van Dyke J. U. & Beaupre S. J. 2011. Bioenergetic components of reproductive effort in viviparous snakes: Costs of vitellogenesis exceed costs of pregnancy. Comparative Biochemistry and Physiology, Part A 160 (4): 504–515.

Van Dyke J. U., Griffith O. W. & Thompson M. B. 2014. High food abundance permits the evolution of placentotrophy: evidence from a placental lizard, *Pseudemoia entrecasteauxii*. The American Naturalist 184 (2): 198–210.

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