Guidelines on the veterinary care of the endangered large felids found in the Russian Far East

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INTRODUCTION

Measures addressing conflict situations that involve wild tigers and leopards have been taken since the late 1990s. Managing of tiger-human conflict situations includes professional veterinary support during the tiger capturing procedure and rehabilitation period.

With unrelenting poaching of Amur tigers and their prey animals, the issue of releasing conflict tigers in the wild has acquired a high priority. Every individual wild tiger is unique and each of theses animals requires special attention.

Human-tiger conflicts are a regular event occurring every year in the tiger range countries. Conflict situations are inevitable, regardless of the level of tiger protection measures. Both adult animals and tiger cubs for some reason abandoned by their mothers can be involved in the tiger-human conflict. Human intervention is necessary, if a tiger cannot physically hunt and kill its prey. In such cases, the way of addressing the problem is to remove the conflict tiger from the wild and transfer it to a special rehabilitation centre.

Rehabilitation of wild tigers and their preparation for the return to the wild includes a number of stages, and the work at each of these stages requires veterinary support. Animal health issues play a key role in the success of any rehabilitation program aimed at the release of young carnivores in the wild. The long-time experience in the work with conflict large cats has shown that the most important problems facing wildlife veterinarians are presented by the following:

1. Traumas: traumas caused by collisions with a cars, gunshot wounds, injuries in the traps and other devices laid by poachers. An injured animal may be in a condition of shock, both hemodynamic and pain shock, or thermal shock.

2. Infectious diseases. An infectious disease may be caused by viruses (feline herpes, calici virus infection, panleucopenia, rabies), bacteria (a wide range of pathogenic agents), or parasites. Infected animals typically suffer from both the underlying disease and associated symptoms, such as the dehydration, hypo- or hyperthermia, and cachexy. Besides, such animals may pose a threat as a source of zoonotic diseases.

3. Exhaustion: this is a common condition in the orphaned tiger cubs. The condition of the patient may be further aggravated by dehydration, cachexia, and hypothermia.

Various combinations of the listed problems may also be observed.

Generally, morphological and physiological organization of wild felids is similar to that of domestic cats (including dental formula and blood groups). The only morphological feature that differs between large cats (Pantherinae) and small felids (Felinae) is an elastic ligament present in the hyoid apparatus in the large cats.

PART 1. IMMOBILIZATION OF WILD ANIMALS

1.1 Evaluation of the animal's condition and preparation for immobilization

The capture of an animal (for both the removal from the wild and immobilization in the rehabilitation centre) is usually carried out with the use of remote dart injection. In most cases, it is extremely difficult to evaluate an animal's condition before the administration of anaesthesia and determine the category of anaesthetic risk and potential complications that may be encountered during anaesthesia. In addition, most often, wild animals have to be removed from the wild because they are not able to survive in their natural environment; such animals usually are "conflict" large cats, injured animals (of various ages), orphaned cubs, or wild cats suffering from infectious diseases. The group of conditionally healthy animals includes large cats removed from the wild for behavioural reasons (animals that do not demonstrate natural caution or fear of people and animals entering human settlements, and do not), though such types of behaviour may be associated with various factors. It should be also noted that information about the age and weight of wild animals is usually quite inaccurate. The lack of comprehensive information about the animal's life history makes the work of the anaesthetist even more complicated.

Assessment of anaesthetic risk. Classification system created by the American Society of Anaesthetists (ASA) is now commonly used for evaluating anaesthetic risks (Fig. 1). The system describes the condition of the patient, based on clinical data and laboratory test results and does not include such factors as personal qualities of the anaesthesiologist, availability of equipment or medications, etc.

Category 1	A normal healthy patient.
Category 2	A patient with mild systemic disease without substantive functional limitations.
Category 3	A patient with substantive functional limitations; one or more moderate to severe diseases (mortality rate up to 5%)
Category 4	A patient with severe systemic disease that is a constant threat to life; the rule of 24 h (the patient is not expected to survive > 24 hours without surgery)
Category 5	A moribund patient who is not expected to survive > 24 hours.

Figure 1. *Anaesthesia physical classification system*

An additional factor affecting the anaesthetic risk is the age of the animal. (*The kidneys and liver in newborn animals begin functioning normally in two days after birth, the heart, in a week after birth, hematoencephalic barrier and autonomic nervous system, in four weeks, and thermoregulation, in six weeks after birth.*) In geriatric patients, slower homeostatic compensatory mechanisms create a greater anaesthetic risk.

Obviously, it is hardly possible to evaluate an animal's condition against these criteria while working in the field, but in any case, they help to assess the risks and decide on the best practical approaches *before* and *during* anaesthesia.

Estimation of weight

The animal weight is an important parameter used for predicting the dosage of anaesthetics. Since weighing of the wild animal in the field before the first anaesthetic episode is impossible, it is very important to estimate its weight by its appearance, age, and body condition. Average weight of a healthy adult male tiger is 140–190 kg, the weights of the female vary from 100 to 130 kg. Adult male leopard weighs 40–60 kg, the female, 30–45 kg. Naturally, the animal may be quite exhausted at the time of its removal from the wild, and this fact should be considered, but most anaesthetics have a wide safety dose margin, which allows for their safe use even in case of weight overestimation. Weights of young animals vary in a wide range and depend on the age and general condition of individuals. It should be remembered that young animals lose weight faster than mature individuals since they are not able to hunt efficiently and have higher metabolic rates than older animals.

Preparation of animals for general anaesthesia in rehabilitation centre

Veterinary procedures carried out in rehabilitation centre are usually scheduled in advance, and the animal should be starved for the period of 24 hours prior to immobilization. To facilitate the capture of animals in spacious enclosures it is recommended to move them to smaller enclosures beforehand. Enclosure design should not include any dangerous structures (pools, covered grots, caves, and other shelters). All enclosures should be easily accessible to special vehicles.

1.2 Physical restraint

Physical restraint technique includes the use of nets, hoop nets and other equipment; this technique should be only used on the animals weighing no more than 10 kg. However, it should be borne in mind that physical restraint poses a great risk for the animal that certainly experiences chronic stress or is in a condition of hemodynamic or thermal shock. All these factors may contribute to the risk of death; therefore chemical restraint should be the first choice to consider as it reduces the risk of mortality caused by stress and allows for the comprehensive examination of the animal.

1.3 Chemical immobilization

Large felids are potentially dangerous for humans, therefore general anaesthesia technique should be used for performing any manipulation in case of large cats. The depth of anaesthesia should be monitored during the whole procedure and additional doses of anaesthetic agents should be given, if movements of the animal are noticed. Various anaesthetic drugs and their combinations are currently available for immobilization of large felids. Considering the situation with drug availability in Russia, the following chemical agents are recommended for the use in the field:

1.3.1 Anaesthetic agents

Ataractic drugs

Diazepam and *midazolam* are benzodiazepine receptor agonists that depress the functions of limbic system (a complex set of structures in the mesencephalon, thalamencephalon, and pericranium, that are responsible for visceral, motivational, and emotional responses), producing sedative effect and acting as muscle relaxants due to the enhancement of the inhibitory effects of neurotransmitter GABA. Diazepam and midazolam bind specifically to the benzodiazepine receptors of GABA, which results in the inhibition of neurological activity. GABA neurons and receptors are not found on the pain pathways therefore these drugs do not produce analgesic effect. *Diazepam* is not water-soluble; propylene glycol is used as a solvent, and intramuscular injections are quite painful. Besides, diazepam is characterized by some hepatic toxicity. *Midazolam* is a water-soluble drug that has a form of unclosed ring at the pH below 5; at pH > 5, the mixture is present in the closed-ring form that is rapidly absorbed by the tissues of nervous system.

Agent	**Clinical importance**	**Dosage (mg/kg)**	**Duration of action**	**Effects**	**Antagonists**
Midazolam	Anxiolysis; Depression of motoric activity; Reduction of the overall dose of anaesthetic agent; Miorelaxation; Control of convulsions.	0.1–0.3	15–45 min	Minimal cardiovascular effects; Mild hypotension; In theory, respiratory depression, but apnea is possible when midazolam or diazepam is used with other drugs (e.g., Propofol)	Flumazenil IV 0.01 mg/kg during 15 seconds; if the effect of the consciousness is not regained, the same dose (0.01 mg/kg) may be injected IV (during 15 min)
Diazepam			60–120 min		
	Intravenous injection may cause anxiety in young animals. Midazolam and diazepam are metabolized in the liver.				

Ketamine is a NMDA receptor antagonist. It is a dissociative anaesthetic and good analgesic agent, which causes an elevation of the values of pulse rate (PR) and arterial blood pressure (ABP) and respiratory depression. Administration of ketamine may result in hypo- and hyperthermia and catalepsy (the loss of ortostatic reflexes, maintenance of cranial reflexes). Ketamine causes muscle rigidity and may increase convulsive activity. It is an effective bronchodilator.

Tiletamine/zolazepam is a combination of tiletamine hydrochloride, a dissociative anaesthetic and a competitive NMDA receptor antagonist, and zolazepam (Flupyrazapon), a benzodiazepine. Tiletamine induces a dissociative state characterized by catalepsy, amnesia, and analgesia. Most important adverse

effects of tiletamine include acute tubular necrosis, neurotoxicity, convulsions, and hypersalivation. Zolazepam is a benzodiazepine producing all the effects typical for this group of drugs, i.e., sedative, anxiolytic, anti-spasmodic, and hypnotic effects. Combination of these two drugs in the form of a lyophilized substance helps to reduce adverse effects of tiletamine (both pharmacologically and due to the reduction of the dosage of tiletamine) and to use solutions of various concentrations.

Alpha-2 adrenergic receptor agonists

Alpha-2 adrenergic receptors are usually found presynaptically in the sympathetic nervous system where they inhibit the release of noradrenaline. They are located in the central nervous system, gastrointestinal tract, uterus, kidneys, and blood platelets. Agonists of the α-2 receptors induce sedation and analgesia effects in the central nervous system due to the blockade of these presynaptic metabotropic receptors. Sedation effect is produced due to the blockade of the receptors in the brain stern (in the area of *locus coeruleus*), and analgesia is induced by the blockade of the receptors in the brain stern, posterior horns of the spinal cord, and peripheral nervous system. Hypothermic effect results from the blockade of the hypothalamic receptors (thermoregulatory centre). Cardiovascular effects have two phases; during the first phase, activation of the α-2 adrenergic receptors in the medullary vasomotor centre results in the inhibition of noradrenaline release and depression of the central sympathetic activity, which is manifested in the reduction in heart rate and blood pressure (Mizobe and Maze, 1995). The second phase of cardiovascular effects is associated with peripheral vasodilatation, which may cause tissue hypoperfusion or hypoxia. Development of bradycardia may result in various associated arrhythmias. Respiratory responses are mild, resulting from direct effects of the adrenergic agonists on carotid bodies found in carotid arteries; nerve cells in the carotid bodies participate in the cardiac output regulation, depending on arterial oxygenation. Noradrenaline acts as a mediator.

Other side effects of α-2 agonists include: hyperglycemia, hypothermia, vomiting, polyuria, reduced motoric and secretive activity of the gastrointestinal tract, reduced salivation, decreased intraocular pressure, mydriasis, enhanced platelet aggregation, and depressed synthesis of steroid hormones. Hyperglicemia is a direct effect of the inhibition of insulin secretion by the islets of Langerhans located in the pancreas, and its severity depends on the dosage of the drug. Polyuria is caused by the inhibition of antidiuretic hormone secretion and the increase of glomerular filtration rate (Adams 2001). Pascoe argues (2009) that this effect may have negative consequences in hypovolemic animals, however, available data are not sufficient to answer this question. Vomiting is a common side effect that usually occurs after intramuscular injection of α-2 agonists (Vainio, 1989; Haskins et al., 1986;).

Since the effects of α-2 agonists depend on the numbers of receptors, these drugs have a restricted therapeutic “window” for sedation: they produce a certain sedation effect, after which the increase of the dose prolongs the sedation but does not increase its depth. Therefore, α -2 adrenergic receptor agonists are not used for maintaining anaesthesia.

Different α-2 agonists differ from each other mostly in the duration of action and also in their specifity and affinity for α-2 adrenergic receptors. For instance, xylazine has a specificity for α-2/ α-1 receptors of 160 : 1, whereas clonidine, detomidine, and dexmedetomidine exhibit a specificity of 220 : 1, 260 : 1, and 1,620 : 1, respectively (Virtanen, 1989). On the other hand, different species demonstrate a wide range of sensitivity to various α-2 agonists. Effects of α-2 adrenergic receptor agonists on the central and peripheral nervous system can be fully reversed by specific antagonists that act as competitive inhibitors (atipamezole is available in Russia). Atipamezole is administered at the dose of 5 mg per 1 mg of medetomidine, 0.5 mg per 1 mg of dexmedetomidine or 50 mg of xylazine.

1.3.2 Choice of anaesthetic agent (maintenance of anaesthesia)

Tiletamine/zolazepam can be used as a sole agent or in combination with α-2 adrenergic agonists. Administration of tiletamine/zolazepam without any other drugs provides smooth and fast induction, long anaesthesia, and prolonged and stormy recovery. It should be remembered that recommended dosages are fairly high and may cause adverse nephrological and neurological effects. Intramuscular injection results in the duration of anaesthesia of 40 to 60 minutes, with the time of full recovery comprising 90 min. It is of note that the use of this combination results in abundant salivation. Hyperthermia may develop at the high ambient temperature. (The normal body temperature for large felids is 38.8^0C.) The sole combination tiletamine/zolazepam is not recommended for the use during hot weather periods (risk of hyperthermia, transient dehydration, and kidney injury), for young animals (hemodynamics is not fully developed), and for geriatric individuals (age-related hemodynamic disorders and kidney diseases).

Recommended dosages of Tiletamine/Zolazepam combination:

Species	**Anaesthetic agent**	**Dosage**
Amur tiger (*Panthera tigris altaica*)	Tiletamine/Zolazepam	4 mg/kg (Kreeger, Arnemo)
Far Eastern leopard (*Panthera pardus orientalis*)	Tiletamine/Zolazepam	5–6 mg/kg (Wack)

However, the most commonly used combinations are those of the NMDA receptor antagonists, benzodiazepines, and alpha-2 adrenergic receptors since they help to reduce both the doses of drugs and their side effects.

Combination of ketamine and α -2 agonists is most commonly used worldwide for anaesthesia of non-domestic cats. The most important benefit of this combination is a short period of recovery after the administration of antagonists. Besides, the anaesthesia can be prolonged with the use of additional doses of ketamine or benzodiazepines. Induction is faster, if both agents are used simultaneously. Vomiting and temporary apnea sometimes occur immediately after induction and usually do not require any special

measures. Significant reduction of the respiratory rate and even apnea may be observed during anaesthesia; if a manual stimulation of the thorax does not provide necessary effect it is recommended to use IV administration of doxapram in the dose of 0.5 mg/kg; another option is IV injection of atipamezole in the dose of 5 mg per 1 mg of medetomidine, 1 mg of dexmedetomidine, or 60 mg of xylazine. There are no general contraindications to the use of this combination in animals because a synergistic interaction allows the use of lower doses of the combination ingredients and, consequently, results in the reduction of their adverse effects.

Recommended dosages:

Species	**Anaesthetic agent**	**Maintenance of anaesthesia**	**Reversal agent**
Amur tiger; Far Eastern leopard	Ketamine + xylazine 4–6 mg/kg + 0.4 mg/kg (Armstrong)	Ketamine 1.0 mg/kg	Atipamezole 0.2 mg/kg
		Diazepam 0.01–0.05 mg/kg IV, slowly	
		Midazolam 0.01 mg/kg IV, slowly	
	Ketamine + medetomidine 2.5 mg/kg + 0.03 mg/kg (Jalanka)	Ketamine 1.0 mg/kg IV	
	Tiletamine/zolazepam + medetomidine 0.8 mg/kg + 0.02 mg/kg (Roken)	Ketamine 1.0 mg/kg IV	

If a general anaesthesia is performed at the rehabilitation centre, the preferable method of maintaining anaesthetic state is inhalation anaesthesia with *2% (by volume) isoflurane and steady oxygen supply at 1–2 L/min* instead of intravenous fluid boluses. This method offers better control of the anaesthetic state, helps to stabilize blood level of oxygen, and facilitates resuscitation measures.

It should be noted that general anaesthesia in the field is associated with more constraints compared with general anaesthesia that is performed at the rehabilitation centre. The primary limiting factor is weather conditions that may create a risk of hyperthermia in case of hot weather (the animal should be cooled by water or ice), a risk of hypothermia in case of cold weather, rain or snow, and wind (the animal should be kept warm with the use of blankets, hot-water bottles, or polythene sheets). Harsh weather conditions impose limitations on anaesthetic monitoring (usually, only auscultation, pulse oximetry, and thermometry), as well as on the use of resuscitation techniques. Another factors affecting the choice of the anaesthetic agent are the age and condition of the animal; combinations of drugs are a preferable choice in case of the animals under 10 months of age or weakened individuals.

Selection of the anaesthetic protocol depends on specific conditions and animal state; the choice should be always made by the anaesthetist.

The anaesthesia record is a highly important document that provides information on the anaesthetic parameters and administered medications, and allows for retrospective analysis of the anaesthetic episode, therefore anaesthesia records should be kept during any general anaesthesia procedure. An example of anaesthetic protocol is presented in the Appendix to this document.

1.4 Monitoring during anaesthesia

Anaesthetic monitoring provides information on the vital signs, allows to see individual variations, and makes it possible to notice and assess sudden changes; the monitoring is a necessary process for constant checking of the patient condition and functioning of anaesthetic equipment. The main monitored parameters are the depth of anaesthesia, the state of the patient, and his/her reaction to administered medications and drugs.

Vital signs:

- Parameters of circulatory system;
- Oxygenation;
- CO_2 clearance;
- Temperature.

1.4.1 Non-instrumental monitoring: clinical observation

Circulatory system:

- auscultation;
- pulse rate measurements;
- CRT – capillary refill time (tissue perfusion);
- conditions of the mucous membranes (tissue perfusion);
- condition of arteries (pulse rate, filling of arteries).

Respiratory system:

- auscultation;
- respiratory rate;
- colour of mucous membranes;

- blood colour.

Non-instrumental monitoring is a quite subjective method, but it is easily available, especially in the field conditions, and in general, allows for fairly accurate assessment of the animal's state. Normal respiratory rate in non-anaesthetized large cats is 17–25 breaths per minute and normal heart rate, 40–60 beats per minute.

Assessment of the depth of anaesthesia

Main indicators that should be used for assessing anaesthetic depth are palpebral reflex and corneal reflex (anaesthetic agent affects CNS, and as the eye is the organ located in close proximity to the brain (0.5 cm) the condition of the eyes usually reflects the state of CNS). **During the surgical stage of the anaesthesia (which is a preferable regiment in the anaesthesia of wild animals), the palpebral reflex is lost, but corneal reflex will remain.** Other parameters indicating the depth of anaesthesia are the jaw and limb tone, secretion of tears, and responses of the sympathetic nervous system (respiratory functions and arterial circulation are stimulated by pain, hypoxia, and sharp decrease of arterial blood pressure). One of the indicators of deep anaesthesia is the turn of the eyeball downward (the upper extraocular muscles relax faster than the lower ones).

1.4.2 Instrumental monitoring

- Esophageal stethoscope;
- Measurements of arterial blood pressure;
- Pulse oximetry;
- Electrocardiography (ECG);
- Measurements of body temperature;
- Capnography.

Esophageal stethoscope is a simple, non-expensive medical device for monitoring respiratory and heart functions, but it can only be used after the intubation of the animal is completed since the stethoscope should be inserted in the endotracheal tube in the area of oesophagus.

Pulse oximetry is a non-invasive method of continuous measuring the oxygenation level in the blood. Pulse oximeter probes use a light source consisting of two light-emitting diodes (LEDs), one emitting red light (660 nm), and the other, infrared light (940 nm). A photodetector placed opposite the LEDs measures the intensity of the transmitted light across the vascular bed. The probe is positioned on any site where the pulse can be measured. The measurement principle is based on the difference in the light absorption spectrum between oxygenated and non-oxygenated haemoglobin, and also on the detection of pulse surges.

Pulse oximetry is used to measure the pulse rate and level of the blood oxygenation; some models allow to obtain a photoplethysmogram *(*PPG*)*. However, the pulse should be additionally monitored by the palpation or through electrocardiography, because there are many factors that may affect the readings of the pulse oximer, including *the position of the probe, compromised tissue perfusion, mucous membrane moisture level, ambient temperature, thickness of body tissue, probe shape, pigmentation and hairiness, movements of the patient, peripheral vasoconstriction (medetomidine), and exposure to bright light.*

Saturation – the oxygen saturation of haemoglobin in the arterial blood – is an average amount of oxygen bound to each molecule of haemoglobin. The results of measurements are presented in the form of visual recording of the saturation level (%) and the audio signal with the frequency of radio waves depending on the situation. Thus, pulse oximeters do not provide information on the oxygen content in the blood and only allow for indirect assessment of the body requirements for oxygen. It may create a false sense of safety: *the blood level of CO_2 may be very high because of impaired ventilation even at the normal values of PaO_2.*

In this situation, the pulse oximeter will provide a warning about potentially fatal complication only a few minutes after it actually happens. Delay in the reaction is caused by the fact that the oximeter records an averaged signal; an interval between the real drop in the saturation and changes in the readings of the oximeter is 5 to 20 seconds.

The importance of the pulse oximeter is that it provides a possibility of continuous monitoring of the oxygen saturation of arterial blood. Interpretation of the pulse oximeter readings is as follows:

The normal blood oxygen saturation level should be around 95%. The level of less than 90% reflects the situation of hypoxemia. Values below 75% refer to pronounced hypoxemia, but readings below 75% are usually outside the range of measurement error. If the oxygen saturation drops below 90% insufflation with oxygen at the rate of 2–3 L/min should be attempted with the use of the nasal catheter or endotracheal tube. In the field, 5-litre oxygen tank equipped with the volumetric flowmeter and reduction gear can be used.

Electrocardiography. It should be borne in mind that the cardiogram only reflects the electrical activity of the heart (cardiogram may be normal in the situation of myocardial depression or decreased blood oxygen saturation). The use of this method during anaesthesia is important for monitoring the rhythm and rate of the heart. Various types of equipment for continuous monitoring of the heart electrical activity in real time are commercially available.

Arterial blood pressure

Arterial blood pressure is an important indicator of tissue perfusion. Low blood pressure can deprive the tissues, brain and other vital organs of oxygen. Arterial blood pressure and central venous pressure (CVP) can be measured by direct or indirect methods. In the field, the direct method normally is not used in wild

carnivores. Differences between the outcomes of direct and indirect methods are statistically significant but not clinically important.

In healthy conscious animals, normal systolic, diastolic and mean arterial blood pressure are 140–160, 85–95, and 100–110 mm Hg, respectively. Blood pressure decreases during general anaesthesia (an exception is the case of mono-component anaesthesia with dissociative anaesthetics), but correction measures must be applied only if the mean arterial pressure drops below 65 mm Hg.

Indirect methods for measurement of arterial blood pressure:

1. Doppler ultrasound: the sensor is positioned on the site of the artery (with the use of ultrasound gel). Piezoelectric crystals produce ultrasound waves that reflect off moving, regularly shaped objects (artery wall and erythrocytes), and the change in the wavelength, the Doppler shift, can be registered through the use of loudspeaker. The blood pressure cuff should be wrapped around the limb distal to the elbow. The cuff is inflated by pumping the squeeze bulb to the level above the systolic pressure; then, the valve of the cuff is opened slightly, allowing the pressure to slowly fall, and as the pressure falls, the readings of the manometer reflect systolic and diastolic pressures. Mean arterial blood pressure (MAP) can be calculated as MAP = DP + 1/3(PP), where DP is diastolic pressure and PP is pulse pressure [PP = (SP – DP), where SP is systolic pressure].

2. Oscillometry: an automatically inflated cuff of an appropriate size is placed onto the limb. Deflating of the cuff results in the change of blood pressure, and arterial pulsations are measured by the transducer. Digital readings of the systolic, diastolic, and mean arterial pressure are shown on the display. The width of the cuff should comprise 40–60% of the limb circumference.

Monitoring of body temperature

Body temperature can be measured by an esophageal or rectal temperature probe.

In healthy animals, the peripheral temperature is 2–4^{0}C lower than the core temperature. General anaesthesia causes the dilation of blood vessels, shifts in the temperature pattern and resulting hypothermia, which may be aggravated by low ambient temperature, treatment of skin with cold solutions, abdominal surgery, or injection of solutions that have a room temperature. Such condition may lead to circulatory depression and general decrease in catabolism.

The main mean of preventing hypothermia is keeping the patient warm during anaesthesia and post-anaesthetic period.

Hypothermia is usually caused by the following factors:

- Overheating;
- Fevers associated with septicemia;
- Thermoregulatory dysfunction of the CNS;
- Thyrotoxicosis;
- Chromaffin-cell tumour;
- The use of tiletamine and zolazepam as anaesthetic agents: this combination causes the hypertonicity of skeletal muscles and significantly increases the rate of the heat output from the muscles.
- Malignant hypothermia – a life-threatening complication of general anaesthesia caused by the increases in intracellular ionized calcium due to the activation of ATP (which generates the excessive heat in the cells, leading to the development of hyperthermia).

1.5 The most common complications of general anaesthesia

The most important life-threatening conditions during general anaesthesia are associated with the depression of **cardiovascular and respiratory functions**. Such conditions include:

- **Hypotension;**
- **Hypoventilation;**
- **Hypoxemia;**
- **Hypothermia;**
- **Hyperthermia;**
- **Arrhythmias.**

Hypotension

Generally, the hypotension is a condition when the mean arterial blood pressure is lower than 60 mm HG and systolic blood pressure is below 80 mm Hg. Most serious complications of hypotension are:

1. Acute kidney failure;
2. Damage of organs caused by ischemia;

3. Acidosis associated with elevated plasma lactate concentrations.

Hypotension is diagnosed with the use of the Doppler ultrasound method or oscillometry. Regular palpation for determining the pulse rate is a less accurate method that allows detecting the drop of the pulse wave indicative of the reduction of the arterial blood pressure.

Main method of the treatment of this complication is intravenous infusion of crystalloids (normal saline solutions or Ringer's solution) or colloids (hydroxyethyl starch solution).

Besides, the depth of the anaesthesia should be decreased (by partial reversal with the use of 1/3 of the dose of reversal agent, if alpha-2 agonists were used as anaesthetic agents); if the anaesthesia is maintained by the use of gaseous anaesthetic, the dose of the used anaesthetic agent should be reduced.

With the persistent hypotension, it is recommended to administer sympathomimetic drugs:

Dopamine – 5–10 μg/kg/min;

Adrenaline – 0.1 mg/kg/h.

Hypoventilation

Apnea is the most serious complication of the hypoventilation.

Apnea is the absence of breathing, which is easily recognized by the absence of thorax movements or breathing noises at auscultation. This condition may be the reason of impaired tissue oxygenation and, in severe cases, causes damage to the heart and brain, the two organs that have the highest requirements to oxygen supply.

The treatment of apnea is to assist or support ventilation:

- **Intubation – the diameter of the endotracheal tubes should be 18–22 mm for large Amur tiger individuals, 10 to 14 mm, for young tigers, and 8–12 mm for Amur leopards. Equine laryngoscopes can be used for intubation procedures performed on tigers and leopards.**
- **Manual ventilation – this procedure is carried out through the use of the anaesthetic circuit or self inflating bag (Ambu bag). Manual ventilation can be also done by massaging the patient's thorax until the restoration of spontaneous breathing.**

Besides, general anaesthesia may entail the risk of hypoventilation, the state when insufficient breathing does not satisfy the requirements of the organism (reduced depth and frequency of the breathing movements, reduced lung function).

In the field, hypoventilation can be detected by the reduced breathing rate. The most accurate method, the capnometry and blood gas test, requires special equipment (including anaesthetic and laboratory equipment). A portable device for colorimetric end-tidal carbon dioxide detection may help to detect the problem; the values of $PaCO_2$ above 45 mm Hg indicate the condition of hypoventilation. Elevated level of CO_2 may result in respiratory acidosis and increased intracranial pressure. The treatment of this condition is presented by the artificial ventilation of the lungs.

Hypoxemia

Arterial *hypoxemia* is the state of low oxygen concentration in arterial blood. Hypoxemia may cause hypoxia that leads to cell damage, lactic acidosis, and compromised functions of various organs.

Methods for detecting hypoxemia:

- Pulse oximetry;
- Blood gas test.

Treatment of hypoxemia should address the cause of this condition and, if possible, eliminate it, for instance, by changing the animal's head or endotracheal tube position and delivering oxygen.

Hypothermia

Classification of hypothermia conditions:

Mild hypothermia: temperature ranges between 32°C and 37°C.

Moderate hypothermia: temperature ranges between 28°C and 32°C.

Severe hypothermia: temperature drops below 28°C.

Body temperature can be measured by esophageal or rectal temperature probe **(peripheral temperature may not be representative).**

The most common causes of hypothermia are direct effects of anaesthetic agents, exposure to cold weather conditions, and prolonged abdominal surgeries.

Significant effects of hypothermia include sinus bradycardia and other arrhythmias, hypotension, and significantly lowered metabolic rate.

Treatment of hypothermia:

- Adjustment of anaesthesia depth;
- Keeping the animal warm and rewarming it with the use of blankets, hot-water bottles, circulating warm water blankets, etc.)

Hyperthermia

Hyperthermia is a state of significantly elevated body temperature.

Classification of hyperthermia conditions:

Mild hyperthermia: temperature between 38°C and 39°C.

Moderate hyperthermia: temperature between 39°C and 41°C.

Severe hyperthermia: temperature rise of over 41°C.

Body temperature can be measured by esophageal or rectal temperature probe **(peripheral temperature may not be representative).**

The most common causes of hyperthermia are direct effects of anaesthetic agents (tiletamine/zolazepam), infections, muscle hypertonicity, convulsions, malignant hyperthermia (a genetic disorder that is triggered by gaseous anaesthetics).

The most important effects of hyperthermia are increased metabolic rate, dysfunction of enzyme systems and, as a result, dysfunction of organs.

Treatment of hyperthermia mainly consists of the measures which will rapidly lower core body temperature.

Arrhythmias

An arrhythmia is an abnormal heart rhythm that can be detected by the ECG.

Arrhythmias leads to the reduced cardiac output, development of asystolia or ventricular fibrillation.

The most common types of the arrhythmia are:

- Sinus tachycardia;

- Sinus bradycardia;
- AV block type II;
- Ventricular extrasystoles (VESs).

These complications usually do not affect the functions of cardiovascular system, if they develop during anaesthesia.

The most common causes of the tachycardia are insufficient depth of anaesthesia and analgesia (during painful interventions), hypotension (as an effect of the hypovolemia), hypercapnia, hypoxemia, electrolytic disorders, and hyperthermia.

The primary treatment during an episode of tachycardia is an adjustment of the anaesthetic/analgesic depth, infusion therapy, artificial ventilation, and treatment of hyperthermia.

The most common causes of bradycardia include excessive anaesthetic depth, hypothermia, and excessive vagal stimulation (surgical interventions).

Bradycardia is treated by reducing the anaesthetic depth, ceasing vagal stimulation, and rewarming the animal.

PART 2. COMPELLED REMOVAL OF WILD ANIMALS FROM THE WILD. MEDICAL EXAMINATION, EMERGENCY VETERINARY CARE, AND TREATMENT OF DISEASES

2.1 Medical examination

Many animals are presented to the rehabilitation centre in a critical condition, especially orphaned large cat cubs. Their survival often depends on the efficiency of the emergency medical care. Experience has shown that the majority of conflict situation occur in winter seasons when the condition of the animal is aggravated by harsh weather conditions.

After the presentation of the animal to rehabilitation centre or during the period of capture and general anaesthesia, it is necessary to carry out a full medical examination of the individual and collect samples of various biological materials, which will be used for both clinical tests and future scientific research.

When the animal is anaesthetized, the full clinical examination should be carried out, including the following:

- Temperature measurement (this indicator is important mostly for maintaining anaesthesia);
- Pulse measurements (monitoring of anaesthesia and detecting symptoms of potential arrhythmias);

- Respiratory examination (monitoring of anaesthesia);
- Heart auscultation (monitoring of anaesthesia and detecting arrhythmias and murmurs);
- Lung auscultation (monitoring of anaesthesia and investigating potential lung problems);
- Examination of hair (detection of the skin or hair defects and ectoparasites);
- Assessment of the mobility of joints and intactness of bones;
- Examination of eyes and appendages (presence of injuries);
- Examination of nasal cavity (traumas, discharges, ulcers);
- Examination of oral cavity (condition of teeth, mucous membranes, tongue, and larynx);
- Palpation of the abdominal area (detection of new growths);
- Examination of reproductive organs and anus (presence of discharges or traumas).

If any pathologies requiring further investigation have been revealed (rales in the lungs, nasal discharge, pathological masses in the abdominal cavity, pathologies of the abdominal and thoracic cavities, traumas of the musculoskeletal system, gunshot injuries) it is recommended, when possible, to arrange X-ray or ultrasonic examination. Examination of the thoracic cavity requires quite expensive equipment that is normally not used in the field.

Most common pathologies found in the animals removed from the wild include *dehydration, hypothermia, exhaustion, traumas of varying degree of severity, and infectious diseases.* Emergency medical care is required in all listed cases.

2.2. Dehydration

Dehydration increases the risk of anaesthetic complications as this condition causes compromised tissue perfusion, hypotension, and kidney damage.

First of all, the veterinarian should assess the level of dehydration depending on the deficit of total body water:

<5% ***No clinical signs.***
5–6% ***Mildly decreased skin turgor.***
6–8% ***Significantly decreased skin turgor; possibly, dry mucous membranes.***
8–10% ***Skin fold persists; possibly, sunken eyes.***
10–12% ***Skin fold persists; sunken eyes; dry mucous membranes; symptoms of decreased tissue perfusion (CRT >2 sec).***
12–15% ***Symptoms of shock. Death.***

Obviously, the presented symptoms of dehydration are not objective; besides, it should be remembered that overweight and young animals look less dehydrated than they actually are. One of the most objective criteria is the weight loss; it can be argued that the loss of 1 kg of body mass leads to the loss of 1 litre of

body fluid. Hyperproteinemia and reduction in hematocrit level are additional reliable criteria of the degree of dehydration.

Considering the pattern of the body fluid distribution among the compartments (for large animals, the fluids in the intracellular fluid compartment (ICF) amount to 40% of the total body mass, fluids in interstitial compartment (ISF), to 15% of body mass, and fluids in intravascular fluid compartment, to 8% of body mass), the options of rehydration therapy are limited because the fluids are injected in the intravascular compartment containing only a small proportion of the total body fluids; besides the capacity of this compartments is restricted to the total volume of blood vessels. In addition, body fluids are the solutions of electrolytes and colloids containing positively or negatively charged ions. They play a vital role in maintaining the homeostasis and fundamental physiological processes (blood clotting, heart and neuromascular functions) due to the regulation of colloid osmotic pressure.

Water balance of the body is controlled by fundamental biochemical principles related to the plasma electrical neutrality, plasma colloid osmotic pressure (created by cations and anions of various salt solutions), and oncotic pressure of colloids (mainly, of plasma proteins).

Common medications used to treat dehydration and blood loss

CRYSTALLOIDS

Crystalloid solutions contain water and electrolytes.

There are 3 types of crystalloid solutions:

- isotonic;
- hypertonic; and
- hypotonic.

Main advantages of crystalloid fluids:

low reactogenecity;

no effects on kidney function;

no effects on immune system;

no significant effects on homeostatic system.

Crystalloid solutions, especially isotonic saline solutions (potassium-magnesium asparaginate, Ringer's or Hartman's solution), help to normalize the balance of electrolytes.

Main disadvantage of crystalloids is that they rapidly distribute into the extracellular fluid compartment; 75–80% of the administered solution is found in the interstitium within one to two hours

after infusion. Because of the short volemic effect, larger volumes of sole saline solutions are required for successful rehydration therapy, which pose a risk of the development of hypervolemia and oedematous syndrome.

Isotonic sodium chloride solution is an aqueous solution of 0.9 percent sodium chloride (NaCl). It is subacidic and slightly hypertonic to the blood plasma. Intravenous injection of large volumes of this solution may trigger hyperchloremic metabolic acidosis, though such cases are rather rare.

Lactated Ringer's solution is considered a more physiologically compatible fluid than isotonic sodium chloride solution. Ringer's solution is a balanced combined solution containing sodium chloride, potassium chloride, calcium chloride, and sodium bicarbonate. Lactate is used as a buffer. The Ringer's solution is widely used for the treatment of patients suffering from traumas of unknown etiology. This solution is isotonic with blood plasma. Anions of the weak lactic acid bind to hydrogen ions; the resulting compound enters metabolic pathways and degrades or is converted into glucose in the liver. These reactions lead to the increase in the pH.

COLLOIDS

There are several synthetic colloid solutions including:

- dextrans;
- gelatin derivatives;
- hydroxyethyl starches (HESs).

Dextran is a complex branched water-soluble glucan. Dextran-based solutions are classified into two categories depending on their properties as plasma volume expanders:

- Low molecular weight dextrans (e.g., Dextran-40): plasma volume efficacy – up to 175%; duration of action – 3–4 hours;
- Medium molecular weight dextrans (e.g., Dextran-70): plasma volume efficacy – up to 130%; duration of action – 4–6 hours.

Main problems associated with the use of dextran-based solutions as plasma volume expanders:

- high reactogenecity;
- narrow therapeutic window.

Infusion of large volumes of dextran-based solutions may cause the following negative effects:

- prolongation of hypocoagulation state caused by heparin-like effects;

- blockade of the mononuclear phagocyte system (MPS);
- “dextran-caused burn of the kidneys”;
- various lung pathologies;
- tissue dehydration;
- renal failure;
- pathologic intra- and post-surgery bleedings.

Dextran-40 is used as a 10% solution; the colloid osmotic pressure of Dexran-40 is about 40 mm Hg. Increase in the circulating blood volume, resulting from intravenous infusion of Dextran-40 may double the volume of the infused liquid. At the same time, it should be remembered that over 50% of the infused solution will be excreted from the organism as soon as within six hours. Dextran-40 may cause increased bleeding associated with the reduced platelet aggregation and inhibited factor VIII activity. Dextran-40 may also facilitate fibrinolysis. Yet, for producing of anticoagulation effect, administration of quite high doses of Dextran-40 is needed (about 1.5 g/kg/day). Intravenous infusion of dextran solutions may cause acute renal failure. Possible mechanism of this phenomenon may be attributed to hyperosmotic properties acquired by glomerular blood, which leads to the reduction in the effective glomerular filtration pressure affecting glomerular filtration rate.

A hydroxyethyl starch is a high molecular weight substance consisting of polymerized glucose residues; hydroxyethyl startches are derived from potato tubers and a variety of corn, wheat, and rice seeds. The hydroxyethyl starch produced from potato and waxy corn contains a fraction of highly branched amylopectin in addition to linear chains of amylase. High concentration of the amylopectin in the potato starch (75–80%) and waxy corn starch (over 95%) makes the starch of these plants a useful source of plasma volume expanders.

Comparison of the hyrdoxyethyl starch with colloidal plasma volume expander produced from different types of materials (albumin, dextran) reveals specific characteristics and advantages of colloidal solutions derived from the hyrdoxyethyl starches:

- safety of the use and extremely rare cases of side reactions (which is attributed to the similarity of the structures between hyrdoxyethyl starch molecules and glycogen);
- rare cases of allergic reactions (compared with dextrans);
- no antigen-antibody reactions (that are typical for dextran-based solutions).

Different preparations of HESs with various structural characteristics have different physicochemical properties governed by three main variables:

- MM – molecular mass;
- MS – molar substitution; and
- DS – degree of substitution.

All HESs are polydisperse and contain molecules of different sizes. Different HES solutions are classified by average weight that is expressed in Daltons (Da). The larger is the molecular weight (e.g., 200,000 – 400,000 Da) and degree of substitution (0.5 – 0.7), the longer the solution will remain in blood vessels.

- HES solutions with the average molecular weight of 200,000 Da and DS of 0.5 are included in the pharmacological group of Pentastarches;
- HES solutions with the average molecular weight of 450,000 Da and DS of 0.7 are included in the pharmacological group of Hetastarches.

These differences should be considered when selecting the HES solution for infusion therapy. The weight-average molecular weight (Mw) is calculated by the fraction of the total weight represented by each type of molecule. The lower is the average molecular weight and the more low molecular fractions are contained in the polydisperse solution, the higher is the colloid osmotic pressure. Hydroxyethyl starches have the ability to seal endothelial *"*pores*"* that develop in the microvessels after different forms of endothelial injuries. The duration of plasma volume expansion for HES solutions is usually 24 hours. HES solutions are mainly excreted by kidneys. Polymers of hyrdoxyethyl starches with the molecular weight of less than 59,000 Da are quickly eliminated in the kidney by glomerular filtration. Elimination by glomerular filtration occurs also after the hydrolysis of larger fragments into the smaller ones. Oncotic pressure exerted by HES solutions does not affect the flow through the large pores, influencing only the flow through small pores that comprise the majority of capillary pores.

Thus, the first stage of the balanced infusion therapy is the infusion of crystalloid solutions for urgent treatment of hypovolemia. At the second stage, the volume of circulating blood should be maintained by the infusion of colloid solutions.

Infusion therapy technique

Infusion therapy involves the use of peripheral venous catheters (the higher the rate of infusion, the larger gauge catheter is required) that should be placed in a peripheral vein (insertion of a central catheter is an extremely difficult and even dangerous procedure in the field conditions).

Flow rates for the catheters of different sizes:

Gauge	Colour code	Flow rate (ml/min)
14G	Orange	250–360
16G	Grey	130–220
18G	Green	75–120
20G	Pink	40–80
22G	Blue	20–40
24G	Yellow	10–20

Volumes of medical solutions

Crystalloids:

A healthy animal, infusion during anaesthesia: 10 ml/kg/h.

An animal suffering mild dehydration or bleeding: 15–20 ml/kg/h.

An animal in the condition of shock: up to 90 ml/kg/h.

Colloids:

10–20 l/kg/d, by slow bolus during 30–60 minutes.

2.3. Hypothermia

Hypothermia is a state of significantly reduced body temperature in homoeothermic animals, which develops when the heat loss rate is higher than the rate of the heat generation in the body.

Hypothermia is defined as a body core temperature below 35.0°C. Hypothermia can be distinguished into three stages – mild (32.0–35.0°C), moderate (28.0–32.0°C), or severe (below 28.0°).

Hypothermia develops when a body loses its ability to maintain the core temperature through internal physiological processes, such as a vasoconstriction, shivering, muscle contractions, or non-shivering thermogenesis. When the shivering ceases and the body is unable to maintain the normal temperature it drops below 35.0°C and hypothermia starts developing.

The heat is generated in all parts of the body due to metabolic processes. The body of the healthy animal regulates temperature by keeping a tight balance between heat gain and heat loss, which allows to maintain a normal body temperature. Heat is lost in different ways including the radiation (55%), evaporation (5% from respiratory tract and 25% from the skin surface), conduction (15%), and convection (5%). Thermal conductivity of water is 20–30 times higher than that of the air, therefore the thermal conductivity increases, when the body is submerged in water.

The balance between heat loss and heat gain is controlled by the hypothalamus. Signals generated by the cold receptors of the skin are transmitted to the preoptic area of hypothalamus through the lateral

spinothalamic pathway. Posterior area of the hypothalamus activates sympathetic nervous system, which results in the increase of the tonicity of skeletal muscles and, consequently, in shivering, and the body heat production increases from 40–60 kcal/h to 300 kcal/h. If the heat losses are still higher than heat gains, total body heat progressively falls. Once the core temperature drops below 30.0°C, shivering usually stops, heat production falls, and the rate of cooling sharply increases.

Sharp body cooling stimulates the sympathetic nervous system, which results in tachycardia, increase in cardiac output, and arteriolar spasm. Drop of the body temperature below 32.0°C results in compromised conductivity of myocardium, heart rate and cardiac output reduction, and often in ciliary bradyarrhythmias; the ventricular fibrillation occurs once the temperature drops below 28.0°C.

Arteriolar spasm causes the blood to flow into the mainstream, which leads to the compensatory increase in diuresis for reducing the circulating blood volume (cold diuresis). During episodes of prolonged supercooling, this effect, together with the fluid flow out of vessel beds and impaired renal epithelial transport resulting in the reduction of sodium and water reabsorption, may cause a severe hypovolemia and arterial hypotension.

In its turn, the hypovolemia causes haemoconcentration and increases blood viscosity, which are the risk factors for thrombosis. At the same time, coagulation disorder, thrombocytopenia, and disseminated intravascular coagulation (DIC) at low temperatures may result in severe blood loss even in case of a minor injury.

The degree of the acid-base and electrolyte imbalances depends on the duration of the persistence of low body temperatures. Ischemia may cause lactate acidosis and, as a result, respiratory alkalosis. The respiratory depression in severe hypothermia leads to respiratory acidosis. Concentrations of electrolytes in serum remain normal, but in the absence of traumas or rhabdomyolysis, an intensive inflow of potassium into the cells may result in hypopotassuimia.

Hypothermia may also cause the bronchorrhea and bronchial spasm. As the hypothermia progresses, the tachypnea is replaced by hypoventilation. The loss of the cough and pharyngeal reflexes combined with the state of unconsciousness may lead to aspiration and pulmonary oedema. At low temperatures, the oxyhaemoglobin dissociation curve is shifted to the left, and oxygen transport to hypothermic tissues is impaired, but the body oxygen requirements are reduced due to the reduced metabolic rate.

Hypothermia reduces cerebral blood flow, and reduced neuronal activity slows down the transmission of nerve impulses. Neurological dysfunctions associated with hypothermia include ataxia and retardation of the papillary and tendon reflexes. Severe hypothermia may lead to coma. In this situation, the EEG may sometimes show no electrical activity in the brain (isoelectric line), though in such cases, it does not mean the brain death and this condition may be reversible.

Hypothermia increases the secretion of corticoliberin and thyroliberin, which activate the secretion of ACTH and TSH. The blood levels of cortisol and catecholamines are also elevated. Blood flow from the

capillaries reduces the release of ADH, which leads to increased diuresis. Moderate or severe hypothermia induces insulin resistance and hyperglycaemia. Severe hypothermia eventually results in the reduced secretion and activity of ACTH and steroid hormones.

Moderate hypothermia often affects the intestinal motility. Half-life of many substances may be reduced because of the depression of liver metabolism.

Treatment of hypothermia

Rewarming can involve active and passive, and internal and external methods.

Passive external rewarming (blankets and heated room) is the simplest and safe method that should be used at the first stage of the treatment on the patients suffering from mild hypothermia. This approach can help the body to use its own thermoregulation mechanisms; the core body temperature may increase at the rate of 0.5–2.0°C per hour.

Active external rewarming includes the use of external sources of heat (electric blankets, electric radiators or heaters). Blankets and other warming means should be only placed on the chest; otherwise, there is a high risk of the following complications:

– Angiestasis caused by the skin exposure to heat eliminates an important mechanism of the maintenance of arterial pressure in the state of hypovolemia and may result in shock;

– Cooled blood from the dilated peripheral vessels flows into the mainstream, which causes a further reduction of body temperature;

– Potential risk of the body surface burns.

Internal rewarming can be achieved in several ways. The simplest method is the inhalation of humidified oxygen heated to 42°C via the mask or endotracheal tube; this approach allows to increase body temperature by 1.0–2.0°C per hour.

Intravenous infusion of fluids heated to 40°C has little effect and is not used, if rapid rewarming is required. Therapy with warm isotonic solutions (e.g., normal saline solution or 0.45% NaCl solution with 5% glucose) is recommended for the patients with hypovolemia. Lactated Runger's solution is not used for the patients with hypothermia because of impaired lactic acid metabolism.

Physical manipulations should be restricted; great care should be taken when performing catheterization of major veins, insertion of the nasogastric tube, or tracheal intubation, as such procedures may cause heart rhythm disturbances.

Acid-base balance usually restores as the body temperature increases; all medications should be used with care because their effects change and their excretion slows down at low temperatures. If septicemia is

suspected, broad spectrum antibiotics should be administered whereas the blood culture results are not available.

Rewarming of the animal decreases the blood glucose levels. Insulin becomes inactive at the temperatures below 32°C, and surplus amount of insulin during rewarming may result in hypoglycemia. Besides, the prolonged shivering leads to the exhaustion of muscle glycogen stores, which also facilitates the development of hypoglycemia.

Potassium levels should be closely monitored during rewarming. Hypopotassuimia usually does not require any special measures as it is eliminated as the potassium is released from the cells, while hyperglycemia may be indicative of the extensive tissue necrosis (rhabdomyolysis) and sometimes of the renal failure.

ECG monitoring is very important in the treatment of hypothermia as rewarming often induces atrial arrhythmias.

2.4 Frostbites

A frostbite is an injury that is caused by exposure of parts of the body to the cold. Frostbite is often combined with the general cooling of the body and commonly affects such animal body parts as the tip of the nose, pinnae, upper limbs, and tail.

When the body is exposed to the cold, tissues undergo complex changes, which depend on the level and duration of the low temperature conditions. At the temperatures below –30°C, most important consequences of the frostbites are the effects of the cold on the tissues where the cell death occurs. At the temperatures between –10°C and –20°C, which typically cause frostbites, key factor is the vascular changes presented by the capillary vasospasm; as a result, the blood flow reduces, tissue enzymes are inactivated, and oxygen transport to the tissues significantly reduces. Ice crystals may form within the cells and in intercellular space, preventing the normal activity of the sodium-potassium pump and breaking cell membranes. Additionally, this condition may cause erythrocyte or platelet aggregation followed by microemboli or thrombosis. As a result of the secondary vascular response to the cold injury, blood flows out of the damaged area, which further affects its perfusion, at the same time increasing the perfusion of other tissues. The size of affected area may vary from small to very large, reflecting structural and functional damages of small blood vessels, nerves, and skin.

The therapy of frostbites involves rewarming of the frostbitten areas (the best way is to use lukewarm water), keeping the damaged areas dry, and administration of local and systemic antimicrobial therapy.

2.5 Exhaustion

Exhaustion is always observed in the wild animals removed from the wild, which are not able to hunt and kill a prey (because of young age, injuries, or diseases).

Cachexia is the state of extreme exhaustion. It is characterized by sharp progressive weight loss, general weakness, slowing of physiological processes, and changes of behaviour.

The purpose of the animal examination is to determine the primary cause of the exhaustion and perform the infusion therapy; it is also advisable to do an X-ray examination to exclude the presence of foreign bodies in the stomach, since hungry animals may swallow large bones or inedible objects.

When the animal is stabilized, it can be fed meat; very young individuals can be fed specialized enteral formulas via nasogastric tube.

2.6 Traumas

The first step should be the assessment of the severity of the trauma to make the decision on the feasibility of the treatment, as in most cases, it is not possible to help the animals suffering severe traumas and even to maintain the quality of their life in zoo environment. If a tiger or leopard has a spinal cord fracture, a fracture of two or more limbs, and open craniocerebral injury, it should be understood that such animal will most likely die.

In general, the tactics of dealing with an injured animal should involve the use of tranquillization and analgesia (the best anaesthetic agent of choice is zolazepam/tiletamine/medetomidine combination), treatment of shock (infusion therapy), and animal transportation to the clinics for comprehensive examination and, if necessary, surgical intervention.

Capture-related traumas

Capture with the use of snares may result in the injuries of skin in the area caught in the snare wires. Non-steroid anti-inflammatory drug ketoprofen in the dose of 2.2 mg/kg can be used for the treatment of pain syndrome and inflammation. If an animal has cuts or skin cracks, long-acting antibiotics can be administered, for instance, amoxicillin suspension (150 mg/1 ml) in the dose of 1 ml per 10 kg of the body mass (IM).

PART 3. TRANSPORTATION OF THE ANIMALS TO THE QUARANTINE FACILITIES

Transportation and associated procedures are the important stress factors for the transported animals; besides, they pose a potential threat to people. All animals should be transported in special transport crates, which will help to prevent both animals and humans from being injured.

If a tiger is in a serious condition, a veterinarian should provide it with an emergency treatment before the transportation. **Long distance transportation may be started only after the patient has been at least somewhat stabilized (due to the fluid and antibiotic therapy, rewarming, etc.).** If there is no veterinarian available, the animal should be transported to the nearest veterinary hospital for stabilization.

- Tigers are able to endure even a long-distance transportation, and the main requirement is the use of the transport crate of appropriate sizes and design, and keeping the animal in the darkness. Carries for large dogs can be used for the transportation of tiger cubs under 6 months of age. The sizes of the transport crate for the tiger cubs aged between 6 and 12 months are 1.0 m (L) x 0.7 m (W) x 0.7 m (H); for the animals aged over 1.5 years, the crate should have the following sizes: 1.0 m (L) x 0.7 m (W) x 1.0 m (H).

- Depending on weather conditions, specific measures should be taken to protect the animal(s) against overcooling or overheating.

- Each individual animal should be transported alone in a separate transport crate.

- A veterinarian should supervise the process of transportation and have all the equipment and medications that may be needed for general anaesthesia and emergency care procedures.

- In case of the transportation of a healthy tiger after anaesthesia to the release site, food and water may be withdrawn during the first 12 hours; afterwards, water should be given to the animal every 5–6 hours. Tiger cubs under 9 months of age should be given food according to their ages.

Animal transportation to the rehabilitation facilities

- Tiger cubs under six months of age and older cubs in serious condition should be kept warm during transportation.

- A tiger in a severe condition should be provided with emergency care before the transportation. If the transportation takes more than 2 hours, it should be started only when the patient has been at least somewhat stabilized (due to infusion therapy, antibiotic therapy, rewarming, etc.). If there is no veterinarian available, the animal should be transported to the nearest veterinary hospital for stabilization.

- Participation of the veterinarian in the transportation of the weak animals is mandatory. If it is not possible, such animals should be urgently transported to the rehabilitation centre; a veterinarian at the rehabilitation centre may be consulted by telephone during transportation.

Animal transportation to the release site

- The transportation time should be as short as possible to minimize transport stress.

- Logistic issues should be given serious attention when preparing the animal transportation. All details should be considered and backup plans developed (e.g., what actions should be taken, if the vehicle has broken down; who will be dealing with the crate unloading on site; how quickly the crate with the animal will be carried to the release site in the forest, etc.). All these matters will affect the duration of the animal's stay in the transport crate.

- The transport crate of appropriate sizes, equipped with the waterer should be used for the animal transportation.
- If the transportation lasts for more than 5–6 hours, it is advisable to regularly evaluate the animal condition and provide the animal with water every 5–6 hours.
- Transportation of animals by plane over the distances of less than 1,500 km is not reasonable because of complex logistics and remoteness of the airport from both rehabilitation centres and release sites. Transportation by helicopters is justified, if the animal can be transported directly to the release site.
- All permits and documents applying to the transported animals, issued under the federal and regional legislation, should be in place during transportation.

PART 4. QUARANTINE

Each animal admitted to the rehabilitation centre should be placed in the quarantine holding area. This measure is mandatory as it helps to prevent the spread of infectious and invasive diseases. This is particularly important in case of young animals or animals that are weakened by the cold or hunger and are at a higher risk of infections because of the weak immune system.

Quarantine procedures:

a) Infectious and other diseases should be identified and treated. It is not recommended to keep the animals in the rehabilitation centre, if identified diseases require a long treatment.

b) The animals should be vaccinated.

c) Any abnormal behaviours that may hamper successful release and survival of the animals in the wild should be identified and a decision should be made on whether these behaviours can be corrected. If it is not possible, wildlife specialists and supervising bodies should be informed about the situation to make a decision on the further actions, and the animal in question should be removed from the rehabilitation centre at the first opportunity.

4.3. Husbandry procedures during quarantine period

Each animal admitted to the quarantine should be examined and all necessary samples should be collected according to examination protocol.

- At the animal admission to the quarantine, the fur colouration pattern (stripes or spots) should be photographed and presence of any injury (torn ears, large scars, etc.) should be recorded to facilitate

identification of the animal; the animal should be fitted with a transmitter or otherwise marked (if the animal has been anaesthetized and if marking is possible in view of the animal's age).

- If the animal is young and exhausted, at the first stage, it should be given soft, high-energy foods containing a large proportion of proteins and fats (commercial diets for cats in post-surgical period can be used), but the animal should be fed on meat as soon as its appetite, stool and activity return to norm.

- The length of the quarantine period should be at least 30 days.

- Release from the quarantine should be dependent on obtaining negative results of the parasitology and virology tests but it should not be earlier than 30 days after the start of the quarantine period.

- If during the 30-day quarantine period any of the quarantined animals developed a disease, the 30-day period must begin again from the date of the detection of the ill animal.

- A professional veterinarian who has an experience in the work with wild cats should be involved in the procedures of anaesthesia, examination, vaccination, and treatment of animals.

- When the animals arrive to the quarantine from different areas, they should be put in the quarantine enclosure in the same groups, in which they were found at the time of their removal from the wild. Group structure can be changed only after the end of the quarantine period.

- During quarantine period, the animals should be kept in specially equipped quarantine enclosures. Sick and exhausted animals or individuals suffering hypothermia should be placed in the heated room with the ambient temperature of 15°C or higher. The construction of such room should be planned at the pre-building stage. The animal should be kept in this room until the full recovery and then gradually moved to the outdoor enclosure.

- Quarantine facilities for each animal group should have separate equipment and disinfection barriers. Disinfection mats are a practical and inexpensive solution to the disinfection of personnel's footwear and vehicles; besides, such mats are durable[1]. Any modern disinfectant can be used for disinfection, for instance, Alamynol, "Caro's acid", or Lysoformin 3000[2]. Disinfectant solutions should be prepared according to the instructions from manufacturers. Disposable shoe covers may be used in winter time.

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[1] http://www.dezkovriki-dezkov.ru/dezkovriki-ekonom.html)

[2] Alamynol (5 % of alkyl dimethyl benzyl ammonium chloride and 8% of glyoxal, "Caro's acid" (Potassium peroxymonosulfate, 50%), Lysoformin 3000 (9.5% of glutaric aldehyde, 7.5% ofglyoxal, and 9.6% of didecyldimethylammonium chloride)
http://www.saniks.ru/catalog/index.php?SECTION_ID=1#view%2Fcatalog%2F%3FSECTION_ID%3D1%26ELEMENT_ID%3D85

- Overalls and footwear used by the personnel working with a certain group of quarantined animals should not be used for the work with other animals kept in the quarantine facilities or other enclosures. Overalls should be kept in a specially designated area of the quarantine facilities. If any infection is suspected, the overalls should be soaked in the disinfectant solution (Alamynol); otherwise, all overalls and other working clothes should be washed once a week.

- Animal observation chart (in electronic or paper-based form) should be kept for every animal during the whole quarantine period, where data about the animal's condition (including consumption of food, physical activity, and behavioural changes) are recorded on a daily basis. Staff members should immediately inform the director of the rehabilitation centre and attending veterinarian about any changes that may require veterinary or zoological measures.

- In the course of any manipulation of the animal under general anaesthesia, the animal should be examined and all necessary samples should be collected according to examination protocol.

- After the end of quarantine period and release of the animal(s) from the quarantine, the enclosure should be cleaned mechanically (removal of food scraps and faeces) and feeders and water bawls should be disinfected.

- If infectious diseases were detected during quarantine period, the enclosure should be cleaned mechanically (removal of food scraps and faeces) and feeders and water bawls should be disinfected; no animals should the placed in this enclosure for at least six months.

- In case of an animal death, an autopsy should be performed with the involvement of the pathologist, veterinarian of the rehabilitation centre, and an authorized veterinarian. All listed persons should sign the PM protocol. Samples for histological and bacteriological tests should be taken (if the animal died within two hours before the autopsy).

- Disposal of biological waste should be carried out according to the current veterinary legislation (through incineration of waste or placement it in special pits). A practical way of dealing with this problem is the conclusion of the agreement on the disposal and utilization of biological waste; more complicated approach includes obtaining a license for the construction and management of the waste pit or incinerator.

- Under the Russian legislation, all staff members working with wild animals should be vaccinated against rabies. If a staff member is not willing to be vaccinated or if there are contraindications to the vaccination he/she must submit a formal written refusal of vaccination to the relevant medical institution.

- No visitors are allowed in the rehabilitation centre.

- Domestic carnivorous animals should not be allowed in the area of rehabilitation centre.

4.2 Vaccination and dehelminthization

Vaccination

Vaccination may prevent the development of some infectious disease or reduce their manifestations, duration or symptoms but it does not produce protection against other types of infection.

Only healthy animals that do not show any signs of diseases, have normal stool, appetite, and urination, and are free from helminths (animal after dehelminthization or those with negative faecal test results) can be vaccinated.

Vaccination should be carried out not earlier than 7 days after dehelminthization.

For developing durable immunity non-vaccinated animals should be vaccinated two times. Animals should be revaccinated not earlier than 21 days and not later than 30 days after the initial vaccination.

Tigers and leopards are vaccinated against infectious enteritis (FIE or feline panleucopaenia), feline rhinotracheitis virus (FRT), feline calici virus (FCV), and rabies. Cases of canine distemper virus (CDV) have been documented but a vaccine for felids has not been developed yet, while the safety and efficacy of the existing dog vaccines in felids have not however been established.

It is preferable to use inactivated vaccines as they are less dangerous for the animals and a viral strain can not mutate into a pathogenic form. Fel-o-Vax IV[3] is usually available in Russia. If it is not available, attenuated Nobivac Tricat Trio vaccine[4] may be used.

- Nobivac Rabies[5] is used for vaccination of felids against rabies.
- Each animal should be vaccinated with a single standard dose prescribed for domestic cats, regardless of the animal sizes.
- Initial vaccination should not include vaccination against rabies, but the vaccine against rabies should be included into the booster.
- Vaccination against rabies should be registered with the state veterinary service.

Dehelminthization

- Dehelminthization is carried out based on the faecal test results.

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[3] Fel-o-Vax IV – contains inactivated feline rhinotracheitis virus, feline calici virus, panleucopenia virus and *Chlamydophila felis*.

[4] Nobivac Tricat Trio – live vaccine; one dose contains no less than 5.2 lg FIGHTS of a virus of feline viral rhinotracheitis (strain of "G 2620A"); 4.6 lg FIGHTS of feline calici virus (strain of “F 9”); and 4,3 lg FIGHTS feline panleucopenia virus (strain of "MW-1").

[5] Nobivac Rabies – an inactivated, adjuvated vaccine against rabies containing inactivated strain Pasteur of rabies virus.

- When sample collection or tests are not feasible, dehelminthization is carried out as a prophylactic measure.

- The following anthelmintics can be used: Caniquantel PLUS (50 mg of Praziquantel and 500 mg of Fenbendazole in one tablet; dosage: 1 tablet per 10 kg of body weight); Polyverkan (40 mg of Oxibendazole and 200 mg of Niclosamide in one cube; dosage: 1 cube per 10 kg of body weight), and Pratel (144 mg of Pyrantel embonate and 50 mg of Praziquantel in one tablet; dosage: 1 tablet per 10 kg of body weight).

- Calculation of the doses of anthelmintics is based on the animal weight that should be determined in the course of the initial physical examination procedure. If weighing appears impossible, animal weights should be estimated on the basis of the following data: a tiger weighs 40–60 kg at the age of 5–6 months, 60–80 kg, at the age of 6 – 8 months, and 80–120 kg, at the age between 8 months and 1.5 years. A final estimation of the dose which will consider individual differences in tiger weights should be done by the specialist responsible for dehelminthization procedure.

- Anthelmentics should be mixed with food and fed to the animals.

4.3 Ectoparasites

All animals should be evaluated for ectoparasites. If the examination reveals large ticks, mites, or parasitic insects (fleas and lice) infected animals should be treated with Fipronol-based (e.g., Frontline[6]) or Tetrachlorvinphos-based (e.g., insectoacaricidal spray "Hartz"[7]) parasitocides.

If any lesions of non-traumatic character are detected on the animal skin or hair, scrapings should be taken and placed on two slides for parasitological tests (the slides with the samples should be kept in special cassettes or in plastic bags at room temperature until their delivery to the laboratory). The treatment regiment will depend on the diagnosis determined by the parasitologist.

PART 5. SAFETY REQUIREMENTS RELATED TO THE WORK WITH LARGE CARNIVORES

Personnel working with large carnivorous mammals should have specific qualifications.

Keepers should have knowledge and experience in the area of the species biology including:

[6] Frontline; active ingredient – Fipronil C12H4CL2F6N4OS, 0.25 %
[7] Spray Hartz – insectoacaricidal spray; acting ingredient – Tetrachlorvinphos, 1.08%.

- Background biological information (morphometry, reproduction);
- Background physiological information (adaptations to the conditions of the Russian Far East, physiological characteristics);
- Behavioural characteristics, shaping of natural behaviour;
- Feeding;
- Safety requirements related to the work with wild animals.

The veterinarian should have:

- Professional qualifications in wildlife veterinary;
- Knowledge in the area of safety requirements related to the work with wildlife.

Safety requirements

Personnel working with large carnivores should closely observe safety standards:

- At least two staff members should work together when cleaning the animal enclosures or providing food.
- Feeding procedure should not pose any risk to humans or animals.
- Sliding doors and gates should be locked immediately after their use.
- The use of special overalls, footwear, and gloves during working procedures is mandatory.
- Before any manipulation of the animal is started, each staff member should be assigned a particular function that he/she will have to implement during the planned procedure. In case of general anaesthesia, there should be one specialist (a veterinarian) responsible for the entire process.

Sanitation requirements

Animals and foods of animal origin can be a source of infections to personnel, therefore strict measure of sanitary safety should be taken to prevent the spread of infections:

- The use of special overalls, footwear, and gloves during working procedures is mandatory.
- All foods should be accompanied by the relevant veterinary documents.
- Feeding sites, feeders and watering devices should be cleaned and disinfected on a regular basis.

- When working with the animals during general anaesthesia the use of disposable overalls and gloves is mandatory.

- Holding facilities for newly arriving animals should be equipped with disinfection barriers.

It is also important to understand that the animals removed from the wild can be a source of infection to personnel, for instance, of rabies, a zoo-anthroponotic infection that may be transmitted to a human through the bite of an infected animal. Strict personal safety measures should be taken, especially if the animal demonstrated any signs of neurological disorder before its removal from the wild. If such signs have been detected, the animal should be kept in isolation and all further manipulations of such animal should be carried out under the supervision of the state veterinary service representatives.

On the other hand, the staff members of the rehabilitation centre can pose a risk of infection for the animals kept at the centre. Such infections are mainly related to the mechanical transmission of pathogens from domestic animals, such as feline infections (calici virus, rhinotracheitis virus, panleukopenia virus, intestinal nematodes), therefore domestic animals should not be allowed within the area of rehabilitation centre and all staff members should comply with recognized sanitary regulations.

PART 6. RECORD KEEPING

Record keeping is an important component of the rehabilitation process, that may provide conservationists and other specialists with valuable retrospective information about this relatively new, complex process.

APPENDIX

1. ANAESTHETIC PROTOCOL

Species_____ **Name** _____

Date _____ **Weight** _____ **Age** _____

Veterinary clinic_____ **Sex** _____

Name of anaesthetist _____

Purpose _____ **Clinical examination** _____

Risk **1** **2** **3** **4**

Previous episodes of general anaesthesia **yes** **no**

Whether the animal was starved **yes** **no**

Pre-surgery condition

HR_____ **RR** _____ **Temperature** _____

Appetite **yes** **no**

Thirst **yes** **no**

Pre-medication

Medications_____ **Sedatives**_____ **Analgesics**_____ **Antibiotics**_____

Dose _____

Time _____ **ET-tube** _____ **Position** _____

Effect _____

ECG _____ **Pulse oximetry** _____

General anaesthesia **AP** _____ **Other monitoring equipment** _____

Anaesthetic agents **Catheter** ____ **Fluid**_____

Doses

Time / Drug												

Notes

Time / Parameter												
SO2												
Heart rate												

2. CLINICAL EXAMINATION PROTOCOL

General information

Date, time

Place

Species

Immobilization method

Physical restraint □

Chemical immobilization □

Anaesthetic agent

Dose

Induction method

Additional information

Physical examination:

1. External examination

Sex ♀ ♂

Age (assessed)

External integuments

Presence of injuries or mutilations

2. General condition of the animal:

Nutritional state (fat)

Degree of hydration

Weight

3. Measurements

4. Oral cavity:

Colour of the mucous membranes

Dental formula:	**Maxilla:**	**I**	**C**	**P**	**M**
	Mandible:	**I**	**C**	**P**	**M**

Injuries, pathological conditions

5. Cardiorespiratory system:

Auscultation

Percussion

6. Musculoskeletal system:

Reflexes

Mobility of bones and joints

7. Gastrointestinal tract: results of transabdominal palpation

8. Reproductive system: examination of the external genitalia; results of rectal examination

9. Eyes and ears

List of biological samples

	Sample type	**Number**	**Description**
Blood			
Faeces			
Swabs			
Photographs			
Ectoparasites			
Other/samples			
Comments			

3. THE LIST OF THE MOST IMPORTANT VETERINARY MEDICATIONS AND EQUIPMENT FOR THE LARGE FELID REHABILITATION CENTER

- **Zolazepam/tiletamine**
- **Ketamine**
- **Medetomidine/dexmedetomidine**
- **Xylazine**
- **Doxapram**
- **Adrenaline**
- **Atropine sulphate**
- **Dobutamine**
- **Furosemide**
- **Prednisolone**
- **Normal saline solution**
- **Ringer's solution**
- **Glucose 5%**
- **Amoxicillin suspension 150 mg/ml**
- **Enrofloxacin 5%**
- **Intravenous catheters**
- **Intravenous dripping systems**
- **Syringes: 1 ml, 2 ml, 5 ml, 10 ml, and 20 ml**
- **Soft patch**
- **Cotton pads**
- **Disposable gloves**
- **Non-sterile bandages**
- **Stetophonendoscope**
- **Pulse oximeter**
- **Bedside monitor with the battery**
- **Portable X-ray machine**
- **X-ray digitizer**
- **Portable ultrasound machine with a set of probes**
- **Thermo blankets (electrical blankets, water blankets, or plastic sheets)**

- **Gas anaesthesia circuit**
- **Laryngoscope**
- **Endotracheal tubes**
- **Ambu bag**
- **Containers for samples**

4. COLLECTION AND STORAGE OF BIOLOGICAL SAMPLES

Biological samples for laboratory tests should be collected during the process of animal examination.

Clinical laboratory tests:

1. Complete blood count:

    - Blood without preservative; at least three 5-ml tubes;
    - Blood with EDTA; at least three 3-ml tubes.

The complete blood count test should be performed as soon as possible (ideally, blood should be stored for no longer than one day). If the blood test is done with the use of automatic analyzer, the results should be verified by the study of the smear on glass slide.

2. Clinical biochemistry should include the following parameters:
    - Total protein
    - Albumin
    - Urea
    - Creatinine
    - ALT
    - AST
    - Alkaline phosphotase
    - Total bilirubin
    - Direct bilirubin
    - LDG
    - Amylase
    - Calcium
    - Phosphorus
    - Potassium
    - Sodium

3. Urinalysis (when possible)

Tests for detecting infectious and parasitic diseases:

- Nasal swabs for molecular genetic study;
- Conjunctive swab;
- Swab from genital organs;

- In case of identified injuries, the swabs should be taken from the injury for bacteriological tests;
- Fecal samples for parasitological tests.

Required tests:

- PCR-method: canine distemper virus (CDV), feline calici virus (FCV),feline herpes virus, feline immunodeficiency virus (FIV), feline leukaemia virus (FeLV), bartonella infections, dirofilariosis, toxoplasmosis.
- Enzyme-linked immunosorbent assay: CDV, FIV, FeLV, dirofilariosis.
- Serology: toxoplasmosis (IgG).

Collected faecal samples should be sent for parasitological test and frozen (in labeled plastic containers).

Genetic testing:

- Hair samples;
- Whole blood (EDTA).

Biological samples should be placed in plastic tubes and zip-log bags labeled with the use of permanent marker (species, sex, local name or number, place of capture, date of sample collection).

Storage of samples:

Serum should be frozen (at least two labeled plastic tubes).

Whole EDTA blood should be frozen (at least two labeled plastic tubes).

Swabs should be fixed in 95% ethanol and frozen.

Faecal samples should be frozen.

All samples should be stored at the temperature $t \leq -19°C$.

5. BLOOD TESTS: REFERENCE INTERVALS

Animal **Test**	**Leopard** **Mean range** (Source: ISIS)	**Tiger** **Mean range** (Source: ISIS)
Total protein (g/L)	59–85	53–83
Albumin (g/L)	26–43	25–44
Creatinine (µmol/L)	54–348	55–370
Blood urea nitrogen (mmol/L)	7.5–19.2	5.7–18.3
Glucose (mmol/L)	3.72–13.04	4.15–14.03
Total bilirubin (µmol/L)	1.6–8.7	0.0–8.6
Direct bilirubin (µmol/L)	0.0–3.2	0.0–1.7
Alanin aminotransferase (U/L)	18–99	18–159
Aspartate aminotransferase (U/L)	14–69	11–63
Alkaline Phosphotase (U/L)	5–66	6–84
Amylase (U/L)	162–1676	182–2737
Cholesterol (mmol/L)	2.31–7.61	3.59–10.25
Calcium (mmol/L)	2.20–2.90	2.17–2.97
Phosphorus (mmol/L)	1.18–2.62	1.19–3.04
Chloride (mmol/L)	109–128	110–128
Sodium (mmol/L)	143– 162	143–159
Potassium (mmol/L)	3.2–4.9	3.5–5.3
Red blood cell count (10^{12} cells/L)	5.18–11.40	4,50–8,95
Haemoglobin (g/L)	76–160	82–166
Hematocrit (%)	24–50	24–52
MCV (fL)	34–55	45.0–69.1
MCH (pg)	11.0–18.1	15.8–21.9
MCHC (g/L)	276–382	281–375
White blood cell count (10^{9} cells/L)	6.14–21.54	5.62–18.66
Platelet count (10^{12} cells/L)	0.124–0.829	0.078–0.511
Neutrophilic band cells (10^{9} cells/L)	0.03–0.12	0.02–1.31
Segmented neutrophils (10^{9} cells/L)	3.20–16.91	3.52–14.47
Eosinophils (10^{9} cells/L)	105–1879	63–703
Monocytes (10^{6} cells/L)	80–1186	74–916
Lymphocytes (10^{9} cells/L)	0.4–4.08	0.42–4.05
Basophils (10^{6} cells/L)	0–243	6–251
Reticulocytes (%)	–	0.0–2.5

6. NORMAL PHYSIOLOGICAL PARAMETERS: REFERENCE INTERVALS

	Amur tiger	**Far Eastern leopard**
Body temperature	36.6–41.3^{0}C	36.9–41.1^{0}C
Normal heart rate (adult animals)	50–100 beats per minute	70–140 beats per minute
Normal respiratory rate (at the normal body temperature)	8–15 breaths per minute	8–24 breaths per minute