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Microcirculatory investigations of nasal mucosa in reindeer *Rangifer tarandus* (Mammalia, Artiodactyla, Cervidae): Rudolph's nose was overheated

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The classic Christmas tale and song 'Rudolph the red-nosed reindeer' evoked several theories about the cause of the remarkable colour of the animal's nose, ranging from (1) a common cold, (2) alcoholic intoxication, to (3) a parasitic infection of the nostrils. Still, there is no conclusive scientific evidence of the nature of Rudolph's red nose. We found a clue in earlier studies that showed reindeer are able to restrict respiratory heat loss by the use of nasal heat exchange. Work that demonstrated a vascular basis for regulation of nasal heat exchange through capillaries and arterio-venous anastomoses inspired us to use Sidestream Dark-Field (SDF) imaging to examine the microvascular flow in the nasal mucosa of Zoo-kept reindeer (Rangifer tarandus fennicus). We found hairpin shaped 'radiator-like' (looping) small blood vessels in the nasal mucosa, which are most likely responsible for the warming and/or cooling of the inhaled freezing arctic air during nasal panting. This led to the following new explanation of the nature of Rudolph's red nose. The exceptional physical burden of flying with a sleigh with Santa Claus as a heavy load could have caused cerebral and bodily hyperthermia, resulting in an overworked nasal cooling mechanism that resembles an overheated cooling radiator in a car: Rudolph suffered from hyperemia of the nasal mucosa (a red nose) under more extreme heat loads during flight with a sleigh.

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INTRODUCTION

Reindeer (caribou), Rangifer tarandus (LINNAEUS, 1758), have a wide circumpolar distribution in the tundra and taiga zones of northern Europe, Siberia and North America. In Europe, wild populations are found in Norway, Finland, Svalbard and Russia. In North America, wild populations roam Canada and Alaska. Semi-domesticated reindeer are widespread in Lapland (northern Sweden and Norway) and Iceland (Henttonen & Tikhonov 2008). The populations are large. Reindeer are divided into two major aroups. tundra reindeer and woodland reindeer. Nine subspecies are recognized. The most common subspecies are the mountain reindeer, Rangifer tarandus tarandus, from the arctic tundra of Eurasia, and the porcupine caribou, Rangifer tarandus granti, which is distributed in Alaska and Canada. The Finnish forest reindeer, Rangifer tarandus fennicus, occurs

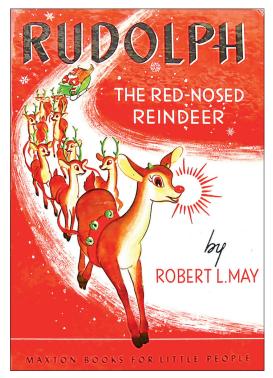


Figure 1 The cover of the first edition (1939) of Robert May's 'Rudolph the red-nosed reindeer'. (Mongomery Ward & Co | Denver Gillan)

in the wild in areas in the Fennoscandia peninsula. We studied individuals of this subspecies.

Reindeer are well adapted to the extreme arctic weather by an isolating fur, but this insulation can also lead to hyperthermia during extreme physical exercise. During such heat stress, cold venous outflow from the nasal mucosa is assumed to be drained to the heart for central body cooling or through the angular oculi vein to the cavernous venous sinus located below the brain for selective brain coolina (Blix et al. 2011). Restina winter-adapted reindeer pant with closed mouths through their noses, whereas when exposed to an increased heat load, reindeer alternate between open- and closed-mouth panting (Aas-Hansen et al. 2000). Evaporative cooling of the nasal mucosa is assumed to be essential for selective brain cooling (Aas-Hansen et al. 2000; Blix et al. 2011). Blix et al. (2011) concluded that, in resting winteracclimatized reindeer under moderate heat loads, the respiratory frequency is increased and the carotid blood flow is increased to supply blood to the nasal mucosa to provide cooled blood via the facial vein for general body cooling (see also: Johnsen et al. 1985).

The relationship between humans and reindeer is well known. Men have herded reindeer for centuries. They are raised and kept for their meat, fur and antlers and for transportation.

The Santa Clause tale

In the Santa Claus tale, a fantasy story is told that flying reindeer pull the sleigh of Santa Claus. The flying reindeer are first mentioned by name in the poem 'A visit from Saint Nicholas' by Clement Clarke Moore in 1822 (Moore 1837). The eight reindeer of Saint Nicholas are named Dasher, Vixen, Dancer, Prancer, Comet, Cupid, Dunder and Blixen. Rudolph, the legendary red-nosed reindeer, was obviously not part of the historical herd. The intellectual father of Santa's 9th reindeer, Rudolph, was Robert L. May (1905-1976), who created the story 'Rudolph the red-nosed

reindeer' in 1939 as an assignment from Montgomery Ward (May 1939). In the first year of the publication of the story of Santa's 9th reindeer, 2,5 million copies were sold (Fig. 1). The brother-in-law of Robert May, Johnny Marks (1909-1985), adapted the story of Rudolph in the song 'Rudolph the red-nosed reindeer', a number-one hit in the US in the weeks around Christmas 1949. The song was sung by Orvon Grover Autry (1907-1998), better known as Gene Autry. Autry's recording sold 2,5 million copies. It is, based on the origin of May's story and Marks' song, most plausible to state that Rudolph and the other eight reindeer kept by Santa Claus belong to the North American subspecies, Rangifer tarandus granti.

The story tells the life of Rudolph, a young reindeer buck who has an unusual luminous red nose (Fig. 1). This luminous nose, once noticed by Santa Claus, could lead the sleigh through the night, which offered Rudolph recognition and acceptance by the other eight reindeer. It is a feel-good story. As Rudolph is a male name, this part of the story is confusing because Rudolph is almost always depicted with full antlers in the winter season. Therefore, Rudolph must be a female animal or a castrated male as male reindeer lose their antlers during the winter season.

Aim of this study

Why the nose of Rudolph is red or has turned red, remains a mystery. There are several theories, ranging from pure fantasy to (semi)scientific. In this article we try to shed new light on the nature of Rudolph's red nose. We review other work on this subject and present the results of our own investigations on the microcirculation in the nose of reindeer from the herd kept at the Rotterdam Zoo in the Netherlands.

Nasal anatomy in reindeer

Both artiodactyls and felids have a complex, frequently anastomosing vascular carotid network or rete that can cool blood flow to the brain in combination with the cavernous sinus receiving cool blood returning from the nasal cavity. Kreutzer-Johnsen (1985) and Mercer (1986) proposed a theory of heat exchange in the reindeer based on casts of the vascular system in the nasal cavity of the reindeer. In situations of heat conservation, blood runs in opposite directions in the arterial and venous rete systems in the nasal mucosa, resulting in a counter-current system of heat exchange. To preserve the temperature gradient necessary for the cooling of expired air, arterial inflow to the rete is supposed to be low in combination with a constriction of the dorsal nasal vein and the anterior part of the venous rete. Venous return from the nasal mucosa is drained through the sphenopalatine veins, and the blood temperature becomes close to that of arterial blood. In a hot environment or during exercise, an increase in respiratory volume combined with an increased arterial nasal flow, open arterio-venous anastomoses, unidirectional anterior flow in both retia plus venous return through the dorsal vein and constriction of the spheno-palatine veins results in heat loss from the mucosal surface and cold venous blood flow to the carotid rete for brain cooling or to the caval vein for more extensive body cooling. This model was later refined by the observation of an increase in the brain temperature when the nasal venous angular oculi were obstructed (Kreutzer-Johnsen 1987) and the confirmation of β -adrenergic receptors in the angular oculi veins in the nasal mucosa and β-adrenergic innervation of the facial veins, resulting in opposite reactions to sympathetic stimulation and increased facial flow with an effect on general body cooling. Reduced sympathetic stimulation results in increased nasal venous dilatation and selective cooling of the brain (Kreutzer-Johnsen & Folkow 1988). Under heat stress, reindeer will change their breathing pattern, maintaining inspiration through the nose and expiring through the mouth, thereby conserving nasal mucosal cooling and thus cooling of the brain (Kreutzer-Johnsen 1987).

Possible explanations of nasal erythema in reindeer

There are several explanations given for the nasal erythema in Rudolph's nose. Most of these are, as is the story itself, pure fantasy and have not reached the serious literature. One example is alcoholic intoxication resulting in rhinophyma. Rhinophyma is the most severe expression of acne rosacea. The condition is much more common in men than in women. There are conflicting reports about the association between alcohol and rhinophyma, but these reports are supported with invalid or no statistical evidence (Curnier & Choudhary 2004). As there is no mention at all of alcoholic ingestion by Rudolph in the original story by May or in the song by Marks and we are not aware of any case report of rhinophyma in a mammalian species other than man (Homo sapiens), we reject this explanation. Other explanations include nasal parasitic infection, nasal viral infection and weather-dependent nasal erythema. These three explanations are worth discussing in more detail.

Nasal parasitic infection Halvorsen (1986) summarizes the epidemiology of reindeer parasites with reference to the story of Rudolph: 'Typically among the semi-domesticated reindeer of north Norway, the respiratory system alone will harbour a parasitic community consisting of the pentastomid, Linguatula arctica in the sinuses, larvae of the nostril fly, Cephenemyia trompe in the nasal cavity and in the pharynx. [...] So far we have not been able to quantify the combined effects of these parasites, but it is no wonder that poor Rudolph, burdened as he is by parasites, gets a red nose when he is forced to pull along an extra burden like Santa Claus.'

Local erythema due to chronic irritation by parasites offers a reasonable explanation for the phenomenon of a red nose in a reindeer. However, a reindeer with such a seriously infected nose, producing a nasal erythema that could be observed at a distance, would not be able to perform physical exercise such

as pulling a sleigh loaded with presents and an overweight Santa Claus. Thus, the hypothesis that the nasal erythema of Rudolph is due to severe parasitic nasal infection is not plausible.

Viral nasal infection (rhinitis, common cold)

There is an anecdote that Rudolph's nose looks red because he has caught a cold. In man, after rhinitis or the common cold, the nose looks red as result of nasal congestion and hyperemia. Could this symptom also be the reason why Rudolph's nose is red? Although bacterial pneumonia has been described in reindeer, viral infections are less known. There is some evidence showing reindeer have antibodies to a group of bovine respiratory viruses (Dieterich & Morton 1990). Gunenkov et al. (2003) studied the properties of the blood serum of reindeer and reported the results of using such serum in virology and biotechnology. They found that the blood serum of reindeer virtually does not contain any antibodies to respiratory viruses that are widely spread among cattle. Stuen et al. (1993) studied 326 Norwegian reindeer for antibodies to the reindeer herpesvirus, bovine viral diarrhea and parainfluenza type 3 virus but found no antibodies against any of these three viruses.

As the common cold resulting in a rhinitis is a viral infection, there is no evidence that Rudolph's red nose could be explained by such an infection.

Weather-dependent nasal erythema

Teece & Foëx (2007) asked themselves the question 'During inclement weather, especially low temperature and fog, is nasal erythema in reindeer (mythical or otherwise) a good indicator of fitness for 24 h trans global navigation and travel?' They conducted a Medline search including all published papers between 1950 and September 2007 using the OVID interface using the following search terms: reindeer, Rangifer tarandus, nose, rhinophyma, rhinitis, snout, snotter, conk, neb, schnoz, and hooter. They found

nine papers that helped answer their question

They concluded that 'there appears no evidence for a specific disease state in reindeer, therefore nasal erythema may be due to increased blood flow to the nose. The evidence suggests that in winter resting reindeer will not have a red nose, but that when frantically circumnavigating the globe their nose will glow as they try to lose heat. A reindeer with a red nose at rest at the North Pole means either deranged temperature regulation, or the use of drugs. Neither of these conditions would inspire confidence for an arduous journey.'

These authors do not explain why the nasal tissue turns red during increased blood flow and do not present any results from studying the nasal epithelium of reindeer. This study prompted us to investigate the nasal mucosa, to find a clue explaining the nasal erythema described in Rudolph.

MATERIAL AND METHODS

Reindeer

Four male and one female reindeer of the subspecies Rangifer tarandus fennicus, the Finnish forest reindeer, were studied at the Rotterdam Zoo in Rotterdam, the Netherlands during three visits:

- 1 Zoo registry number 107590, 7-yearold male, studied November 3, 2009 and October 8, 2010;
- 2 Zoo registry number 107591, 3-yearold male, studied November 3, 2009 and October 08, 2010;
- 3 Zoo registry number 107592, 4-yearold male, studied November 3, 2009 and October 08, 2010;
- **4** Zoo registry number 107965, 2-year-old male, studied October 8, 2010.

The experimental protocol was approved by the Animal Experiments Committee under the national Experiments on Animals Act (of the Netherlands) and adhered to the rules laid down in this national law that serves as the implementation of the 'Guidelines on the protection of experimental animals' by the Council of Europe (1986), Directive 86/609/ EC (DEC-Consult nr 142-10-02).

5 The nasal mucosa of a 15-year old female (born December 25, 1996) was obtained (and studied) after the death of the animal on July 19, 2012.

Study methods

The studied reindeer were anesthetized for seasonal antler removal. The nasal mucosa of the animals was studied (Figs. 2, 3, 4) under anesthesia and without any chemical influence at an approximately 10-15°C air temperature and a normal body temperature.

The microcirculation was directly visualized in the nasal mucosa using a Sidestream Dark-Field (SDF) imager (Microscan, Microvision Medical, Amsterdam, the Netherlands) (Figs. 3, 4) (Goedhart 2007). The SDF imager contains a 5x stroboscopic light-emitting diode that produces light absorbed by the hemoglobin in red blood cells, making the capillaries of the microcirculation visible in different tissues. This modality filters out the surface reflection and permits the visualization of subsurface structures. The light from the concentrically positioned light-emitting diodes (530-nm wavelength) penetrates 1 mm into the tissue, thereby illuminating the microcirculation and its components. Because hemoglobin absorbs this wavelength, erythrocytes can be clearly observed as flowing cells. After the gentle removal of saliva and other secretions with gauze, the device was applied without pressure on the lateral side of the left nasal cavity in an area approximately 1-3 cm from the tip of the cavity. Steady images of at least 20 seconds were obtained and stored on digital videotape, avoiding pressure artifacts. Subsequently, the images were captured in 5 to 10 s representative video clips in the avi format, which were suitable for analysis.

Quantification of these images was performed using the appropriate software



Figure 2 External nostril of one of the study subjects. (Erwin Kompanje)



Figure 3 Obtaining SDF video frames in the nostril of one of the study subjects using a Sidestream Dark-Field (SDF) imager (Microscan, Microvision Medical, Amsterdam, Netherlands). (Erwin Kompanje)



Figure 4 First (bold headed) and second author (in front) obtaining the SDF images of one of the subjects. (Erwin Kompanje)

(AVA 3.0 package; MicroVision Medical, Amsterdam, the Netherlands) and was scored per quadrant (Dobbe 2008). The vessels were separated into small vessels (< 20 µm; mainly capillaries), medium vessels (20 to 50 µm), and large vessels (51 to 100 µm). The microcirculatory analysis was confined to the capillaries (diameter ≤ 20 µm) because they are the most sensitive to microcirculatory changes in both human and experimental studies (van Genderen 2012). Accordingly, we analyzed the video images semiquantitatively, as described previously (De Backer 2007; Boerma 2005).

• The overall score, called the microvascular flow index (MFI), was defined as:

0 = no flow,

1 = intermittent flow,

2 = sluggish flow, and

3 = continuous flow.

 Vessel perfusion was defined as the proportion of perfused vessels (PPV) (%), calculated as the number of vessels continuously perfused during the observation period divided by the total number of vessels of the same type. The perfused capillary density (PCD) (n/mm) was calculated as the capillary density X the proportion of perfused vessels.

RESULTS

The functional microvascular convection (MFI) and diffusion-related (PCD, PPV) indices observed with the SDF imager showed adequate nasal microcirculatory perfusion. Accordingly, the microvascular flow index (MFI) was 3.0, the perfused capillary density (PCD) was 20,95 n/mm, and the proportion of perfused vessels (PPV) was 94,78%. Figure 5A shows a single video frame of the nasal microvascular architecture of a reindeer demonstrating typical hairpin shaped 'radiator-like' capillary loops in the nasal mucosa. Figure 5B shows a still of a microcirculatory image during analysis using the AVA 3.0 software.

The histological examination of the material obtained after biopsy of the mucosa of the dead reindeer does not provide additional data to help answer our question concerning the origin of possible nasal erythema in reindeer.

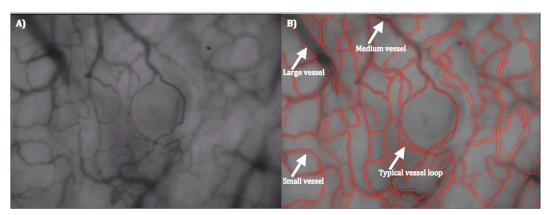


Figure 5 Single video frame of the nasal microvascular architecture of one of the study subjects demonstrating ($\bf A$) a raw image during the measurements and ($\bf B$) the image during analysis using the AVA 3.0 software, showing small (< 20 µm), medium (20 to 50 µm), and large vessels (51 to 100 µm). The image reflects a tissue area of 0.98 × 0.73 mm; Objective 5×, on screen 325×.

DISCUSSION

Reindeer are well adapted to annual changes in ambient temperature by their change in fur, which provides extremely effective insulation. This insulation can cause overheating during physical exercise. Reindeer have some physiological and anatomical adaptations for heat loss during physical exercise to prevent pathological hyperthermia. They have an adaptation for selective brain cooling (Aas-Hansen et al. 2000; Blix et al. 2011). They can regulate this 'radiator-function' by different ways of panting (closed-mouth nasal panting, open-mouth panting). Blix et al. (2011) found that reindeer regulate body and brain temperatures under heavy heat loads by a combination of panting, first through the nose and later through the open mouth. We agree with Teece & Foëx (2007) that if nasal erythema occurs in reindeer, it is most likely due to physiologically increased blood flow to the nose. Other explanations, such as viral or parasitic infection, are not plausible.

The red or luminous nose of Rudolph could be caused by increased blood flow during the inhalation of extremely cold air during nocturnal flights in the month of December at high speeds. In this manner, Rudolph would have inhaled much colder (freezing) air than reindeer at rest on the ground. Furthermore, the panting combination (closed-mouth and openmouth) for effective core and brain cooling might fail to be effective during this abnormal behavior.

Despite the heterogeneous character of the microcirculation, the sublingual microcirculation could reflect capillary perfusion behavior in different organ tissues and provide an easy-to-assess measurement site (Dubin 2009). Capillaries originate from the terminal arterioles, are covered by a thin endothelial surface, and are mostly responsible for O2 and nutrient exchange as well as the elimination of cellular waste products. Although events occurring at capillary and even venular sites may affect microcirculatory perfusion similarly in different organs, in some organs, the specific architecture may favor counter current

exchange mechanisms (Haglund 1993).

Accordingly, our SDF imaging showed 'radiator-like' (looping) small vessels in the nasal mucosa, which are most likely responsible for the warming and/or cooling of the inhaled freezing arctic air during nasal panting and are 'organ-specific' for the nasal microcirculation. Additionally, semi-quantitative and quantitative methods were used for the evaluation of microcirculatory changes. These methods showed adequate microcirculatory perfusion in the nasal mucosa, based on density and flow (Boerma 2005; De Backer 2002). Following the theory of Blix et al. (2011), an explanation of the nasal erythema in some reindeer is that Rudolph, during the extreme physical burden of flying, has to cool his overheated brain by diverting cooled venous blood, which comes from the nose, away from the body and up into the head, where it enters a network of heat exchanging blood vessels to cool the hot arterial blood destined for the brain to protect it from overheating (Knight 2011). Rudolph's exceptional physiological response appears to be visible as an increased erythema of the nose as if it was a cooling radiator in an overheated car engine.

CONCLUSION

There is a scientific explanation for the observation that the nose of Rudolph is red. The exceptional physical burden of flying with a sleigh with Santa Claus as a heavy load could have caused cerebral and bodily hyperthermia, resulting in an overworked nasal cooling mechanism that resembles an overheated cooling radiator in a car: Rudolph suffered from hyperemia of the nasal mucosa (a red nose) under more extreme heat loads during flight with a sleigh.

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Accountability of the authors

BvdH wrote the paragraph on the nasal anatomy of the reindeer, conducted the SDF imaging, was responsible for obtaining permission from the Animal Research Committee at Erasmus University and discussed the content of the discussion/conclusion sections with the other authors; EK conducted the SDF imaging and discussed the content of the discussion/conclusion sections with the other authors; MvG conducted the SDF imaging, interpreted the obtained SDF imaging results, wrote the paragraph on SDF imaging and discussed the content of the discussion/conclusion sections with the other authors; WS was the veterinary surgeon responsible for the studied subjects at the Rotterdam Zoo and discussed the content of the discussion/conclusion sections with the other authors; DvD conducted the SDF imaging and discussed the content of the discussion/conclusion sections with the other authors; LdV processed the biopsy material and made the micrographs; and EJOK wrote the introduction and paragraphs on the possible explanations of nasal erythema, discussed the content of the discussion/conclusion sections with the other authors and was responsible for the overall coordination during the writing of this paper.

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