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Modified technique for surgical implantation of transmitter device for radio telemetry in levantine vipers (*Macrovipera lebetinus*) in Kashmir region, India

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ABSTRACT

India harbours 23 species of viper among which Levantine viper (*Macrovipera lebetinus*) inhabits the regions of Jammu and Kashmir. In and around the vicinity of Srinagar, Jammu and Kashmir, human-snake conflict has been observed majorly involving *M. lebetinus*. With the capability of hemotoxic venom, the envenomation adversely impacts the victim's cardiovascular system. Wildlife SOS team frequently receives calls from locals to rescue Levantine vipers. With requisite equipment and protective gear, the rescuers are deputed to rescue these snakes and to release them back into safer habitat as a part of human-snake conflict mitigation. In order to understand the habitat ecology, and activity pattern of the species in the released natural habitat telemetry study was planned. The authors successfully accomplished the implantation of SI-2 transmitter device in *M. lebetinus* for radio telemetry studies. After a post operative observation of 14 days, the vipers were released back into suitable natural habitat. The detailed surgical interventions and anaesthetic procedures are discussed in this article.

Key words: Conflict mitigation, human-snake conflict, levantine viper, radio telemetry, surgical implantation

INTRODUCTION

Reptiles are ubiquitous but cryptic. It's always challenging to understand their behavioural pattern and ecology in natural habitats as well as the adaptation of those snakes rehabilitated in the suitable habitat as a part of conflict mitigation. Thus, the need of developing some special devices arises and herpetology scientists started to design suitable device based on their study need by considering the peculiar anatomy and physiology of the various reptiles including snake species. Radiotelemetry has been used for locating snakes by numerous previous researchers (Fitch and Shirer, 1971; Parker and Brown, 1972; Landreth, 1973; Brown and Parker, 1976; Nickerson et al., 1978; Henderson et al., 1980; Jacob and Painter, 1980; Reinert and Cundall, 1982). In most cases, a miniature transmitter was force-fed to the snakes. This method is unsatisfactory for long-term tracking of free-ranging snakes because, the effective range of the transmitter is small due to the necessary absence of an appreciable antenna; and the presence of the transmitter in the stomach may cause changes in foraging behavior (Fitch and Shirer, 1971). The transmitter may be regurgitated after a few days to several months, making planned long-term studies difficult. Brown and Parker (1976), Henderson et al. (1980), and Jacob and Painter (1980) used surgical implantation of transmitters, but the methods were not described in detail and one of the most valuable benefits of surgical implantation, i.e., increased range, was not obtained as no antenna was used.

Passive integrated transponder (PIT) tags have been established as an effective method of uniquely marking animals across a wide range of taxa (Elbin and Burger, 1994; Gibbons and Andrews, 2004). In the year 2016, the PIT tag telemetry in studying a population of Queen snakes (*Regina septemvittata*) was done by Oldham et al., 2016 in Jessamine County, Kentucky (USA). Furthermore, newer tracking technology (i.e., satellite or global positioning trackers) often cannot be used for snakes due to size or shape constraints. In 1982, an improved surgical implantation method for radio-tracking snakes was developed by Reinert & Cundall, 1982 with commercial SM 1 and SB 2-IV transmitters (AVM Instrument Co., 6575 Trinity Ct., Dublin, CA 94566 with 1.4-volt Hg batteries (Eveready E675, E625, or E640) and 30-40 cm whip antennas of 32 AWG 7 x 40 Teflon insulated hook-up wire in venomous snakes (Crotalus horridus and Agkistrodon contortrix). Under the study, the authors used SI-2, VHF (138-235 MHZ) transmitters with VIP ANTENA in nine Levantine vipers which were rescued from conflict situation in and around Srinagar, Kashmir, India as a part of conflict mitigation. In this article, the authors discussed the adopted technique for induction of anaesthesia, transmitter implantation, and post-operative care in detail.

MATERIALS AND METHODS

Wildlife SOS is an NGO working successfully in many parts of India and is actively involved with the rescue and rehabilitation of wild animals including various kinds of conflict mitigation projects. With the collaboration of the Jammu & Kashmir wildlife protection department, Wildlife SOS is running rescue and rehabilitation centres for black and brown bears at Dachigam and Pahalgam. Those centres also perform reptiles rescue and rehabilitation in and around the Srinagar region of Kashmir. Levantine viper (*Macrovipera lebetinus*) is one of the major conflict species and receives frequent rescue calls from Srinagar city. So, it needs to be rescued and rehabilitated in a suitable habitat. To understand the ecology and activity pattern of these snakes, it was planned to have a radio telemetry study. The rescued snakes were selected as per the size and body weight (Table 2). Then a radiographic examination was performed to evaluate the skeletal damage if any and to eliminate the gravid females.

Radio transmitter specifications

For the above study, the authors used SI-2 transmitters from Holohil Systems Ltd., Canada (Table 1).

Anaesthetic approach

Immobilization of snakes is mandatory to implant transmitter beneath the skin and it needs versatile expertise as snakes have peculiar breathing physiology. The freely movable trachea of snakes is narrow and long, making up to one-fifth of the length of the body and the opening remains at the front portion of the mouth (Lillywhite et al., 1976). In snakes, the right lobe of the lung is developed to the two-fifth of the total body length whereas, the left lobe is either missing, vestigial, or well developed but is smaller than right lung (van Soldt et al., 2015). The cerebral regulation is weak as snakes don't have a diaphragm. Metabolism in snakes is generally low and it may vary according to environmental temperature. As the metabolism is low, the influence of the anaesthetics also builds slowly and so as the awakening. To achieve complete immobilization, the spinal reflex must be inhibited especially in the case of largely vegetatively neuroregulated animals. The respiration of a snake may also stop completely due to anesthesia as it doesn't have a diaphragm and the anesthesia blocks the

Table 1. Detailed specification of transmitter

Frequency range	138 – 235 MHz
Transmitter	Crystal-controlled two-stage design pulsed by a CMOS multivibrator
Pulse Width (standard)	24 milliseconds
Pulse Rate (standard)	35 pulses per minute (ppm), Available from 20-120ppm
Power Output	Set to use available battery power over the required transmitter life
Activation	Removing an external magnet starts the transmitter. Replacing the magnet stops the transmitter
Housing	The battery and transmitter are hermetically sealed in a brass case. A small groove is added to the unit to allow for suturing during implantation. To prevent tissue reactions, the case is coated with multiple layers of a biologically inert butyl rubber compound. For subcutaneous placement, asymmetrically tapered ends can be added, increasing the length by 5mm at each end
Antenna	Finely stranded stainless-steel cable, covered with a clear Teflon coating. Clear silicone tubing reinforces the base
Total weight of the transmitter with the VIP antenna	 7.8 g

muscle between the ribs (Betz, 1962). General anaesthesia is paramount to ensure minimal invasive surgical procedure as the transmitter needs to be implanted inside the celomic/peritoneal cavity. Earlier reports on the anaesthesia of snakes were published during 1930s and were all connected to venom glands and venom production of snakes (Tait, 1928; Clark, 1937; Kellaway, 1937). In all these studies, as an inhalation anesthetic agent, majorly chloroform and later a mixture of ether and air was used which made the snakes motionless during venom gland operation. After a decade, a new volatile fluid called Fluothane or Halothane came to the limelight which is neither inflammable nor irritates the respiratory tract (Hackenbrock and Finster, 1963). This procedure of snake anaesthesia was followed in several studies such as in Rattlesnake (Reinert and Cundall, 1982), in Meadow viper (Ujvari and Krosos, 1997; Ujvari and Kosos, 1999) and also in Common Grass snake (Nagy and Korsos, 1999). However, particularly in venomous snake species chamber induction is

Table 2. List of levantine viper (*Macrovipera lebetinus*) undergone the telemetry procedure

Sl. No.	Snake ID	Gender	Weight (in kg)	Total length (cm)
1	LV-1	Male	0.505	100
2	LV-3	Male	0.680	100
3	LV-4	Male	0.455	100.3
4	LV-5	Male	0.480	91.4
5	LV-6	Male	0.930	102
6	LV-2	Female	0.460	96.5
7	LV-7	Female	0.415	91.4
8	LV-8	Female	0.425	83.8
9	LV-9	Female	0.370	80

found to be effective and widely used (Mader and Divers, 2014). The authors adopted a modified method in Levantine vipers i.e., instead of using a separate inhalation mask/chamber, the anesthetic delivery system was made within the snake restraining tube itself. Isoflurane with oxygen was used to induce general anesthesia after restraining the snake inside the transparent restraining tube, and provision was made to fix the oxygen supply directly into the restraining tube and the measured volume of isoflurane-soaked cotton ball was kept separately in a glass chamber which was fixed to the cranial end of the restraining tube (Fig. 1). A Doppler probe was attached to the ventral surface at the cardiac location of the snake's body to monitor the heartbeat throughout the procedure.

Surgical procedure

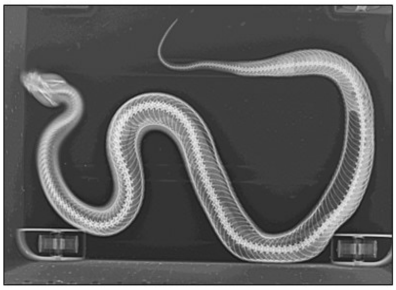
The surgical plane of anaesthesia was assessed by the symptoms of nil tongue flicking motion, and disappearance of righting reflex. After the surgical plane of anesthesia was attained, the morphometry, and sexing were performed. The exact body weight measured with a digital weighing balance. The snake was kept to mediolateral position, and the surgical site; six to seven inches above the cloaca was sterilized with antiseptic lotion. The position for implantation for radio transmitter varies with the size of the snakes. A scalpel blade (number 11) was used to make the dermal incision (less than an inch) where the dorsal and ventral scales adjoining to expose the abdominal muscle. A small nick was made on the abdominal muscle, then that was extended a little bit gently with scissor and forceps. The pre-sterilized transmitter was inserted inside the coelomic cavity with gentle pressure by holding the same at the antenna side. Tiny nick was made on the dorsal aspect of the snake 2 inches below the heart or, towards the head; to insert the endoscopic forceps subcutaneously for pulling and fixing the VIP antenna under the skin. The muscle layer was opposed separately with 3-0 / 4-0 absorbable needled suture material by a simple interrupted suturing technique. The dermal incision was opposed separately with simple interrupted suture materials, and tissue adhesive glue was also applied in the dermal incision to ensure the complete apposition (Fig. 2).

Recovery monitoring -postoperative care

Wound dressing was done and Dressol bandage spray (Vamso Biotec. Pvt. Ltd.) was applied on the incision sites. Maintaining the snake's body temperature is essential for quick recovery and improving their metabolic rate. In order to prevent hypothermia it is paramount to maintain the patient within the POTZ of the species throughout the preanesthetic, perianesthetic and postanesthetic (Mader and Divers, 2014). The snake was kept inside the observation box with warm water bags. The infrared lamp was also used as a heat source till the animal starts recovering and frequent tongue flipping. The snakes were kept under observation for a period of 14 days prior to release (Fig. 2).

RESULTS AND DISCUSSION

The authors made slight modifications in the surgical procedure as described earlier by (Reinert and Cundall, 1982). Instead of using a needle for fixing the antenna by multiple pricking the skin, endoscopic forceps were used through a tiny incision to make tunnel under the skin to pull and fix the flexible VIP antenna subcutaneously. Most of the investigators recommend a transmitter that does not affect the normal mobility of the snake. A limit value of about 4-5% of the body mass is generally given (Weatherhead and Anderka, 1984; Reinert, 1992), but in the case of smaller species length and width of the transmitter can also be important. For a small, slender snake the transmitter should be accordingly to the slender (Weatherhead and Anderka, 1984). Even if the transmitter is less than 4% of the body mass of snake, having a large diameter object under the skin can cause intolerable inconvenience to the snake. Thus, it is important to select slender sized transmitter and snake with prescribed body mass and length (approximately the length of snake should be four times the length of the transmitter with antenna) for convenience. The transmitter here used in the procedure was slender and fit. No complications were found during and after the implantation of SI2 transmitter in *M. lebetinus* and the diameter and length of transmitter was found fit for the species.



Step 1: Radiogarphic examination of Levantine viper (*Macrovipera lebetinus*) prior to surgery

Step 2: Induction of L. viper in a snake tube followed by anaesthetic chamber to attain general anaesthesia

Step 3: Measurement of body weight through digital weighing balance

Step 4: Determination of gender through probing

Step 5: Monitoring of cardiovascular performance

Step 6: Morphometric analysis and markings at mediolateral aspect for implantation of transmitter

Fig. 1 (Step 1-6). Step wise illustration of attaining general anaesthesia, morphometry and monitoring

Step 7: Dermal incision at mediolateral aspect and insertion of transmitter in coelomic cavity

Step 8: Endoscopic forceps inserted subcutaneously to pull and fix the VIP antenna under the skin

Step 9: Simple interrupted suture at dermal incision and application of tissue adhesive glue

Step 10: Radiographic examination showing implanted transmitter

Step 11: Recovery monitoring with maintaining POTZ of the species

Step 12: Completion of post operative care and found fit to release back in suitable natural habitat

Fig. 2 (Step 7-12). Surgical procedure and recovery monitoring

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