



Sveriges lantbruksuniversitet  
Fakulteten för veterinärmedicin och husdjursvetenskap

Swedish University of Agricultural Sciences  
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## **Pregnancy detection in reindeer (*Rangifer tarandus tarandus*): Comparison of transrectal ultrasound, abdominal palpation and herders' visual cues in late gestation**



by  
**Elisabeth Paul**

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## Abbreviations

<b>Acc</b>	Accuracy
<b>FCaf</b>	Females with Calf-at-foot
<b>n.a.</b>	not available
<b>APP</b>	Apparent pregnancy prevalence
<b>Se</b>	Sensitivity
<b>Sp</b>	Specificity
<b>- PV</b>	negative predictive value
<b>+PV</b>	positive predictive value
<b>FP</b>	False Positive (barren females tested incorrectly as “pregnant”)
<b>FN</b> result)	False Negative (pregnant animals with an incorrect negative testing
<b>TN</b>	True Negative (barren females tested correctly as “non-pregnant”)
<b>TP</b>	True Positive (pregnant animals with correct positive testing result)
<b>PALP</b>	Palpation
<b>US</b>	Ultrasonography
<b>VIS</b>	Visual examination
<b>BW</b>	Body weight
<b>MBW</b>	Mean body weight
<b>LMS</b>	Least Mean Square
<b>SD</b>	Standard Deviation

## **Abstract**

Pregnancy detection during late gestation (last trimester) in domesticated reindeer (*Rangifer tarandus tarandus*) gives the possibility to select and enclose the pregnant reindeer during calving as protection against predation. The aim of the present study was to compare transrectal ultrasonography and palpation, and also to describe visual examination as traditionally used by herders as pregnancy detection methods in reindeer. In all, 1107 female reindeer were tested for pregnancy with ultrasound in April 2013 and April 2014. 207 from these females were tested with palpation in April 2014. In 2013 ultrasound showed an apparent pregnancy prevalence of 83.1% and a calving rate of 88.3%. In 2014 two tests, ultrasound (US) and palpation (PALP) were conducted independently. Females with an unclear or negative pregnancy result were re-examined by both methods. Observed calving rate was 93.0%. Accuracy for both evaluations was 94.2% (US) and 97.7% (PALP), with sensitivity of 95% (US) and 98.8% (PALP). Both methods showed good results, however palpation was slightly more accurate than ultrasound. Both test outcomes increased to a specificity of 100% and a positive predictive value of 100% when combining the two methods. Hence, combination of both methods and re-examination were found to increase correct test outcomes. Two herders were asked to describe their indicators for visual evaluation of pregnancy. The indicators differed between the herders, but both looked for changes in abdominal and inguinal anatomy. Accuracy was not calculated. However, herders seemed to associate high body weights with pregnancy.

## Introduction

Husbandry of domesticated reindeer (*Rangifer tarandus tarandus*) has a long tradition in Fennoscandia. For more than 1000 years reindeer herding has been closely linked to the livelihood of the Sami people and other northern indigenous populations like Nenets and Selk'up in the Arctic and Sub-Arctic Region in Eurasia (Williams 2003). Being an important part for subsistence for the indigenous Sami population for centuries, reindeer use changed gradually from hunting wild animals to an extensive production system (pastoralism), becoming the main source of income for many Sami families (Bjørklund 2013). In the present management system reindeer are kept in big herds, migrating freely between summer and winter pastures, some with a remarkable distance covered, followed by the herders (Skarin et al., 2010, Bergerud et al. 1990). Reindeer are gathered in corrals several times a year for counting, controlling and sorting for slaughter (Williams, 2003). Mating as well as calving takes place without any direct control of herdsman. The reindeer graze freely in remote areas in northern Fennoscandia but are rounded up at least twice a year for marking of the calves, and culling through slaughter. Thereby a survey of calving success usually takes place. In comparison to other ruminant production systems, management of reindeer herds does not include reproductive management, like artificial insemination etc. Hence, little is known about such practicalities in reindeer production systems (Ropstad, 2000, Savela et al. 2009).

Some of the main aspects of extensive production are the physical mobility of reindeer herders with helicopters and snowmobiles. During winter the herders attend the herds daily, move the herds and even scare away predators. The common way of protection of animals is not through surveillance but campaigning against predators as for example brown bears (Ingold, 1980). The numbers of predators, like lynx (*Lynx lynx*), brown bear (*Ursus arctos*), wolverine (*Gulo gulo*) and golden eagle (*Aquila chrysaetos*) have increased during the last decades and showed their effect on increased calf mortality due to predation (Jernsletten and Klovov, 2002; Nybakk et al. 2002; Nybakk et al, 1999; Swenson, 2013). Pregnancy detection in reindeer herds has economical and health related advantages: not only to fence pregnant females as safety measure against predation during calving, but to facilitate supplemental feeding of pregnant reindeer, and to cull barren or non-reproducing animals to control fertility of the herd (Savela et al, 2009; Vahtiala et al., 2004). Ropstad et al. (2005) predicted pregnancy detection as a useful tool for further intensification of reindeer husbandry in the future. However, accurate and cost-effective pregnancy detection at a later stage of pregnancy in the field is difficult. Several methods have been compared in relation to their reliability, costs and applicability in field conditions (Savela et al. 2009, Ropstad, 2000, Vahtiala et al, 2004). Practical methods like Palpation of the abdomen or visual examination as part of traditional knowledge has so far been disregarded.

A project run by the Wildlife damage center in Sweden, is currently evaluating fencing of the females before, during and after parturition to protect newborn calves against predation. Pregnancy detection by transrectal ultrasonography has been used for the testing of animals within the last 3 years of the project. The testing has been performed during late pregnancy stage in late march to mid April (26<sup>th</sup> March -18<sup>th</sup> April). However, its accuracy, specificity and sensitivity have not been analyzed within the study in the preceding years.

This thesis aims to investigate the sensitivity and specificity of abdominal palpation method on reindeer in comparison to the ultrasound method and pregnancy detection by visual cues as proposed by two Sami reindeer herders. An overview on possible pregnancy detection methods is given in the literature review.

## Literature Study

### Reindeer husbandry in Sweden

Successful reproduction and high survival rates of calves within their first days is crucial for a stable reindeer production and the economic stability of over 2.500 Sami, whose income directly depends on reindeer production (Sametinget, 2013). The reindeer husbandry is regulated according to the “Swedish Reindeer Herding Act” that grants the rights on reindeer herding primarily to Sami population, with some regional exceptions. Reindeer herding territory covers 54% of Swedish surface, providing space for 250.000 reindeer (Sametinget, 2013). In northern Eurasia there are two types of reindeer herding still practiced. The intensive herding systems is characterized by a close proximity of the herder to his animals, keeping them together especially in spring and summer season, where herd size is small or medium, from fifty to several hundred head (Williams, 2003). In Sweden extensive herding systems evolved in the 1960s. It is characterized by the use of better technology (snowmobile, helicopters and lately monitoring using GPS-collar) and free roaming of herds in large areas. Since then the number of reindeer herds has decreased significantly in the last three decades, whereas the size of the herds has increased (Ingold, 1980). The animals choose resting grounds and group constellation at their own. Apart from daily surveillance the reindeer are gathered at least twice a year for ear tagging and culling for slaughter. Reindeer meat is a highly appreciated product among Swedish consumers and according to Jernsletten & Klovov (2002) production of reindeer goods is developing positively. Due to high predation and increased cost for technological equipment, reindeer herders experience high production pressure. Herders are supported by governmental organizations to offer value-added products, however development has been slow.

In reindeer management, the animals choose their diet freely by grazing on pastures according to seasonal availability of feed, mainly ground and arboreal lichen, leaves and grasses, mushrooms, dry shrubs and herbs (Williams et al., 2003; Mathisen et al., 2002). Reindeer therefore use different areas depending on season. In winter for example, they mainly use areas towards the Baltic Sea coast and they are able to find forage such as ground lichen under the snow by using hoof to break the snow crust. In extreme conditions however, supplemental feeding consisting of hay and pellets may be provided by the herder (Thompson & Barboza 2013; Wiklund et al. 2003).

### Breeding cycle of reindeer

Reindeer reproduction has many similarities to other cervid's reproduction processes, being seasonal and influenced by decreasing light conditions in autumn (Ropstad, 2000). Rut starts in mid-September until end of October, and gestation length can vary from 203 to 240 days according to Rowell and Shipka (2009), depending on the nutritional status of the female, genetic influences, and possibly photoperiod changes in spring. Thereby shorter gestation occurred when insemination took place in late stage of rut (Ropstad et al. 2005). Mean gestation time is  $214 \pm 3$  days, thirty to thirty-one weeks on average (Shipka et al., 2007, Ropstad et al., 2005), although earlier studies suggested longer gestation means from 229days up to 235days (Ropstad, 2000; Bergerud, 1975). During

pregnancy hormonal changes occur in the reindeer, making pregnancy detection possible at several stages, summarized by Ropstad (2000).

#### Description of pregnancy stages with importance for pregnancy detection

After ovulation and fertilization, the growing blastocyst is transported into the uterus. The fetus is floating relatively free in the amniotic fluid surrounded by the fetal membranes *amnion* and *chorion* (Sjaastad et al., 2010). The attachment of the fetal outer membrane-chorion to uterus (*endometrium*) occurs through cotyledons organized in six to eight placentomes that are shaped like buttons (as described by veterinarian H Sirkkola, performing the ultrasound detection test in the present study). During growth and weight gain of the fetus the distances of fetal parts to the amniotic wall decrease and the uterus will sink closer to the abdominal wall due the stretching of the uterus and its ligaments. Six weeks before parturition the chorion comprises a volume of approximately 28x28x15cm (corresponding to 11.8 liters).

#### Hormonal pregnancy detection in blood, saliva or feces

Pregnancy related physical changes are controlled through changing hormonal balances detectable in blood, saliva and feces. Pregnancy detection through analysis of compounds in blood, saliva, urine or feces samples has been thoroughly researched for ruminants. As with deer, detection methods differ in accuracy, time of detection and costs of application. The most common research method used is blood sampling. This is however an invasive procedure that requires not only trained staff but also the technology for analysis. General pregnancy markers for the related tests have been progesterone (P4), and pregnancy-associated glycoproteins (PAG's) both detectable through radioimmunoassay (RIA) of blood plasma; and pregnancy specific protein B (PSPB) quantified by a double antibody RIA (Savela et al., 2009; Willard et al., 1999). Within the study of Shipka et al. (2007) and Ropstad et al. (2005) progesterone concentrations within pregnant and cyclic non-pregnant females have been seen as overlapping during the first months of gestation, and therefore do not qualify as a secure test method. P4 and PSPB are however recommended as valid indicators by Russell et al. (1998) for pregnancy detection in caribou from November onwards since they are specific enough. Also Savela et al. (2009) found that the testing for progesterone (threshold level of 5.0 nmol/L) showed an accuracy of 94.9%. In the same study pregnancy-associated glycoproteins in plasma reached overall accuracy of 96.9% at a cut-off 1 ng/mL. In studies on deer from Willard et al. in 1999 radioimmunoassay of pregnancy specific protein B (PSPB) has been able to detect pregnancy from 27-33 days in roe deer.

Pregnancy detection by reproductive hormones have been vastly researched also by Ropstad et al. in 2005 and Ropstad et al. 1999. They measured plasma progesterone (P4), estradiol (E2) and estrone sulphate (E1SO4), 15- ketodihydro-PGF2a (PG- metabolite) and pregnancy-associated glycoproteins (PAG) in 13 reindeer during gestation. Thereby PAG's were found to be a reliable testing marker for pregnancy detection, using a discrimination level of 2 ng PAG/mL plasma, after one month of insemination. Estrone sulphate, as tested by Ropstad et al. (2005), Shipka et al. (2007) and Flood, et al. (2005) was found not reliable for pregnancy detection, due to its late increase only at the end of the gestation.

Another non-invasive method for pregnancy detection is the measurement of progestins in collected feces of the animals, as shown on caribou by Messier et al. (1990). Their study concluded that after 50 days of gestation measurable differences in progestins in pregnant barren caribou became evident, with progestin values above 208ng/g of dried feces as indicator for pregnancy ( $P < 0.05$ ).

### Ultrasonography

Ultrasound methods have been used in several studies for pregnancy detection on reindeer, having the great advantage of delivering an immediate results through imaging and being conducted by a transportable device, applicable during fieldwork (Savela et al. 2009). Using ultrasonography for production purposes in reindeer husbandry is quite recent, and superseded the method of estimating conception and calving time through observation of mating (Vahtiala et al., 2004). Two ultrasonographic methods are possible in reindeer, transrectal or transabdominal examinations depending on the advancement in gestation.

Transrectal ultrasonography, a non-invasive endoscopic detection method, is commonly used for pregnancy detection in ruminants (Barrett, 1981; and Buckrell et al., 1986 in sheep, Hermes 1997 and Willard et al. 1999 in fallow deer, Sempere et al. 1989 in roe deer, Hoare et al. 1997 in muskoxen). Within the deer family (*Cervidae*) tests are conducted with frequency ranges of 3.5 MHz to 7.5 MHz depending on the resolution and depth of imaging (Hermes, 1997; Willard et al., 1999). Thereby a linear ultrasound transducer is inserted in the rectum of the standing and retained reindeer to examine the uterus to detect the fluid-filled fetal membranes or fetus of a pregnant animal. One expert in field ultrasonography and one assistant, to retain the animal, are required for the test. Accuracy of ultrasonography have been evaluated on 195 reindeer by Savela et al. (2009) and compared to hormonal and pregnancy-associated glycoprotein tests. Overall accuracy of ultrasounds done in late December and early January (in week 7-16 of gestation) was 99.5% with a positive predictive value of 100% and a negative predictive value of 96.4%, which means that there is still a small chance that a pregnancy goes undetected, in this case one reindeer. However ultrasound was significantly more specific than progesterone and PAG's detecting methods (Savela et al. 2009).

Good results in accuracy of transrectal ultrasound have also been achieved by Vahtiala et al. (2004) in their detection study at early pregnancy stages in 13 reindeer, double checked with PAG and plasma progesterone in the blood. Examining the reindeer every week via transrectal ultrasound, accuracy was 15% at the third week and up to 92% within the sixth week of gestation. Unfortunately no specificity of the test itself could be measured since all reindeer got pregnant during this study. Vahtiala et al. (2004) concluded that mating season lasts until November. In order to obtain a good result, tests should not be made earlier than December to leave a 6 weeks buffer from the last mating possibility. Revol and Wilson (1991) presented similar findings for red deer (*Cervus elaphus*), where accuracy was 35% in week 3 and 98% between week 5 and 6, with the same method of pregnancy testing with 5MHz linear transducer. Other ultrasonographic tests on fallow deer (*Dama dama*) were made by Willard et al. (1999) in December after an 8-week-breeding season, where ultrasonography was in agreement with the pregnancy-specific protein B test in the serum of the deer in 93% of the cases. However as Ropstad

et al. (1999) observed in Norwegian and Svalbard reindeer, ultrasonography shows its limitations in comparison to hormonal detection especially in late gestation at the end of April, leading to an elevated amount of false negative diagnoses. Partial explanation for the inability to detect the fetus can be the digestive anatomy and activity (intestinal gas formation) in these ruminants as disturbing factors on the resulting ultrasonographic image.

#### Rectal palpation

According to Ropstad et al. (1999) rectal palpation in reindeer is not possible. Vahtiala et al. (2004) stated in their ultrasound study that rectal palpation is not commonly used for detection in semi-wild ruminants. However there is a study from Greer and Hawkins Jr. in 1967, which describes rectal palpation in elk (*Cervus canadensis*) as highly reliable and cost-effective method to determine pregnancy status of elk. Tests have been conducted on 38 elk by an experienced veterinarian in early to mid-gestation period in late December and showed a positive predictive value of 100%, with 3 cases where pregnancy status was falsely negative.

#### Abdominal palpation

As per my existing knowledge no research has been published on ventral abdominal palpation of reindeer for pregnancy detection. Although not extensively researched, the method is used in veterinary practice in ovine and bovine species (Pratt & Hopkins 1975; Ishwar 1995; Kutty 1999). This detection possibility should however get increased attention, since skilled breeders could apply this technique on reindeer without further technology and minimal costs, after being instructed on how to perform the examination and confirm pregnancy by feeling the contact with a fetus.

#### Antler Shedding

Time of antler shedding is traditionally associated with the status of pregnancy in female reindeer. Some researchers (Reimers et al., 2013, Espmark, 1971, Bubenik et al., 1998; Blake et al. 1998; Lent, 1965) have found connection between pregnancy and late antler shedding, occurring only after calving, whereas in barren females this takes place already several weeks earlier. *Rangifer* is the only cervid species where both sexes wear antlers, and their growth and casting is regulated through endocrine fluctuations (Reimers et al., 2013). The growth of female antlers starts in the first weeks of life, finishing within four and a half months, and casting occurs in late March until May. In females, estradiol levels are assumed to regulate the timing of the processes and insulin-like growth factor 1 (IGF1) as the main growth stimulator within the antlers (Blake et al., 1998; Bubenik et al., 1998). Findings in these studies however vary greatly and prediction on pregnancy status of reindeer by antler shedding is questioned recently by Reimers et al. (2013) showing that time span of latest non-pregnant female casting is overlapping with pregnant females who shed antlers already prior to calving. They assume that the occurrence is more common than formerly known and mention several factors that could influence time of shedding other than pregnancy: ecotype, age, nutrition and mineral intake. Therefore antler shedding as a sign for pregnancy should not be taken as a secure method for prediction. More research has to be conducted on function of estradiol and

other antler shedding hormones during pregnancy, also in relation to nutrition and weight gain within the different ecotypes.

#### Body Condition related to pregnancy

Body condition of reindeer in the different seasons depends largely on population density and climate conditions, since these are determining the individual feed intake and hence nutritional status (Couturier et al., 2009). Winter imposes nutritional stress situations combined with high-energy expenditures in mobility and thermoregulation to reindeer (Barboza & Parker, 2006). Available lichen is high in carbohydrates but a poor nitrogen source. Pregnant reindeer will not only have to sustain themselves during difficult feeding periods (for example with occurrence of ice-crusts at the cratering areas) but they also risk the malnourishment and health of the growing fetus (Barboza & Parker, 2006).

Visual assessment of pregnancy status by reindeer herders could be based on body condition during gestation in the winter months. Direct correlations have been proposed by Gerhart et al. (1997a) on back fat depth and weight differences and pregnancy in caribou, where pregnant animals had significantly higher body weight and back fatness in early pregnancy, compared to barren animals. Pregnancy probability is directly correlated to body energy storage, a non-lactating status and a heavy body weight, since all three facilitate fecundation and subsequent gestation (Gerhart et al. 1997a; Gerhart et al. 1997b, Rönnegård et al. 2002). Body weight varies greatly between herds from different regions, depending on access to forage. Different mean body weights have been recorded by following researchers (Nieminen & Leppäluto, 1986; Rönnegård et al. 2002).

As Lyver & Łutsël K'é Dene (2005) observed while monitoring body condition in wild caribou in northern Canada, aboriginal caribou hunters and elderly are able to distinguish different body conditions in spring, when female caribou are selected for slaughter. Within the study elders described barren caribou females as fattest in late winter (January till February), and identified this time as perfect moment for culling. It is unclear however if trial people have been able to distinguish barren from pregnant reindeer by visual cues only.

Elevated rump fatness as an indicator for pregnancy was also observed by Cuyler et al. (2012), in the study on warble fly infestation on caribou, “pregnant adult cows (age > 3-years) possessed significantly greater rump fat than non-pregnant [ $P_1 < 0,0001$  and  $P_2 < 0,0005$ ]”. Another observation from this study is the decreased occurrence of warble larvae under the skin of the animal, visible from outside as little 1-3cm bumps on the back of the animal. Cuyler et al. (2012) found significantly less warble fly infestations on pregnant caribou than on barren females or males at the end of the winter. In Scandinavia, many herds are nowadays treated with anti-parasites and thus show no parasitic infestation.

## Material and Methods

### Study Area

The study area is located in the County of Norrbotten in the province of Lapland, Northern Sweden (map in Annex A). The study was performed in two reindeer herds in Udtja and Gällivare reindeer herding community. The first group was tested in the Udtja group one of two herding groups in Udtja herding community, which is the southern of the two forest communities. Udtja community comprises twelve registered reindeer herders and at most 2,800 reindeers in the winter herd that stay in the forest area all year round (Sametinget, 2013). The Udtja nature reserve is a wide hilly woodland area with marshes and scattered lakes and a rich fauna, dominated by heaths and spruce forest (Länsstyrelsen, 2014). The second reindeer group to be tested was in the Purnu Group in Gällivare herding community. Gällivare community comprises an area of 8321 km<sup>2</sup> and is managed by 35 registered reindeer herders (Sametinget, 2013). Maximum number of reindeer during winter season is 7000 animals (Sametinget, 2013). This area is woodland and dominated by spruce and heath and is predominately flat with scattered marshes.

### Data Collection

The data collection comprised pregnancy evaluation through ultrasound (US), palpation (PALP) and visual examination (VIS), weighing of the females and observation females with calves during calf marking (Table 1). The weight was measured to the exact kilogram and was registered together with the collar number of each animal prior to testing. In addition walk along interviews were conducted with the herders in order to document the factors of visual pregnancy detection during examination and pictures of fertile females have been made for documentation purposes.

The data collection took place between 7<sup>th</sup> April and 8<sup>th</sup> June 2013 and between 1<sup>st</sup> April and 30<sup>th</sup> June 2014 in two fieldwork phases. The animals were gathered from the winter pastures into large corrals one to two days before testing. Tests were conducted within the 21 - 27 week of pregnancy, (from fifth to seventh month). On the examination day, reindeer herds were divided in smaller groups allowing easier separation of individual animals. The tests were conducted consecutively on each animal, in separate fenced areas. Interferences of the different testing methods were avoided through visual shielding. The experimental setup however was different in each herd since the herder provided it. Pregnancy test was given as either 'pregnant' (subsequently referred to as 'positive) or 'negative'. The latter comprising females that have been declared 'unclear' as well as 'non-pregnant', since no indicators for pregnancy was found in these animals. The negative females were submitted to a second round of tests at the end of the day.

### *Transrectal Ultrasonography method*

The ultrasound pregnancy test was conducted by veterinary Heikki Sirkkola, Hämeenlinna, Finland. For ultrasonography a linear transducer with 7.5 MHz and 8 cm viewing depth attached to a helve for better insertion and handling (Fig. B4) was used. According to the veterinarian, Heikki Sirkkola the transrectal ultrasound had following procedure:

1. Fixation of the animal in weighing box to create a stable testing situation with access to the pelvic region.
2. Removal of feces from the rectum, if this was necessary. Due to handling stress prior to examination, however many animals had already defecated.
3. Lubrication of the ultrasound head with soap solution or plant oil in order to increase contact and facilitate insertion of the probe. Insertion of an ultrasound detector type Agrosan L (ECM Echo control Medical, 126, Boulevard de la République, 16000 Angoulême, France) into the rectum with the right hand, facilitating entrance through lifting of the tail with the left hand.

A positive pregnancy assessment ('pregnant') was given with the visibility of parts of the fetus (membranes or parts) (Fig. B2), placental cotyledons (*placentomi*) (Fig. B1), and the form of the uterus (*cavus uteri*) bending downwards. A clearly visible separation of two fluid filled chambers, the amniotic cavity and urinary bladder (*vesica urinaria*), has also resulted in a positive pregnancy evaluation. The chambers were divided by the wall of the urinary bladder and the mesometrium, which is attached to the uterus borders (*margo uteri*). When none of these sign were visible the animal was registered as 'negative' (meaning no indication for pregnancy found). Ultrasonographic images of pregnancy indicators are found in *Annex B*, kindly provided by Heikki Sirkkola.

#### *Palpation method*

The palpation pregnancy test was conducted by veterinary Erik Ågren, from the Swedish National Veterinary Institute (SVA), Uppsala, Sweden. The examination was conducted on standing reindeer females. In order to restrain the animal, two co-workers held it by the antlers or by the plastic collar, if present. Movements, high muscle tension due to nervousness, and sitting or lying down would disturb the pregnancy detection by influencing the anatomical location of the area being examined on the animal. The palpation method is based on the detection of the fetal bone structures as hard, free-floating (movable) structures within the abdomen where an expanded pregnant uterus was expected to be found. This area is primarily in the lower caudal quadrant of the abdominal wall, usually on the left side of the animal. Thorough manual palpation of the ventral and lateral surfaces may be needed in the area between the Xiphoid region (*Regio xiphoidea*), the middle abdominal region (*Regio abdominis media*) and the Caudal abdominal region (*Regio abdominis caudalis*). The examiner performs the palpation either with gentle pushes with the open hand using the palm as main contact surface, or stronger and rapid consecutive nudges at the abdominal wall with the hand (Fig. C1&C2) (side depends on handedness) to locate hard bone structures of a fetus, usually the skull, the leg or digits with hoof (*Ungula*), or the spine (*Columna vertebralis*). When a bone structure was detected at least twice, the female was noted as 'pregnant'. When no structures could be identified, the female was classified as 'unclear'. Since the fetus is located in the *Amnion* (Fig. C3) surrounded by amniotic fluid acting as additional buffer, a confirmed localization can be difficult. At the time of testing the fetus, within the *chorion*, had the approximate size of 27 x 27 x 15 cm (Fig. C5) with a head size of 14 x 8 x 5 cm.

### *Visual examination according to traditional knowledge inquiry*

During pregnancy tests, semi-directed walk-along interviews have been conducted with two reindeer herders, Lars Henrik Stokke (Udtja) and Anders Persson (Gällivare). Several factors for visual detection of pregnancy have been described and shown on the animals. In a second step pregnancy was evaluated by the latter interviewee in a total of 121 reindeer.

### *Calf observation*

The pregnancy outcome was judged as calf-at-foot at calf marking or earlier registration due to calf-death. In this way presence of calf-at-foot during observation in corral or at calf marking was the gold standard of the test. The results from the calf-observation data at calf marking between 4<sup>th</sup> and 8<sup>th</sup> June 2013 were matched with the test results from April 2013. For the tests in 2014, observations took place from 3rd to 30th June 2014 in order to confirm pregnancy in both reindeer herding districts. Thereby observations on the presence or absence of calves-at-foot were performed through at least 2 observers at each round-up of the females. Minimum two observations indicated presence or absence of calf. When there was only one observation, a positive calf-at-foot was noted if confirmed by suckling or other strong visible signs of bonding. Observations were recorded with collar code and observation verdict. Data was gathered subsequently in excel data sheets. Conclusion on pregnancy outcome however was only available on animals present at calf marking. Therefore the final sample size depends on the sample availability during calf marking. Only partial data collection was possible in Udtja during calf marking due to time limitation for calf mother-observation in the month of June 2014. Calf observations have been conducted on two round-ups on 22<sup>nd</sup> and 28<sup>th</sup> June 2014. A summary of collected data is presented in Table 1.

**Table 1.** Data collected in herds in Purno and Udtja on pregnancy testing in 2013 and 2014.

Year		Udtja	Purno (Gällivare)
2013	<b>pregnancy detection method</b>	ultrasonography	ultrasonography
	<b>data collected</b>	female ID, body weight, calving success, calf sex, calf body weight	female ID, body weight, calving success, calf sex, calf body weight
	<b>tested animals</b>	<b>337</b>	<b>684</b>
2014	<b>pregnancy detection method</b>	ultrasonography, palpation, visual examination	ultrasonography, palpation
	<b>data collected</b>	female ID, body weight, calving success	female ID, body weight, calving success, sex, calf body weight, sex, calving date
	<b>tested animals</b>	<b>121</b>	<b>86</b>

The pregnancy testing on reindeer, weighing and marking is covered by the Ethics approval from Ethics Committee Uppsala as part of the reindeer predation project within the Animals Nutrition and Health Department, SLU, Uppsala.

#### Data analysis

The Data was analyzed in Microsoft® Office Excel 2011 and stored in Microsoft® Office Access 2010. Apparent pregnancy prevalence (APP), sensitivity (Se), specificity (Sp), positive predictive value (+PV) and negative predictive value (- PV) have been calculated for the data from 2013 and 2014. Formulas for statistical measures of the tests, shown below, have been adapted from Karen et al. (2007). Sensitivity thereby is the percentage of animals, tested rightfully as pregnant in relation to all pregnant animals, whereas specificity indicates the percentage of animals tested rightfully as non-pregnant in relation to all actually non-pregnant animals. The positive predictive value indicates the ratio of correctly identified positive results to falsely positive identified results. The negative predictive value denotes the ratio of correct negative results to falsely negative. Accuracy denotes the amount of correctly diagnosed animals in relation to all tested animals (Karen et al., 2007). The results of testing have been assigned as follows: correct positive verdict as true positive (TP), incorrect positive verdict as false positive (FP), correct negative verdict as true negative (TN) and incorrect negative verdict as false negative (FN).

**Sensitivity** (true positive rate) =  $TP * 100 / (TP + FN)$

**Specificity** (true negative rate) =  $TN * 100 / (FP + TN)$

**Precision** (positive predictive value) =  $TP * 100 / (TP + FP)$

**Negative Predictive Value** =  $TN * 100 / (TN + FN)$

**Accuracy** =  $(TP + TN) * 100 / (TP + FN + TN + FP)$

These values were calculated for Udtja and Purnu in 2013 and for Purnu in 2014.

In order to compare specificity and sensitivity of the three pregnancy testing methods Pearson's **Chi-square test** has been applied, as proposed by Kaps & Lamberson (2004).

$$\chi^2 = \sum_i z_i^2$$

The confirmation of actual pregnancy was the observation (minimum two observations) of the female with calf at foot during calf marking in June 2013 and June 2014. During calf marking newborn animals were counted and matched to the respective female during several observations. Females that have been observed with two different calves were marked and kept included in the tables since it did not result in a wrong statement on pregnancy. Furthermore, the mean body weight (MBW) of the tested animals according to the test-outcome (TP, FP, TN FN) has been compared in order to support the visual detection method and indicate pregnancy probability according to weight. Mean body

weight, standard deviation of body weight and Least Square Means (LMS) were calculated for animals within each herd in 2013 and the Purno herd in 2014.

To test the relationship between pregnancy detection, body weight at testing and calf weight and calving date an Analysis of variance (ANOVA) has been performed, with standard error, least square mean for body weight of female and the test outcome. The test determined whether there was a statistical difference in evaluation depending on the weight, or not. Significant differences were declared with  $P < 0.05$  and up to  $P < 0.1$  a tendency was presumed.

The frequency of occurrence of test outcomes within 6 different weight categories (from 45 to 100kg body weight) has been calculated.

## Results

### Results from pregnancy testing 2013 - 2014

In 2013 and 2014 a total of 1107 reindeer were tested with ultrasound in late gestation. In total 925 out of 1107 females were tested pregnant with ultrasound, resulting in an apparent pregnancy prevalence (APP) of 83.6%. In 2014 207 animals out of these were tested through palpation. Re-examination was conducted on 13 animals that got tested unclear or non-pregnant in the first run. Additional 121 animals were evaluated by visual examination through the herder.

The analysis shows 186 animals assessed 'pregnant' by both methods (Table 2). This is an APP of 89.85% for the animals in Purnu and Udtja. The percentage of females with calf-at-foot for these animals has been 93% (Purnu) and 59.2% (Udtja) (Table 3). In the herd of Udtja in 2014 confirmation of calving success was not possible due to unregistered calving and absence of animals during the first two roundups in end of June 2014. Since absence of calves could indicate a non-pregnant reindeer or death of calf through sickness or predation, results from Udtja herd 2014 values were excluded from further analysis of herds and years and specificity, and negative predictive value as well as accuracy not calculated.

**Table 2.** Reindeer pregnancy testing with transrectal ultrasonography (US), palpation (PALP) and visual examination (VIS) in 2013 – 2014

	2013		2014				
	Purnu	Udtja	Purnu		Udtja		
	US	US	US	PALP	US	PALP	VIS
Tested animals	337	684	86	86	121	121	121
Verdict 'positive'	259	589	77	80	111	112	115
Verdict 'negative'	78	95	9	6	10	9	6
Re-examination:	-	-	13	13	-	-	-
Females with doc. calf (incl. dead)	248	671	80	80	76	76	76
No. of dead calves	4	n.a.	4	4	n.a.	n.a.	n.a.
No. of barren females	89	n.a.	6	6	n.a.	n.a.	n.a.

All animals in 2013 and 2014 (except Udtja 2014) were kept in corrals until calving. Dead fetus and calves were included in the calculations, in order to account for all pregnant female animals.

Within the herd of Purnu (n=86) 80 animals gave birth, six reindeer were barren, the observed CR was therefore 93.0%. Thirteen reindeer were tested 'negative' at the first round and were tested a second time in order to minimize incorrect negative values (Table 4). For these animals the last verdict of both detection methods was binding. Four calves died before calf marking, one due to drowning, two from calving complications and one

out of yet unknown reasons. They have been however included in the calculation as this did not change the verdict of the two methods.

As can be seen in Table 3, the females with calf-at-foot and sensitivity of tests were similar throughout the years and herds. Sensitivity and specificity of transrectal ultrasonography increased from 2013 to 2014. Highest rate of sensitivity was reached by palpation with 98.8% in Purnu. Specificity of ultrasound within the Purnu herd increased as well from 59.05% in 2013 to 83.3%, reaching the same specificity as palpation. Test values from palpation had slightly but not significantly better results than ultrasonography in both herds in 2014, according to  $\chi^2$ -Test ( $P < 0.001$ ).

Larger differences are found within the specificity of the ultrasound within the years as a result of high numbers of animals tested FP, animals that got a negative outcome however were observed with calf-at-foot.

**Table 3.** Comparison of test values in two herds in 2013 and 2014 in percent (%). With apparent pregnancy prevalence (APP), Females with calf-at-foot (FCaf), Sensitivity (Se), Specificity (Sp) and Accuracy (Acc) for the detection methods transrectal ultrasonography (US), palpation (PALP) and visual examination (VIS).

	2013		2014						
			Purnu			Udtja			
	Purnu	Udtja	US+			US+ PALP			
	US	US	US	PALP	PALP	US	PALP	VIS	+VIS
FCaf (%)	75.4	94.7	93.0	93.0	93.0	59.2	59.2	59.2	59.2
APP (%)	76.9	86.1	89.5	93.0	93.0	91.7	92.6	95.0	90.9
Se (%)	88.6	89.0	95.0 <sup>A</sup>	98.8 <sup>A</sup>	95.0	n.a.	n.a.	n.a.	n.a.
Sp (%)	59.0	66.7	83.3 <sup>A</sup>	83.3 <sup>A</sup>	100 <sup>A</sup>	n.a.	n.a.	n.a.	n.a.
Acc (%)	81.3	87.9	94.2 <sup>A</sup>	97.7 <sup>A</sup>	95.3 <sup>A</sup>	n.a.	n.a.	n.a.	n.a.
+ PV (%)	86.9	98.0	98.7	98.8	100	n.a.	n.a.	n.a.	n.a.
- PV (%)	62.8	25.3	55.6	83.3	60.0	n.a.	n.a.	n.a.	n.a.

#### Re-examination of 'negative' tested animals in Purnu 2014

Animals with and unclear or negative verdict by one of the methods were tested a second time. Table 4 shows the ID of the females and the first and second verdict for the two pregnancy detection methods, and the observation with or without calf-at-foot. With the combination and agreement between both methods and the re-examination in 'unclear' cases, a specificity of 100% was reached.

As shown in table 4 each method changed its verdict to the correct outcome during re-examination in three cases, in a total of six animals, meaning a correction of verdict in 46% of the re-examined cases. There were four TN evaluations in each round and one

false positive evaluation by each method. Ultrasound has failed to detect pregnancy in three cases in each evaluation round. These females were all in the upper body weight class, in average 8.3kg heavier than the MBW of pregnant females (78.7kg). They were however not significantly different from the positively tested animals (Table 4). With the palpation method there were no pregnant females later observed with calf, which were tested not pregnant at both rounds. Three of the pregnant animals were not detected pregnant in the first evaluation, but were found pregnant in the second evaluation. They all had a high MBW ( $86.3 \pm 7.8$ kg) in comparison to correctly tested pregnant animals ( $MBW = 78.7 \pm 8.1$  kg).

**Table 4.** Pregnancy verdict in ultrasonography and palpation method in reassessment of previously tested ‘unclear’ or ‘non-pregnant’ tested reindeer. Verdict ‘0’ denotes unclear or non-pregnant tested and ‘1’ pregnant tested animals, with ID and body weight in Purnu (Gällivare) 2014.

Reindeer - ID	Body weight	Observed with /without calf	Verdict by method			
			US I	US II	PALP I	PALP II
A80	73	with calf	0	1	1	1
A38	78	with calf	0	1	1	1
A37	80	with calf	0	1	1	1
A731	80	with calf	1	0	0	1
A774	82	with calf	0	0	1	1
A748	84	with calf	0	0	0	1
A794	95	with calf	0	0	0	1
A721	59	without calf	0	0	0	0
A724	63	without calf	0	0	0	0
A722	67	without calf	0	0	0	0
A652	73	without calf	0	0	0	0
A671	77	without calf	0	0	0	1
A653	82	without calf	1	1	0	0

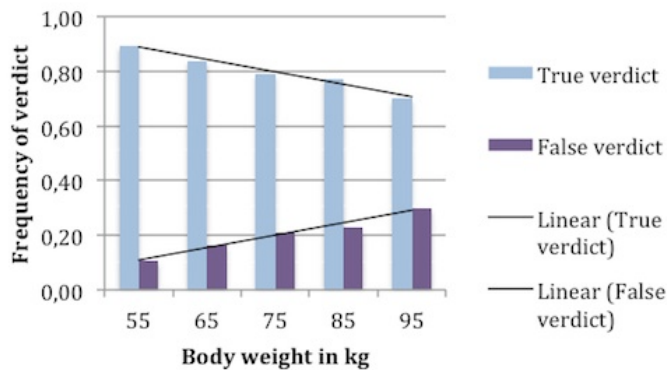
#### Ultrasound in relation to calving date and calf body weight in Purnu 2014

In the control group of Purnu high body weights of calves at calf marking were indirectly proportional to calving dates in both years, having earlier parturition and heavier weights at time of calf marking. MBW of calves from TP tested reindeer (by both methods) was 12.1 kg and of FN (by US) was 12.6 kg.

#### Evaluation of pregnancy detection in relation to the female body weight

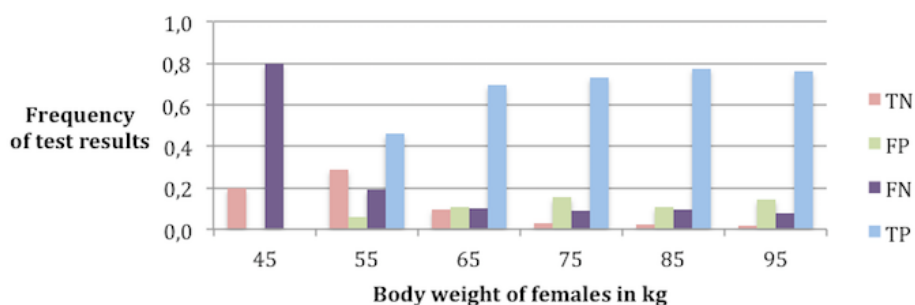
Difficulty in US detection can be related to body weight, body volume and anatomical localization of the foetus. In order to identify body weight ranges with a high risk of misjudgement in ultrasound detection, specificity and sensitivity were matched to different body weight categories (Figure 1). In 2014 the risk of false negative diagnoses

increases with increased weight especially in US testing. First misjudgement occurred at 80kg body weight.



**Figure 1.** Frequency of pregnancy testing outcomes according to weight for ultrasonography testing in reindeer in Purnu 2013.

Body mass has less effect on the accuracy of the PALP detection in comparison to US. However, only animals tested TP and TN differ significantly in body weight with mean body weights of 79.02 kg for TP and 68.8 kg for TN ( $P=0.05$ ). Comparing the risk for wrong judgements and weight, similar results to palpation are obtained. Due to the very low occurrence of false verdicts, few can be concluded. However the one false negative judged animal has a body weight of 84, hence a similar body weight as the false negative judged with ultrasonography. Overall judgements by US in 2013 and by US and PALP in 2014 can be seen in Figure 2. Noteworthy is a high frequency of FN tested animals in the lower body weight categories, which is mainly due to missed detection of pregnancies in 2013.



**Figure 2.** Frequency of occurrence of test outcomes for ultrasound detection in reindeer in 2013 in Purnu and Udtja) and in 2014 (Purnu) by weight categories.

Mean weight depending on pregnancy outcome is shown in Table 6. FN-tested animals by US and PALP have a very high MBW of 85.7 kg in 2014. However in 2013 in Udtja the opposite was the case with a lower than average MBW for FN-tested animals in

comparison to TP tested animals. Analysis of test outcomes for transrectal US in relation to body weight in both years shows that least square mean (LSM) is 78.4 for TP and 78.5 for FP, which are not significantly different. However both means differ significantly from TN (LMS= 66.5) and FN (LMS= 75.6) according to the Student's t-Test.

**Table 5.** Comparison of mean body weight (MBW) and standard deviation in kg of reindeer in late gestation according to ultrasonography pregnancy evaluation done in Gällivare reindeer herding communities in 2013 and 2014

	TP	FP	TN	FN
Gällivare Purnu 2014	78.7 ±8.1 <sup>A</sup>	82 ±0 <sup>A,B</sup>	67.8 ±8.0 <sup>B</sup>	85.7 ±5.8 <sup>A</sup>
Gällivare Purnu 2013	76.6 ±8.3	76.2 ±8.9	67.3 ±8.2	78.7 ±8.4
Udtja 2013	76.3 ±9.0	76.6 ±8.9	65.0 ±12.6	70.9 ±11.5

Letters A,B next to body weight denote statistical differences between weight ( $P > 0.05$ ).

#### Visual examination for pregnancy detection by herders

Visual evaluation of external anatomical changes indicating pregnancy was used on 121 females in Udtja (Table 6). Pictures of features described by the herder are found in the *Annex D*. Factors that are important for the visual examination for pregnancy have been described as follows by the two herders in Purnu and Udtja. Both methods vary greatly in the areas and zones. However no direct comparison of both with calving was possible this year.

#### Interview 1, conducted in Purnu (GVA) on 02/04/2014:

- ‘uplifting of body weight of the female from the upper hind leg towards the thigh’: the raise of the *Regio supramammaria* up towards the *Regio urogenitalis* (Fig. D1, D3) and connected to that, the impression of higher convexity of *Regio femoris* (consisting of *M. semimembranosus* and *M. semitendinosus*) and less expressed convexity at *Regio cruris* and *Regio popliteal*.
- A clearly visible line of divergent hair (*Linea pilorum divergens*) between *R. supramammaria* and *R. urogenitalis* (Fig. D3, D4 & D6) and falling hair protruding in an inverted V-form.

#### Interview 2, conducted in Udtja on 06/04/2014:

- more pronounced concavity visible in regions of *fossa paralumbalis* and *Regio inguinalis* (Fig. D4b & D8b)
- ‘rounds of hips more flat in comparison to the belly’: less pronounced convexity of muscles around the hip (*os coxa*) on the iliac crest/ ilium of *M. gluteus medius*
- ‘the stomach is more pronounced on the lower back’: convexity of the *Regio abdominis lateralis*

Since the females in Udtja 2014 were excluded from the analysis due to missing data on calving success, only accordance between the different pregnancy tests can be analyzed. A total of 121 animals in Udtja have been tested. At calf marking 76 calves were observed and 45 females without calf-at-foot. Accordance between the methods on verdict in Udtja is 95.9% (n=116). In 90.9% (n=110) cases all three agreed on 'pregnant', whereas in 5% of the animals the methods agreed on 'negative'. Disagreement between the three methods occurred in 4.1% (n=5) of the tested animals. According to the Chi-square test, there is no significant difference between the diagnostic value (TP, TN, FP, FN) of the three evaluation methods US, PALP and VIS ( $P < 0.001$ ).

**Table 6.** Pregnancy testing on 121 reindeer in the Udtja herd using three different detection methods in 2014

	Ultrasonography	Palpation	Visual examination
Verdict "pregnant"	111	112	115
Verdict "unclear"	10	9	6
Apparent pregnancy prevalence	91.74%	92.56%	95.04%

## Discussion

Little research has been published on pregnancy detection in late pregnancy stages of reindeer. While early detection is useful for decisions on culling or supplemental feeding (Vahtiala et al. 2004), late pregnancy detection offers the possibility to confine females directly prior to calving. The outcome of the pregnancy testing in this study was similar to prior studies. Females with calves at foot within reindeer herds were 82.5% (Udtja) and 73.6% (Purnu) in 2013 as well as 93.2% in Purnu in 2014. This was comparable to findings from Savela et al. (2009) with a pregnancy rate of 89.9% in Salla in Northern Finland and observations by Bjärvall et al. (1990) from Jåkkåaska community next to Udtja community observed a calving rate of 89%. The calving rate in Udtja 2014 can however not be estimated due missing observations of calf-at-foot.

An advantage of transrectal ultrasonography is the application in the field hence the portability, although batteries need possibility to recharge when used for prolonged periods. This can become complicated when applied in remote areas. The method gives immediate results and it is possible to measure fetus size. However, transrectal ultrasound has some limitations. From week 20 of gestation and onwards it can be difficult to detect the fetus, since the uterus is shifting and becomes unreachable for the ultrasound probe (Figure B3 & B4). In this case a possible sign for pregnancy in reindeer is the downwards-bended uterus, visible as fluid filled chamber and divided from the bladder by clearly visible membranes, as described in the methods. Fetus size varies greatly due to nutritional conditions of the female (Skogland, 1984; Reimers, 2002), which influences the location (deepness) of the amnion. Smaller fetuses will remain in the upper part of the uterus and only shift down, when space is needed or fetus body weight pulls the amnion and uterine horn downwards. In this case the ultrasound signal does not reach deep enough in order to locate a fetus. Prediction can be made with the uterus bending down, however even this seems difficult in very heavy females. Size and position of the fetus also depend on the length of gestation at testing. Females may have cycled several times prior to mounting and fecundation, and will hence carry small fetuses at pregnancy testing and thus have a late birth. Reportedly in Purnu 2014, a few females had not yet calved at calf marking in early June.

Moreover, weight extremes (both high and low) of the female influence the sensitivity of the ultrasound examination. A fetus has more space to go further away from the pelvic area in larger sized animals than in smaller ones. Ropstad (2000) mention that rectal ultrasound is good in early pregnancy stages but yields increasing FN in the later stages. Especially relevant is a low rate of FN results in this late stage of pregnancy, in order to confine and protect as many pregnant females as possible. All test values for ultrasonography, such as sensitivity, specificity and accuracy increased from 2013 to 2014. Sensitivity increased in Purnu from 88.6% to 92.5% and in Udtja from 89.0 to 98.7% in 2014. The increase can partly be explained with difficulties of examination due to supplemental feeding in the both herds prior to testing in 2013. An actual increase of sensitivity (to 95.0%) in Purnu was reached by re-examination of females, in case of an initial 'negative' verdict. When testing for the second time the position of the fetus, as well as digestive activity of the female can have changed, so that the pregnancy features become more visible.

As for the relation between body mass and ultrasonography, transrectal ultrasonography seemed to be more difficult in animals with a lower body weight in 2013. The frequency of FN tested animals was highest in females with a body mass under 60 kg. No explanation is found for this, other than a larger influence from supplemental feeding prior to testing in 2013. In 2014, there was an opposite relation (more FN for females above 80 kg). During reexamination, the shifting from a negative to a positive outcome if animals were pregnant occurred more often in females with lower body mass than the ones tested negatively twice but pregnant, however no statistical difference could be proven. During re-examination the ultrasound method changed from true outcome to a false outcome only once.

Calves body weights at calf marking in June were slightly higher for animals whose mothers have been tested FN in comparison to calves from mothers, that got tested TP in April. Although they are not significantly different, it could show a relation of higher calf weight, early birth date and an advanced pregnancy stage during testing, with the fetus already relocated deeper in the abdomen. Although this is not provable due to low numbers, it would be a good explanation for detection difficulties with ultrasonography. Detection of pregnancy signs via transrectal ultrasonography requires a skilled and experienced veterinarian. The veterinarian in this study, performed transrectal ultrasonography for pregnancy detection at the herds in Udtja and Purno in 2010, 2011 and 2012, prior to the two years testing discussed in this thesis. Apart from the problem with intestinal gases in 2013 the very low specificity indicating many false positive tested animals needs to be seen in relation to the gold standard for the testing, which is the observation of calf-at-foot at calf marking or dead calf found in the corral. However not all dead calves could have been linked to the respective female. Additionally abortions might have occurred during the corralling phase, increase the amount of positive tested females without a calf, and hence decreasing specificity.

The relation of female body weight to the probability of pregnancy has been discussed by Ropstad (2000), Rønnegård et al. (2002), Tyler (1987) and Lenvik (1990). In this study there was a difference between mean body weight and calving rate of the two herds in 2013, as a result of supplemental feeding of the animals during winter. If not fed extra, the animals of Purno may have been generally lighter than the animals from Udtja (GVA). Common reasons for weight differences of herds are population density and feed availability (e.g. poor grazing in late winter months) in the respective areas as described by Skogland (1990).

Reexamination of the negative tested females increased sensitivity of ultrasonography but not specificity. Sensitivity increased from 91.2% to 95.0%. The decision to re-test animals with a negative verdict according to one of the two methods, and then use both methods again, seems to increase the probability to diagnose correctly, even if not significantly measurable. As mentioned earlier, reasons might be relocation of the fetus, and changes in visibility due to changing digestive activity in the intestinal tract.

As predictive values for palpation for *Rangifer* have not been published, no comparison with other results is possible. However, test values like accuracy (93%) are greater for palpation than for ultrasonography. 'Negative' cases for ultrasonography were found

more easily through palpation. One reason could be the fetus body weight and size, forcing the uterus to shift deeper into the abdominal cavity. This indicates a trend that successful detection of fetus through palpation is positively related to a higher average body mass in cases when ultrasonography was not able to detect pregnancies. Another factor influencing detectability is the size of the uterus and position of the fetus. Difficulties in pregnancy detection through palpation are tense abdominal muscles, contraction due to stress and animals sitting down, so that the region cannot be reached. A great advantage of the palpation method is that only experience and people to hold the animal are needed in order to conduct the testing. Hence, reindeer herder themselves could learn to apply the method once instructed.

Combination of ultrasonography and palpation increased specificity and precision of both methods to 100%. Palpation proved to be complementary to ultrasound detection, increasing also overall sensitivity and accuracy, as ultrasound could identify small fetuses that palpation could not register, and palpation noted the larger fetuses that could not be visualized on the ultrasound screen. Both methods are easily applicable as a large-scale examination in reindeer production. Using the combination of methods seems to be a good way to ensure a low number of false verdicts.

Two herders were available to share knowledge on visual indicators for pregnancy. As presented above, these indicators are used personally and differ to a great extent. It is to discuss to which regards hair form and length between the *R. urogenitalis* and *R. supramammaria* is changing due to age (growth up to full maturity) as is the rise of probability to become pregnant. Due to the pre-set study setup done by herder, distance between palpation-examiner and visual testing herder for uninfluenced examination could not always be guaranteed for the visual examination. The evaluator could have been initially influenced through loud repetition of palpation verdict prior to visual testing through close proximity of the testing space. Testing procedure was changed after the first ten animals. A change in accordance in progression of the testing series has not become evident. Due to the low frequency of negative verdicts the independence of the testing method cannot be demonstrated. Visual evaluation for body condition through herders has been described by Lyver & Łutsël K'é Dene (2005) showing that herders have good experience to distinguish different body weight conditions and estimate body weight. Visual evaluation is highly dependent from the experience of the observer. It requires not only the cues that need to be evaluated but also for the general condition within the herd, because body condition, weight and body volume vary between herds, years and areas. As shown in the interviews with the herders, big differences in the evaluation were apparent. Due to these differences a general visual testing method would be difficult to develop.

Field studies in reindeer production pose difficulties that are particular to that kind of production system and are worth mentioning in order to clarify field conditions. One limitation is for example the very high pregnancy rate in *Rangifer*, making it difficult to analyze the pregnancy outcomes statistically, i.e. we have very few animals not pregnant during testing that could be compared with the testing of pregnant animals. Additionally it should be discussed to which extent personal experience of the examiner is influencing

the testing outcome. Only one examiner conducted each test, and a comparison of different examiners would have contributed to the validity of the study.

Practical in-field reindeer pregnancy testing has yet no standardized method. Since highly accurate tests (like blood tests) are not suited for in-field testing and are also more costly, other methods, as those tested in the present study have to be considered. The combination of transrectal ultrasonography and palpation methods seems an optimal choice for practical evaluation of pregnancy during late gestation in reindeer.

## **Conclusion**

In the comparison of ultrasound and palpation in 2014 sensitivity and specificity did not differ significantly between ultrasonography and palpation method. Both methods are equally good. The combination of ultrasonography and palpation increases specificity and precision to 100%. Palpation could become another standard method for pregnancy testing in the field in late gestation. Due to the significant reliance on experience in both testing methods, variance of outcome with different examiners needs to be evaluated. Further testing with younger animals, with a lower apparent pregnancy prevalence should be conducted in order to gain more balanced results for the testing methods. Visual examination was not possible to evaluate on a statistical level, this is however interesting for further research.

## References

- Barboza, P.S. & Parker, K.L. (2006). Body Protein Stores and Isotopic Indicators of N Balance in Female Reindeer (*Rangifer tarandus*) during Winter. *Physiological and Biochemical Zoology* 79 (3): 628-644.
- Barret R.H. (1981). Pregnancy Diagnosis with Doppler Ultrasonic Foetal Pulse Detectors. *Wildlife Society Bulletin* 9 (1): 60-63.
- Bergerud, A.T. (1975). The reproductive season of Newfoundland caribou. *Canadian Journal of Zoology* 53 (9): 1213 – 1221.
- Bergerud, A.T., Ferguson, R., & Butler, H.E. (1990). Spring migration and dispersion of woodland caribou at calving. *Animal Behaviour* 39: 360-368.
- Björvall, A., Franzén R., et al. (1990). Renar och rovdjur. Rovdjurens effekter på rennaringen. *Naturvårdsverket Förlag*, Sweden.
- Bjørklund, I. (2013). Domestication, Reindeer Husbandry and the Development of Sámi Pastoralism. *Acta Borealia* 30 (2): 174-189.
- Blake, J.E., Rowell, J.E. and Suttie, J.M. (1998). Characteristics of the first-antler growth in reindeer and their association with seasonal fluctuations in steroid and insulin-like growth factor I levels. *Canadian Journal of Zoology* 76: 2096-2102.
- Bubenik, G.A., Schams, D., White, R.G., Rowell, J., Blake, J., Bartos, L. (1998). Seasonal levels of metabolic hormones and substrates in male and female reindeer (*Rangifer tarandus*). *Comparative Biochemistry and Physiology Part C* 120: 307-315.
- Buckrell, B.C., Bonnett, B.N., Johnson, W.H. (1986). The Use of Real-Time Ultrasound rectally for early Pregnancy Diagnosis in Sheep. *Theriogenology* 25 (5): 665-673.
- Couturier, S., Côté, S.D., Huot, J., Otto, R.D. (2009). Body-condition dynamics in a northern ungulate gaining fat in winter. *Canadian Journal of Zoology* 87: 367-379.
- Cuyler, C., White, R.R., Lewis, K., Soulliere, C., Gunn, A., Russel, D.E., Daniel, C. (2012). Are warbles and bots related to reproductive status in West Greenland caribou? *Rangifer* Special Issue 20: 243-257.
- Espmark, Y. (1971). Antler Shedding in Relation to Parturition in Female Reindeer. *The Journal of Wildlife Management* 35 (1): 175-177.
- Flood, P.F., Tyler, N.J.C., Read, E.K., Rodway, M.J., Chedrese, P.J (2005). Ovarian and placental production of progesterone and oestradiol during pregnancy in reindeer. *Animal Production Science* 85: 147-162.
- Gerhart, K.L., Russell, D.E., Van DeWettering, D., White, R.G., Cameron, R.D. (1997a). Pregnancy of adult caribou (*Rangifer tarandus*): evidence for lactational infertility. *Journal of Zoological Society of London* 242: 17-30.

- Gerhart, K.L., White, R.G., Cameron, R.D., Russel, D.E. and Van DeWetering, D. (1997b). Pregnancy rate as an indicator of nutritional status in *Rangifer*: implications of lactational infertility. *Rangifer* 17 (1): 21-24.
- Greer, K.R. and Hawkins Jr., W.W. (1967). Determining Pregnancy in Elk by Rectal Palpation. *Journal of Wildlife Management* 31 (1): 145-149.
- Hermes, R. (1997). Sonographie der Trächtigkeit beim Europäischen Reh (*Capreolus capreolus*) und Quantifizierung endometrialer Veränderungen während der Diapause mittels computergestützter Graustufenanalyse. PhD. at *Freie Universität Berlin - Journal der Veterinärmedizin* Nr. 2151.
- Hoare, E.K., Parker, S.E., Flood, P.F. and Adams, G.P. (1997). Ultrasonic Imaging of Reproductive Events in Muskoxen. *Rangifer* 17 (3): 119-123.
- Ingold, T. (1980). Hunter, Pastoralists and Ranchers: Reindeer Economics and Their Transformation. *Cambridge University Press*, New York.
- Ishwar, A.K. (1995). Pregnancy diagnosis in sheep and goats: a review. *Small Ruminant Research* 17: 37-44.
- Jernsletten, J.L., Klovov, K. (2002). Sustainable Reindeer Husbandry. *Centre for Sámi Studies – University of Tromsø*: 1- 164.
- Kaps, M., Lamberson W.R. (2004). Biostatistics for Animal Science. *Cabi Publishing*. UK: 65-107.
- Karen, A., Darwish, S., Ramoun A., Tawfeck, K. et al. (2007). Accuracy of ultrasonography and pregnancy-associated glycoprotein test for pregnancy diagnosis in buffaloes. *Theriogenology* 68: 1150-1155.
- Kutty, C.I. (1999). Gynecological Examination and Pregnancy Diagnosis in small Ruminants using Bimanual Palpation Technique: A Review. *Theriogenology* 51: 1555-1564
- Länsstyrelsen (2014). Djur och natur – Naturreservat arjeplog – Udtja. Accessed: 20<sup>th</sup> July 2014. <http://www.lansstyrelsen.se/norbotten/Sv/djur-och-natur/skyddad-natur/naturreservat/arjeplog/Pages/udtja.aspx>
- Lent, P. C. (1965). Observations on Antler Shedding by Female Barren-Ground Caribou. *Canadian Journal of Zoology* 43: 553-558
- Lenvik, D. (1990). Flokkstrukturing – tiltak for lønnsam og ressurstilpasset reindrift – Herdstructure and Production. *Rangifer* Special Issue 4: 21-35.
- Lyver, P.O'B., Łutsël K'é Dene First Nation (2005). Monitoring Barren-Ground Body Condition with Denésq̓liné Traditional Knowledge. *Arctic* 55 (1): 44-54.

- Mathisen, J.H., Landa, A., Andersen, R. & Fox, J.L. (2002). Sex-specific differences in reindeer calf behaviour and predation vulnerability. *Behavioral Ecology* 14 (1): 10-15.
- Messier, F., Desaulniers, D.M., Goff, A.K., Nault, R., Patenaude, R. and Crete, M. (1990). Caribou Pregnancy Diagnosis from Immunoreactive Progestins and Estrogens Excreted in Feces. *Journal of Wildlife Management* 54 (2): 279-283.
- Nieminen, M., Leppäluoto J. (1986). Relationships between body weight and body measurements of reindeer. *Rangifer* 1 Appendix: 100.
- Nybakk, K., Kjelvik, O., Kvam, T. (1999). Golden Eagle Predation on Semidomestic Reindeer. *Wildlife Society Bulletin* 27 (4): 1038-1042.
- Nybakk, K., Kjelvik, O., Kvam, T., Overskaug, K. & Sunde, P. (2002). Mortality of semi-domestic reindeer *Rangifer tarandus* in central Norway. *Wildlife Biology* 8 (1): 63-68.
- Pratt, M.S. & Hopkins, P.S. (1975). The Diagnosis of pregnancy in sheep by abdominal palpation. *Australian Veterinary Journal* 51: 378-380.
- Reimers, E. (2002). Calving time and foetus growth among wild reindeer in Norway. *Rangifer* 22 (1): 61-66.
- Reimers E., Nieminen M. & Tsegaye D. (2013). Antler casting in relation to parturition in semi-domesticated female reindeer. *Rangifer* 33 (1): 17-24.
- Revol, B. & Wilson, P.R. (1991). Foetal ageing in farmed red deer using real-time ultrasonography. *Animal Reproduction Science* 25: 241-253.
- Rönnegård, L., Forslund, P., Danell, O. (2002). Lifetime patterns in adult female mass, reproduction, and offspring mass in semidomestic reindeer (*Rangifer tarandus tarandus*). *Can. J. Zool.* 80: 2047-2055.
- Ropstad, E. (2000). Reproduction in female reindeer. *Animal Reproduction Science* 60-61: 561-570.
- Ropstad, E., Johansen, O., King, C., Dahl, E. et al. (1999). Comparison of plasma progesterone, transrectal ultrasound and pregnancy specific proteins (PSPB) used for pregnancy diagnosis in reindeer. *Acta Veterinaria Scandinavica* 40 (2): 151-162.
- Ropstad, E., Veiberg E., Säkkinen, H., Dahl, E., Kindahl, H., Holand, Ø., Beckers, J.F., Eloranta, E. (2005). "Endocrinology of pregnancy and early pregnancy detection by reproductive hormones in reindeer (*Rangifer tarandus tarandus*).*" Theriogenology* 63(6): 1775-1788.
- Rowell, J.E. and Shipka, M.P. (2009). Variation in gestation length among captive reindeer (*Rangifer tarandus tarandus*). *Theriogenology* 72: 190-197.
- Russel, D.E., Gerhart, K.L., White, R.G., and Van DeWetering, D. (1998). Detection of early Pregnancy in Caribou: Evidence for embryonic Mortality. *Journal of Wildlife Management* 63 (3): 1066-1075.

Sametinget, (2013). Statistik Renhjorden. *Sametinget*. Accessed on 22<sup>nd</sup> June 2014.  
<http://www.sametinget.se/statistik/renehjorden>

Savela, H., Vahtiala, S., Lindeberg, H., Dahl, E., Ropstad, E., Beckers, J.-F. and Saarela, S. (2009). Comparison of accuracy of ultrasonography, progesterone, and pregnancy-associated glycoprotein tests for pregnancy diagnosis in semidomesticated reindeer. *Theriogenology* 72: 1229-1236.

Sempere, A.J., Renaud, G. and Bariteau, F. (1989). Embryonic Development Measured by Ultrasonography and Plasma Progesterone Concentrations in Roe Deer (*Capreolus capreolus* L.). *Animal Production Science* 20: 155-164.

Shipka, M.P., Rowell, J.E. and Sousa, M.C. (2007). Steroid hormone secretion during the ovulatory cycle and pregnancy in farmed Alaskan reindeer. *Journal of Animal Science* 85: 994-951.

Sjaastad, K.H., Øystein, V. (2010). Physiology of domestic animals. *Scandinavian Veterinary Press*, Oslo.

Skarin, A., Danell, Bergström, R., Moen, J. (2010). Reindeer movement patterns in alpine summer ranges. *Polar Biology* 33: 1263 – 1275.

Skogland, T. (1984). The Effects of Food and Maternal Conditions on fetal Growth and Size in Wild Reindeer. *Rangifer* 4 (2): 39-46.

Skogland, T. (1990). Density dependence in a fluctuating wild reindeer herd; maternal vs. offspring effects. *Oecologia* 84: 442-450.

Swenson, J. (2013). Bear – Sweden. *Status, management and distribution of large carnivores – bear, lynx wolf & wolverine – in Europe*. Editors: Kaczensky P., Chapron, G., von Arx M., Huber, D., Andrén, H. and Linnell J., European Commission Report (Part 2): 59-60.

Thompson, D.P. & Barboza, P.S. (2013). Responses of caribou and reindeer (*Rangifer tarandus*) to acute food shortages in spring. *Can. J. Zool.* 91: 610-618.

Tyler, N.J.C. (1987). Fertility in female reindeer: the effect of nutrition and growth. *Rangifer* 7 (2): 37-41.

Vahtiala, S., Säkkinen, H. et al. (2004). Ultrasonography in early pregnancy diagnosis and measurements of fetal size in reindeer (*Rangifer tarandus tarandus*). *Theriogenology* 61(4): 785-795.

Wiklund, E., Johansson, L., Malmfors, G. (2003). Sensory meat quality, ultimate pH values, blood parameters and carcass characteristics in reindeer (*Rangifer tarandus tarandus* L.) grazed on natural pastures or fed a commercial feed mixture. *Food Quality and Preference* 14: 573 – 581.

Willard, S.T., Petty, S.J., Sasser, R.G., White, D.L. and Randel, R.D. (1999). Pregnancy detection and the effects of age, body weight, and previous reproductive performances on pregnancy status and weaning rates of farmed fallow deer (*Dama dama*). *Journal of Animal Science* 77: 32-38.

Williams, S.M. (2003). Tradition and Change in the Sub-Arctic. Sàmi Reindeer Herding in the Modern Era. *Scandinavian Studies*. 229-256.

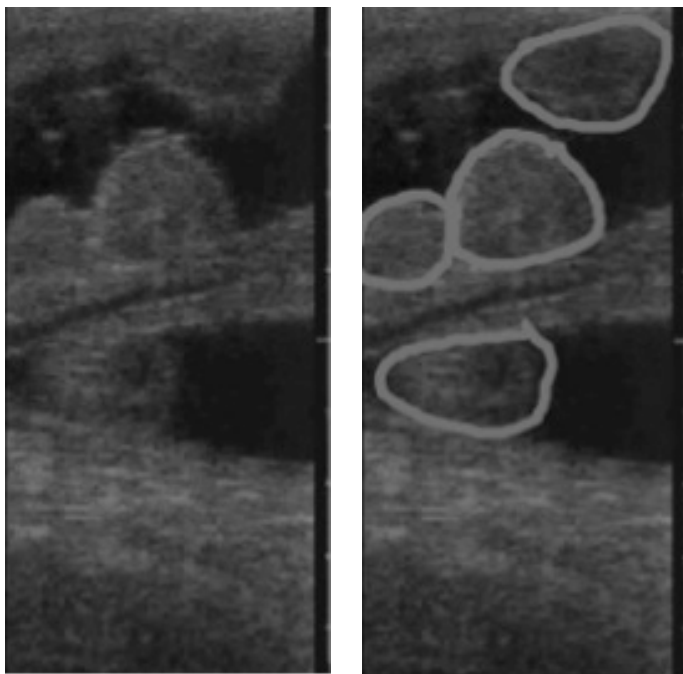
Annex

Annex A – maps

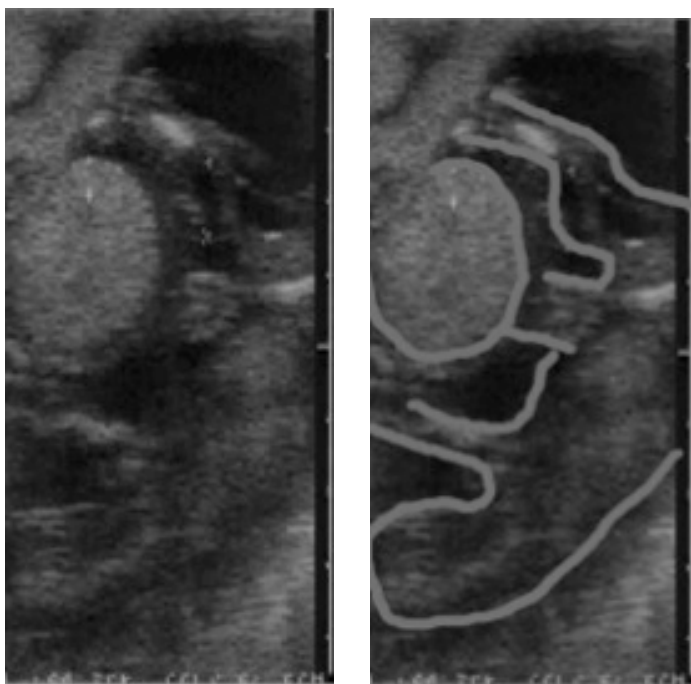


Figure A. Map of reindeer herding area in Sweden. (© Sametinget, 2013)

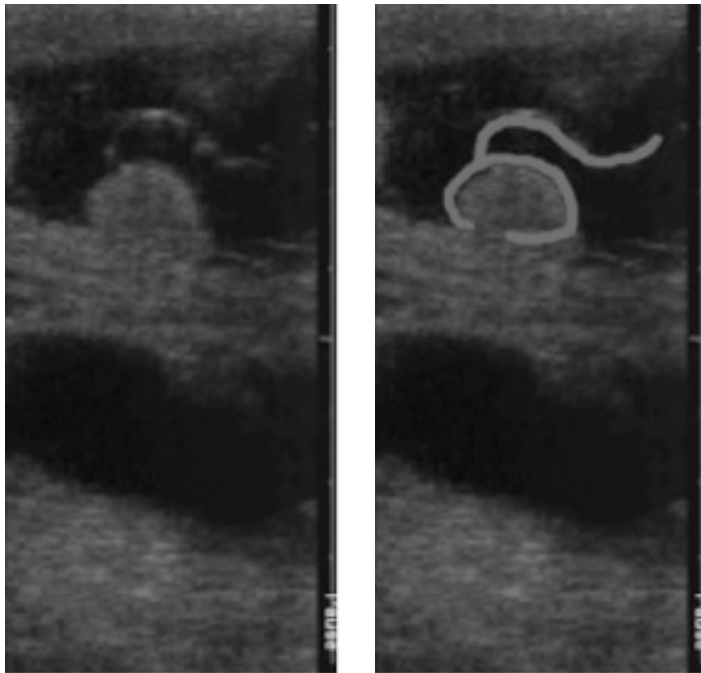
## Annex B - Ultrasonography



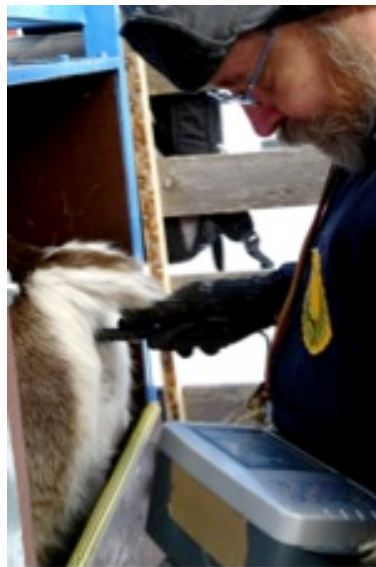
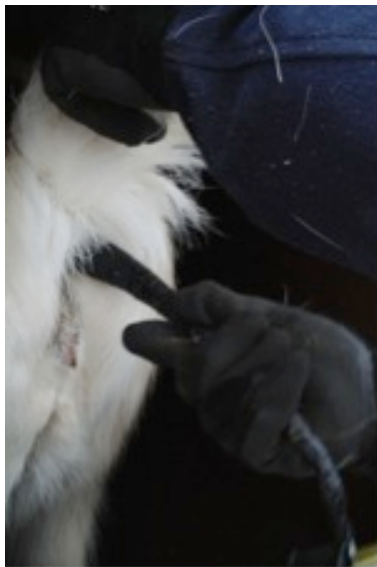
**Figure B1.** Placentomes in pregnant reindeer in January, Purno (© H.Sirkkola).



**Figure B2.** Placenta with fetus in January, Purno (© H.Sirkkola)

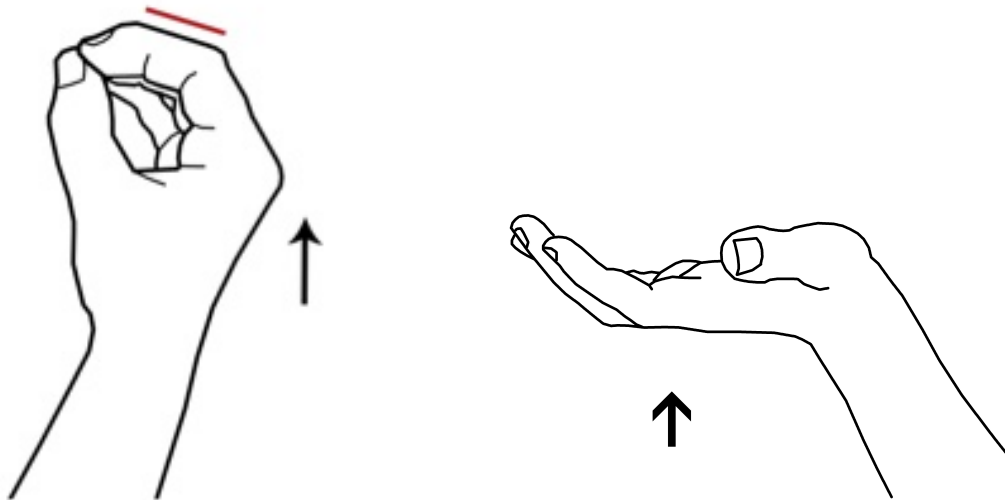


**Figure B3.** Placentome with umbilical chord. (© H. Sirkkola)

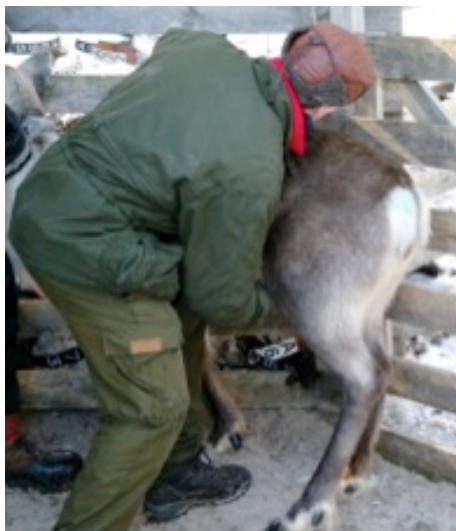


**Figures B4 & B5.** Pregnancy testing with ultrasound probe. (photo E.Paul)

## Annex C - Palpation



**Figures C1a and b.** Hand form and movement direction for abdominal palpation - Contact surface on abdomen marked in red. (photo by E.Paul)



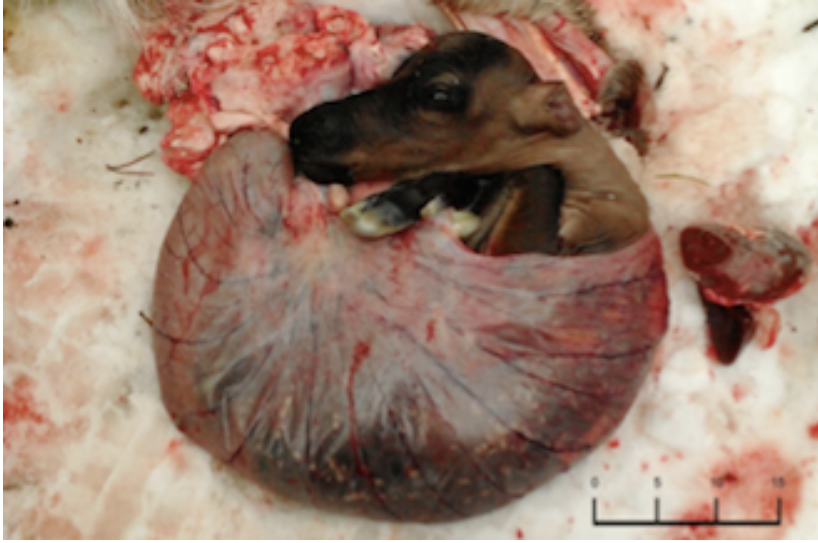
**Figure C2.** Palpation of fixated female (photo by E.Paul)



**Figure C3.** Reindeer Fetus, approximately 24 weeks old. Scale in cm. The female was euthanized due to a traumatic injury at the spine. The same fetus is shown in the following pictures. (Photo by E.Paul)



**Figure C4.** Reindeer Fetus, approximately 24 weeks old. Scale in cm. (Photo by E. Paul)



**Figure C5.** Reindeer Fetus, approximately 24 weeks old. Scale in cm. (Photo by E. Paul)

#### Annex D – Visual examination



**Figure D1.** Female A355 - positive evaluation outcome unclear.



**Figure D2.** Reindeer with collar A313 positive verdict, outcome unclear



a)



b)

**Figure D3.** Reindeer B254 - positive verdict with calving success.

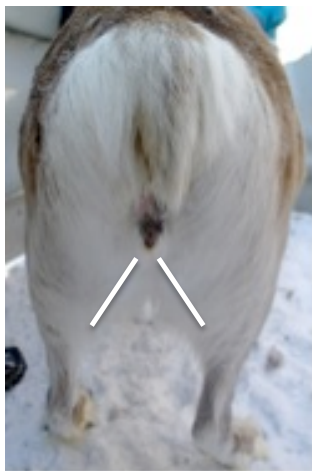


a)



b)

**Figure D4.** Reindeer C202 – positive verdict with calving success



a)

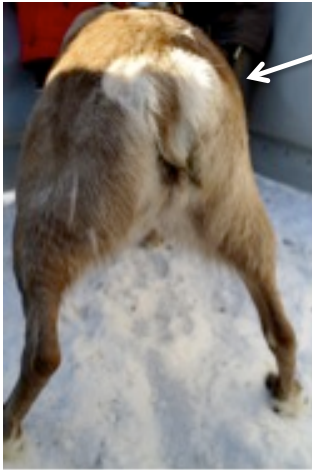


b)

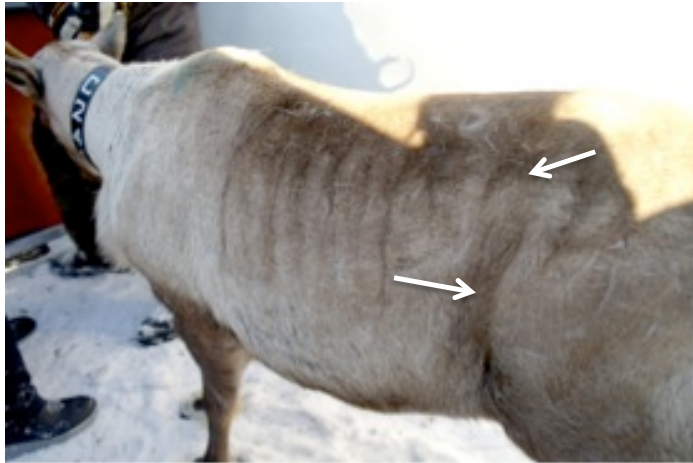
**Figure D6.** Reindeer C239 – positive verdict with calving success.



**Figure D7.** Reindeer C010 – negative verdict no calf-at-foot at calf observation



a)



b)

**Figure D8.** Reindeer C241 – positive pregnancy verdict with unclear calving outcome.

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