Review on Ovariohysterectomy: Surgical approach, Post operative Complications and their Management in Bitches

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Abstract

Ovariohysterectomy (OHE) is a surgical removal of ovaries and uterus conducted for elective sterilization of female dogs to control over population of stray dogs thereby preventing inter-and intra-species disease transmissions. It is indicated in cases of pyometra, uterine tumours, or other pathologies. Females in heat will actively search out male dogs and may attempt to escape from the house or yard, fights with other animals, and contracting disease like, infection with Brucella canis, rabies, toxoplasmosis and other parasites which can cause disease in animals and human beings. These diseases can be transmitted during breeding season. Hence, to avoid such incidences and prevalence, Bitches should be spayed. All surgical patients should receive a preoperative examination by the veterinarian before spaying is conducted like withholding of feed, vaccination and infectious disease control. Surgical Preparations (animal, surgical material and surgical team preparations) are crucial for good prognosis of the surgery. It is essential to choose anaesthetic agents with minimal toxicity. Once anaesthetized, the bitch is positioned in dorsal decubency with her front legs pulled forward and tied to the table, and the back legs tied back. After premedication and anesthesia, OHE is started by a median coeliotomy extending from the umbilicus to approximately halfway between umbilicus and os-pubis. Post-operative managements are vital for ease recovery. Post operative complications due to Ovariohysterectomy should be managed accordingly.

Keywords
Bitch, Ovariohysterectomy, Surgical techniques, Zoonosis.

Introduction

Ovariohysterectomy (OHE) is the most common surgical technique in small animal surgery performed in some countries and is unethical or illegal in other countries (Gunzel, 1998 and Greenfield et al., 2004). Some recognized reasons for the promotion of Ovariohysterectomy are societal in nature, some refer to populations of animals, and some are pertinent to health of individual animals and human beings. There is also great variability in number of studies supporting some of the findings described, and veterinarians are encouraged to be aware of the amount of evidence to support any given claim. Finally, breed predispositions exist for some of the concerns described and must be included in decisions about suitability of OHE in a given animal (Root Kustritz, 2010 and Reichler, 2009).
Advantages of OHE are, decrease in reproductive tract diseases, including pyometra and mammary neoplasia in bitches; decrease in pregnancy and parturition-related disorders including metritis, mastitis and dystocia; decrease in hormone-associated disorders such as vaginal prolapse in bitches and decrease in undesirable sexual behaviors (Romagnoli, 2008).

Disadvantages of OHE are surgical and anaesthetic complications, increased risk of neoplasia of various organ systems, obesity and urinary incontinence in bitches. Furthermore, several surgical techniques of OHE and their benefits and complications have been widely discussed for decades (Spain, 2004).

OHE consists of removing the source of hormones that control reproduction and that determine the typical physical and behavioral characteristics distinguishing males and females. In dogs, this is most commonly accomplished by surgically removing the ovaries (spaying), though there are a variety of surgical techniques as well as non-surgical methods of contraception to prevent reproduction without removing the source of gonadal hormones (Kutzler and Wood, 2006).

There is significant variation among countries, regions and communities in specific neutering practices, in the proportion of owned dogs that are neutered, and in the attitudes among pet owners and veterinarians towards neutering (Perrin, 2009 and Spain et al., 2002). Therefore, the objectives of this seminar paper are:

- To review on surgical techniques of Ovariohysterectomy in bitches
- To recognize OHE as sustainable stray dog population control and its roles in reducing Zoonosis

**Literature Review**

Canine Ovariohysterectomy, commonly referred to as a spay procedure, is the removal of the ovaries and uterus from the female dog, in order to prevent pregnancy and certain types of diseases. This procedure should only be undertaken if the bitch is in a fit state to withstand general anesthesia. However, the procedure is contraindicated if the bitch presents a generalized condition with hypothermia and dehydration. Elective sterilization of female dogs is one of the most common procedures performed in veterinary practice and Spaying pet entails surgical removal of the reproductive organs. In females, these are the ovaries and uterus, and the procedure is called an ovariohysterectomy, or spaying. For routine spaying, the best age for dogs is before puberty.

Spayed animals do not go through heat cycles or produce unwanted puppies. Dogs have their first heat at 5 to 12 months of age (Blender, 2012).

OHE is generally performed via the linea alba. Per-vaginal hysterectomy can also be performed in the event of uterine prolapse, if the latter cannot be reduced or if has been traumatized to such an extent that it cannot be replaced safely. Specific and nonspecific complications can occur as hemorrhage, adherences, urinary incontinence, return to oestrus including repeat surgery. After an ovarioectomy, bitches tend to put on weight, it is therefore important to inform the owner to reduce the daily ration by 10 % (Devil Bencharif, 2010).

The two behaviors affected by spaying of female dogs are “aggressive dominance” toward guardians and indiscriminate eating. In this case, the aggressive behavior increased after spaying. This effect was only noted in female dogs’ ovariohysterectomized before 12 months of age, and that already demonstrated aggression. Bitches ovariohysterectomized after 12 months of age presented no risk of increased aggression and dogs that are in a gestational state for two months following an estrus period and spaying them during that time creates a sudden removal of the source of progestins (which tend to have a calming influence on animals). It is proposed that this removal of progesterone may promote irritability or aggression in some individuals (Blender, 2012).

**Indications**

The main indications of OHE include population control, prevention of diseases of the reproductive tract, and elimination of undesirable behaviors associated with hormonal cycling. Elective sterilization of female dogs is important to reduce euthanasia of unwanted dogs (Ruttema et al., 2001).

**Pathological Conditions**

Ovariohysterectomy in the bitch is a surgical procedure consisting of laparotomy with ablation of both ovaries and the uterus. This procedure is indicated for Uterine tumours, serious uterine lesions, whether traumatic or infectious in origin; the most common cause being dystocia during whelpings. Treatment of metritis, pyometra, cystic endometrial hyperplasia (CEH), neoplasia, injury,
neglected dystocia and congenital abnormalities. OHE is also performed in young dogs up to 2.5 years to decrease the incidence of mammary gland tumors. The relative risk for developing mammary gland tumors decreases when neutering is performed before first estrus (0.5%), between first and second estrus (8%), and between second estrus and 2.5 years of age (26%)(Root Kustritz, 2007).

Prevention of Estrus and Its Consequences

Prevention of estrus and problem associated with bloody discharge, attraction of male dogs, accidental mating, pregnancy and unwanted puppies. Females in heat will actively search out male dogs and may attempt to escape from the house or yard, fights with other animals, and contracting disease like, infection with Brucella canis, rabies, toxoplasmosis and other parasites which can cause disease in animals and human beings. These diseases can be transmitted during breeding season. Hence, to avoid such incidences and prevalences, bitches should be spayed (Fontbonne et al., 2007).

Contraception

Although there are birth control pills and medications manufactured specifically for use in dogs most of these oral products can have serious unwanted side effects, are expensive, and usually cannot be used for long periods of time. Since birth control pills are not a viable option as a practical permanent form of sterilization, the only option is surgical sterilization (Ovariohysterectomy) (Ruttema et al, 2001). Until now, OHE is the contraceptive technique of choice. As it is irreversible, spay programs are widely accepted for population control. In spite of worldwide efforts to reduce pet overpopulation, the number of dogs is still increasing (New, 2006). Spaying of dogs is still recommended to avoid high population densities, animal suffering and the spread of Zoonosis (Levy et al., 2008).

Risk of dog reproduction

Reproduction itself has potential risks which can be eliminated by neutering. Dogs of both sexes are susceptible to infection with Brucella canis, rabies, toxoplasmosis & other parasites which can be transmitted to and human beings during breeding or acquired from contact with aborted fetuses and other material from infected females (Robertson, 2008).

Reason for dog population control

Animal welfare is given as reasons for controlling street dog numbers. Spreads of zoonoses (diseases that can spread from animals to people) are particular problems where high densities of dogs and human beings live in close association in which rabies is a major concern. 99% of human cases of this fatal disease are transmitted in the saliva of infected dog bites. Although other animals, such as herbivores, are also susceptible, they do not have the propensity to bite and are hence less of threats to human beings (WHO, 1988). Dog welfare is likely to be poor if the population is not controlled. This is due to competition for limited resources leading to malnourishment in some individuals, particularly in pregnant and lactating bitches and also a high rate of spread of infectious disease and parasites (Bojrab, 1998).

Animal birth control (ABC) programmes have been advocated as a humane method of stabilizing the dog population and rabies control. Street dogs are captured, surgically neutered, vaccinated against rabies and re-released in the same area where they were caught. The aim is to produce a smaller, healthier, more stable dog population in which rabies is better controlled. ABC may be considered in conjunction with rabies vaccination as a complementary tool to reduce the density of dog populations and rabies incidence (Bojrab, 1998). During the heat cycle there are behavioral and hygienic problems that develop. Females in heat will actively search out male dogs and may attempt to escape from the house or yard, putting them in the danger of traffic, fights with other animals, etc. Often there is a sudden Influx of male dogs around the home and yard, these dogs leave numerous droppings and spray plants and trees with urine in an attempt to mark their new found territory. Owners also need to contend with the vaginal bleeding that typically lasts for 4 to 13 days (Blender, 2012).

Pre-Surgical care

All surgical patients should receive a preoperative examination by the veterinarian. Dogs that are in their heat cycle may have the surgery postponed until the cycle is over. If the dog is an older animal, the veterinarian may take blood tests and possibly chest x-rays to check for any underlying diseases (Djemil Bencharif, 2010). Though operation can be done at almost any age and at any phase of reproductive cycle but it is best performed either before puberty or during
anoestrus. Some prefer to wait until the animal has passed through one heat period. Six to eight months of age is generally considered best. The surgery may be most hazardous during estrus or pregnancy and in old obese female. Most favorable time to spay a mature bitch is 3 to 4 months after estrus (Concannon and Meyers-Wallen, 1991). Vaccination is always recommended prior to surgery, but preoperative vaccination is acceptable when necessary (Childers et al., 2006). As is typical for any surgery, standard procedures for controlling potential infectious diseases should be practiced. In particular, spay-neuter programs should include the following when possible. All equipment, that has direct patient contact, should be thoroughly cleaned and disinfected between patients with agents known to destroy common veterinary pathogens (Clutton, 1999).

**Surgery**

**Surgical anatomy**

Ovaries lie close to the caudal pole of the corresponding kidney, ventral to the 4th lumbar vertebra, and half way between last rib and the crest of the ilium. The ovaries are completely enclosed by the bursa and is attached to cranial end of the uterine horn by ovarian ligament continuous with it is suspensory ligament of ovary. Ovaries receive the blood supply through ovarian artery and vein. The uterus has a very short body and extremely long narrow horns. Broad ligament is attached to the anterior part of vagina (Concannon and Meyers-Wallen, 1991). The genital apparatus of the bitch is primarily located in the abdominal cavity, with the exception of the vagina, which lies in the pelvis. The neck of the uterus is relatively short, it measures 1-2 cm long, and lies a few centimeters in front of the anterior border of the pubis; it is followed by the body of the uterus, which measures 3–5 cm in length in the intrabdominal position, and which starts from the anterior straight of the pelvis then divides after a few centimeters into two divergent horns, which lie on the floor of the abdomen on either side of the linea alba, then travel back up towards the ovaries; The uterus receives its blood supply from the right and left uterine arteries. The body of the uterus that lies closest to the oviduct is irrigated by the uterine branch of the ovarian artery, whilst the neck and remainder of the body are supplied by the uterine branch of the vaginal artery (Bojrab, 1998).

**Animal preparation**

Food is withheld for at least 8-12 hours before the operation. After proper premedication, the general anaesthesia is achieved by using parenteral or inhalant anaesthetics (Concannon and Meyers-Wallen, 1991). Food should be withheld from all animals for an appropriate period prior to surgery; however, withholding water is neither necessary nor recommended. For pediatric animals (animals between 6 and 16 weeks old), a small meal should be fed 2 to 4 hours before surgery, and food should not be withheld for > 4 hours before surgery (Howe, 2006).

**Control and anaesthesia**

The animal is controlled in dorsal recumbency. The operation table may be slightly tilted so as to allow the abdominal viscera to move forward (Concannon and Meyers-Wallen, 1991). It is essential, for the success of the procedure, to choose anaesthetic agents with minimal toxicity. Various protocols are available, these include: IV premedication with valium and morphine at 0.25 mg/kg and 0.1mg/kg, respectively, followed by induction of anaesthesia with propofol at a dose of 5 mg/kg, the volume is increased slowly until the animal is sufficiently well anaesthetized to enable intubation. Isoflurane gas is then used to maintain anaesthesia; a flow rate of 2% is normally sufficient to maintain a good level of anaesthesia until the end of the procedure (Kyles et al., 2006). To control perioperative pain, morphine can be administered at the same dose as for premedication, to a maximum of 10 injections/hour to avoid exceeding the threshold of toxicity. Once anaesthetized, the bitch is positioned in dorsal decumbency with her front legs pulled forward and tied to the table, and the back legs tied back; the bitch is then put on a drip with previously warmed normal saline (0.9%) with glucose. After the animal has been anaesthetized, the urinary bladder is expressed and the ventral wall of the abdomen is prepared for surgery (Stockner, 1991).

**Surgical techniques**

A 6-8 cm long midline incision is made on the ventral aspect of the abdomen beginning over the umbilicus and extended caudally. The skin is incised along the linea-alba, that is, the sheath of the rectus abdominus, starting from the umbilicus and ending a few centimeters in front of the pubis. Using a pair of scissors, the subcutaneous connective tissue, which may contain a substantial amount of fatty tissue, is
bluntly dissected to visualize the linea alba. Using rat-tooth forceps, the linea alba is grasped in the middle and tented up before being incised with a pair of scissors. Haemostasis is performed before opening the abdominal cavity. If simple swabbing proves insufficient, any bleeders should be ligated or twisted to obtain a very clean surgical field. To find the uterine horns easily, the operating table is tilted so that the animal’s head is below its feet, to move the abdominal organs towards the diaphragm; this is known as the Trendelenburg position. To locate the genital apparatus with ease, the bladder is retracted laterally; cranial to the bladder, the body of the uterus and bifurcation of the horns are easily locatable. One of the horns is then followed cranially up to the ovary, which is hidden in the fat-filled ovarian bursa. The ovary is not visible but can be felt through this ovarian bursa. It is a 1-2 cm long mass, which is exposed after incision of the bursa (Djemil Bencharif, 2010).

The suspensory ligament is stretched, broken, or transected by electro coagulation or scissors, to improve manipulation and observation of the pedicle. The arteriovenous complex within the pedicle, arising from the ovarian artery and vein is ligated with absorbable suture material, depending on pedicle size, after which it is transected. The uterine artery and vein are ligated at the cranial tip of the uterine horn, 5 mm caudal to the proper ligament, using absorbable suture material, and transected at the proper ligament. After excision the ovarian bursa is opened and inspected to confirm complete ovarian removal (Evans, 1993). OHE can also be performed through flank incision into the abdomen. A midline approach is easier, less traumatic and is associated with less post-operative pain than the flank approach, which involves incising three muscle layers. Also, any intra-operative hemorrhage is more easily controlled. However, although the midline approach is commonly used in dogs the ABC programme often chooses a flank approach because flank incisions have a lower risk of herniation if dogs are active sooner after surgery, whereas the opposite is true for mid-line incisions (Fontbonne et al., 2007). Mid-line incisions require stronger stitching, preferably using synthetic absorbable sutures, such as vicryl and polydioxanone sutures which are too expensive. Catgut, which is fine for stitching flank incisions, is much cheaper and more readily available. Cheaper, non-absorbable, alternatives to catgut, such as monofilament nylon, are better avoided where there is a high risk of post-operative infection, as is the case when operative sterility is compromised. Conditions for which the lateral flank approach for ovariohysterectomy is indicated include excessive mammary gland development due to lactation or mammary gland hyperplasia (Bojrab, 1998). The ovary is grasped and Babcock forceps placed. The latter are handed to an assistant who holds the ovarian pedicle taught out of the abdomen to facilitate placement of a ligature as close as possible to the root of the pedicle to ensure haemostasis of the ovarian artery. The broad ligament is then punctured with a clamp to grasp the suture material and a ligature is placed in the ovarian pedicle as close as possible to the lumbar wall. Once this ligature has been placed, the ends of the threads are kept long so that the ovarian pedicle can be found with ease in the event of hemorrhage. A clamp is then placed between this ligature and the ovary, and the pedicle is sectioned between the two. The ovarian pedicle is held throughout this procedure with a clamp. The quality of the haemostasis is checked; the long ends of the suture material on the ovarian pedicle are then cut. In some cases, such as in the event of hypertrophy of the vascular bundle, it may be advisable to place two ligatures, one around the artery and one around the ovarian vein. Never hold the ligature itself with the clamp, as it might slip off the pedicle when being released back into the abdomen (Djemil Bencharif, 2010). If the broad ligament is seen to contain large vessels, they should be ligated prior to being cut. However, if the vessels are invisible and buried under fat, the ligament can simply be torn in the middle above the uterine artery by exerting traction between two swabs with the fingers to tear it from front to back to the level of the cervix, and as close as possible to the lumbar wall. Another technique for sectioning the broad ligament involves the placement of a row of overlapping mattress sutures along the length of the ligament before making the section with a scalpel or a pair of scissors. Once the ovarian pedicle has been sectioned, the second horn is located and the corresponding ovarian bursa grasped with Babcock forceps. The ovarian pedicle and broad ligament are sectioned as described previously. Finally, the two uterine horns are replaced back onto pelvis (Hedland, 1997).

The prolapse of intestinal loops through the incision can cause significant heat and fluid loss, which can have very serious consequences, especially if the bitch is already suffering from deterioration in general status due to severe pyometria, for example. It is therefore advisable to suture the anterior portion of the laparotomy wound before continuing the surgery. However, if the homeostasis of the ovarian pedicles or
broad ligaments is a source of concern, the placement of a few forceps should suffice to provide temporary closure of the anterior portion of the laparotomy wound (Laznicka, 1995). The uterine vessels are ligated on each side and cut. Transfixation double ligature is used to encompass the entire cervix. The uterus is severed just cranial to the ligatures. Uterine stump is checked carefully for haemorrhage and returned into the abdomen. Care should be taken to remove as much uterine body as possible (Pearson, 2011). Once both uterine horns have been flipped back onto the pelvis, the uterine cervix is sectioned, following ligation of the uterine arteries and veins. The veins can be visualized passing on either side of the cervix. The arteries run under the veins in the musculosa of the cervix, which is why the haemostatic sutures should transfix the lateral walls of the cervix. However, if the uterine artery is perforated during ligation, a wider transfixion is needed, more caudal to the previous attempt (Stone, 2006). Once both of the ligatures have been placed, the cervix is crushed at their level with an intestinal clamp. Another clamp is then placed just above the first and the contents of the uterus are pushed back towards the horns; two other clamps are placed in the same way above the 2nd clamp. The second and third clamps are removed, thus leaving a secretion-free zone. Once both intestinal clamps have been placed, the anterior section of the cervix is performed; the cervix may be normal or pathological (Stone, 2006). The cervix is simply sectioned with a scalpel between the two clamps. For pathological cervixes, the serosa is dissected just caudal to the clamp that is placed on the uterus; the serosa is then retracted caudally. The muscles are then sectioned cranial to the intestinal clamp placed on the cervix; if the clamps have been placed correctly, no fluid should leak from the cut ends. The stump is simply replaced in the abdominal cavity. It is however advisable to suture it or bury it in a fold of momentum (Roberts, 1990). The sutured stump is returned to the abdominal cavity and the abdominal wall is closed using cross-matters interrupted sutures with absorbable sutures. If the subcutaneous connective tissue is very abundant, a simple continuous subcutaneous suture is performed. The skin is preferably sutured with cross matters suture pattern with non-absorbable suture materials (Djemil Bencharif, 2010).

**Post-operative management**

Most dogs are extremely tolerant of pain and show no signs of discomfort from the procedure. Unfortunately, as a result, they may attempt to resume their normal level of activity immediately after surgery, and this puts them at risk for complications. Strict monitoring is important. The dog should not be allowed off the leash for seven to 14 days after surgery, and should be prevented from licking the incision, running, jumping, and climbing stairs. Outdoor dogs should be kept inside for two weeks. The incision should be checked daily for any signs of swelling, redness, or heat. If the dog tries to lick the incision, an Elizabethan collar or similar device can be used to prevent the behavioral change (Owen et al., 2004). Firstly, advise perioperative oxygenation if the surgical shock is very great. The animal is warmed, especially if the female was in poor condition prior to the procedure, she must be rolled in a blanket and placed in a heated kennel. IV fluid therapy is administered with isotonic saline along with an injection of Vitamin C, k and corticosteroids. The bitch is then placed under antibiotic therapy for at least 5 days. The sutures are removed after 10 days. Any stagnant uterine secretions in the cervix and vagina will be eliminated in the days following and then cease complete. If female dog is in heat (estrus) at the time of surgery, it is a must to keep them away from un-neutered males for at least two weeks. If a male tries to mate with her serious bleeding and trauma to the reproductive tract may occur, possibly leading to death (Salmeri et al., 1991). The operative site should be checked for swelling or discharge and the operative incision should be dressed with antiseptics. The patient should receive antibiotics and analgesics for seven and three days respectively as well as liquid diet should be given for the first 63 days and the patient should be observed for proper urination and defecation. Cutaneous sutures should be removed after 8-10 days of operation or after complete healing (Concannon and Meyers-Wallen, 1991).

**Post operative complications and their management**

**Body weight gain**

OHE adversely affects the ability to regulate food intake and thus predisposes these animals to obesity. Inactivity and increased food intake contributes to weight gains up to 38%. When dogs are exercised regularly and their food intake is controlled, there is no significant increase in weight. After OHE; bitches
tend to put on weight, it is therefore important to inform the owner to reduce the daily ration by 10% (Le Roux, 1983).

**Intra-abdominal hemorrhage**

Hemorrhage is the most common complication (Morris *et al.*, 1998). Concurrently; hemorrhage has been determined to be the most common cause of death after OHE in large breed dogs. Clinically important hemorrhage primarily occurs from the ovarian pedicles, the uterine vessels, or the uterine wall when ligatures are improperly placed, and rarely occurs from vessels that accompany the suspensory ligament or within the broad ligament so that proper ligation of blood vessels during surgery is crucial (Kyles *et al.*, 2006).

**Vaginal bleeding**

Single non absorbable multifilament ligatures around the uterine body can predispose to erosion of uterine vessels, resulting in intermittent vaginal bleeding. Vaginal tamponade or exploratory celiotomy may be indicated, if the bleeding becomes severe. Vaginal hemorrhage may also be associated with infection caused by contamination during surgery, use of infected suture material, or from transfixation ligatures that enter the lumen of the uterus or cervix (Pearson, 2011).

**Ovarian remnant syndrome (ORS)**

Recurrent estrus occurs after OHE when the ovaries are incompletely removed and residual ovarian tissue becomes functional. Collateral circulation to the ovarian tissue can develop even though the ovarian arteriovenous complex has been ligated and interrupted and attempt to remove the ovaries complete (Miller, 1995).

**Stump granuloma**

Inflammation and granuloma formation can be caused by ligatures of non absorbable suture material, poor aseptic technique, or excessive residual devitalized tissue (at the uterine body). Braided non absorbable suture materials, such as silk, nylon, or linen, and nonsurgical self locking nylon bands have been implicated in most patients. Thus, it is better to obliterate all devitalized tissues completely (Werner *et al.*, 1996).

**Conclusion and Recommendations**

OHE is a surgical procedure that is mostly conducted in veterinary practice. It is indicated when the bitches are encountered with various reproductive tract pathological conditions, dystocia and undesirable sexual behaviors. It is also important to induce irreversible sterilization of bitches thereby controlling overpopulation of dogs and hence preventing disease (rabies) transmission among different species of animals and human beings. During the estrous period, the bitches show undesirable behaviors associated with hormonal cycling that enforce them to displace their surrounding environment in search of male dogs. In this period they play pivotal role for the transmission and distribution of various animal and human diseases from area to area by acting as vehicle for the agent. When the dog population is very high, it is better to reduce to significant level by conducting OHE in bitches rather than euthanasia of normal unwanted dogs which is unethical. It is mandatory to maintain all aseptic procedures (pre-, intra-& post-procedures) which mean that all surgical equipments operation room must be checked for their cleanness, preparation of the animal for OHE and preparation of the surgeon.OHE is started median coeliotomy from the umbilicus to os pubis. Finally, suturing the surgical wound and providing appropriate postoperative care by realizing postoperative complications. Based on the above conclusions the following recommendations are forwarded:

- Ovariohysterectomy in bitches should be common surgical practice in veterinary medicine.
- The government of the country should implement dog registration and certification rules and regulations.
- OHE in bitches should be considered as crucial for controlling dog over population and preventing Zoonosis.
- Post surgical complications should be managed accordingly (if any) for good prognosis.

**References**


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