—Full Paper—

Videodermoscopic examination of the skin and its products in purebred Arabian horses in the summer season

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Videodermoscopy is a method that enables the examination of many parameters of the skin and its structures. The aim of this study was to assess specific dermoscopic parameters in purebred Arabian horses during the summer. The study involved 21 clinically healthy purebred Arabian horses (18 mares and three stallions) that had not been used for breeding and were 1 to 25 years old. The videoderoscopic evaluation was performed on seven selected areas of the body: forehead, mane, neck, chest, flank, rump, and tail. The tests were carried out with Vidix and Olympus cellSens specialised software. Videodermoscopy revealed that the skin was pigmented in all of the bay horses, in one of the seal brown horses, and in the 10 grey horses. Only one grey horse and one chestnut horse had unpigmented skin. Hair thickness ranged from 44.82 µm (neck) to 75 µm (mane). Regarding the amount of hair in the field of view, the highest and lowest numbers of hairs were found on the neck (3,004) and mane (990), respectively. A valuable insight obtained from our research is that it is possible to use digital image analysis for precise quantitative and qualitative evaluations of the skin and its structures.

Key words: *hairs, horses, skin, videodermoscopy*

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There are many diagnostic methods currently used in veterinary dermatology. For several years, non-invasive methods of skin condition assessment have been gaining more and more recognition, both in research and clinical practice. Such methods include, for example, the measurement of skin biophysical parameters (such as trans-epidermal water loss, hydration, and skin reactions) or videodermoscopy [18–20, 23]. Videodermoscopy is a method that enables the examination of many parameters of the skin and its products. This method is recognised and widely used in medicine for the study of human skin. It can be used to assess the thickness, density, pigmentation, and other features of hair [22] and to assess degree of exfoliation, pigmentation, pigment changes, and other features of the epidermis. It is also used to examine sweat glands and visible capillaries [22]. The most important application in human medicine is for the diagnosis of pigmented lesions, primarily neoplastic skin of melanocytic origin. In addition, this method allows for the differentiation of both the causes of folliculitis as well as other lesions [22].

One of the most important applications of videodermoscopy is for improved (magnification and registration) trichoscopic examination (referred to as trichoscopy), which comprises the visualisation of hair structures invisible to the naked eye and quantitative assessment of hair features such as thickness, density, arrangement, intensity of pigmentation, possible distortions, and irregularities [2, 5, 7, 12]. Trichoscopy is also a non-invasive research method that enables the assessment of hair follicles, which is helpful in identifying the causes of alopecia [22].

To date, videodermoscopic examination has been used to assess the skin of dogs, cats, and horses, both in healthy

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animals and in animals with various skin diseases (dogs and cats only). With regard to the assessment of dermoscopic features in various disease states, the information on this subject is very fragmentary. These features have been described in cases of dermatophytosis, demodicosis, folliculitis, and diseases associated with the formation of alopecia, both non-inflammatory alopecia and alopecia associated with itching [1, 3, 13–16, 22, 24–26].

In horses, videodermoscopy is not particularly popular yet, and there are only a few publications available that describe it [6, 10, 21].

To date, this type of research has been conducted by Legnani *et al.* (2018) in Italy and Tomich *et al.* (2018) in the United States and concerned randomly selected horses in terms of race, gender, age, and method of use. Only the studies of Pomorska *et al.* focused on a group of animals that was relatively homogeneous in terms of race and gender.

This method will undoubtedly become more popular in the coming years, as horses are increasingly treated as accompanying and diagnosed animals and treated with the same care as dogs or cats.

Due to the extension of the lifespan of horses, previously unrecognised diseases have appeared. This applies, inter alia, to skin diseases, which are now the second most frequently reported problems by horse owners after diseases of the locomotor system (lameness). This brings with it the need to develop research on videodermoscopy and other topics for the diagnosis of dermatological problems in the species. To make full use of the diagnostic potential of videodermoscopy in horses, it is necessary to fully understand the dermoscopic features of the skin and its products.

The aim of this study was to assess specific dermoscopic parameters (hair thickness and number and evaluations of hair follicles and capillaries) in seven selected body areas of purebred Arabian horses during the summer.

Similar studies have already been conducted during the winter, but no information is available about such work performed during the summer. This work is novel because the research was performed in one breed of horses in one season.

As a result, we expect the results to be more repeatable. In the future, it should be possible to compare results obtained in the summer and winter.

Materials and Methods

The study involved 21 clinically healthy purebred Arabian horses that had not been used for breeding and were 1 to 25 (median 6) years old: 18 mares that were 1 to 25 years old and three stallions that were 1 to 5 years old. The study group contained 11 grey horses (three stallions and eight mares), eight bay mares, one chestnut mare, and one seal brown mare. The animals came from a breeding farm located in the Lubelskie Voivodeship. They were kept in a common stable and were fed and used in the same ways. The research was conducted in August 2019.

Written consent to participate in the study was obtained from the horses' owners before examinations. All procedures in this study were non-invasive and did not require institutional approval. The examinations were performed in the corridor of the stable where the animals were housed. The horses were haltered and held by their handlers, and none required any sedation or other methods of restraint during the examinations.

Videodermoscopic evaluations were performed on seven selected areas of the body: the forehead, mane, neck, chest, flank, rump, and tail [6, 10]. During the study, the thickness of primary hair and the number of hairs (density) were quantified. Pigmentation, skin colour, and visibility of hair follicles and capillaries were qualitatively assessed.

The tests were carried out with Video-Dermascope 7 (with 3 VIDIX 5 Mpx cameras and a VX1 overlay − contact-type cap Ø 3.5 cm; Medici Medical SRL). The test sites were prepared by cleaning them with a brush and then applying an immersion oil to the hair and skin in order to improve the quality of contact with the camera head and image quality. The hair was combed to the sides, parting the hair to reveal the epidermis and dermis. The videodermoscope head was only applied after this was done. Photos were taken at two magnifications, 20× and 30×, using polarised light. The use of polarised light minimises reflections from the skin surface and facilitates the visualisation of structures located deeper below the dermal-epidermal junction, i.e., the superficial layers of the dermis [8, 9].

All photos were saved at high resolution (2560 px × 1920 px). The pigmentation, skin colour, and hair follicle and capillary visibility parameters tested were assessed at the patient's side in real time and registered in the appropriate test form. The other parameters, including hair thickness and number of hairs (density), were calculated in a later evaluation.

Photos taken at 30× magnification were used to calculate the average thickness of primary hair. The diameters of 10 randomly selected hairs were measured three times at intervals equivalent to one third the length of the hair. The average of these measurements was used for further calculations. The Olympus cellSens specialised software was used to analyse microscopic images and perform the above measurements. Identical hair thickness tests were performed in each of the seven selected body areas.

Hair density in the examined areas was calculated automatically based on the above-mentioned software from the entire areas of individual photos (9.62 cm^2) at 30× magnification. In total, 147 photos were subjected to this analysis.

Statistically significant differences in hair thickness and hair density were calculated using the Mann-Whitney U test. Statistical analysis was performed using the Statistica 10 software (StatSoft, Tulsa, OK, USA). $P<0.05$ was considered significant.

Results

In all areas of the body, longer overhair predominated, and underhair was practically absent.

Quantitative assessment

Hair thickness

Hair thickness ranged from 44.82 μm (neck) to 75 μm (mane). The results for hair thickness are presented in Table 1. Statistical analysis showed statistically significant differences in hair thickness between different parts of the body. Differences were noted between the forehead and neck (P=0.007), forehead and breast (P=0.01), forehead and mane (P=0.0002), neck and mane (P=0.000008), breast and mane (P=00002), flank and rump (P=0.00004), rump and mane (P=0.00007), and mane and tail (P=0.04).

Density

Regarding the amount of hair in the field of view, the highest and smallest numbers of hairs were found on the neck (3,004) and mane (990), respectively. The results regarding the amount of hair are presented in Table 2. Statistical analysis showed statistically significant differences in the amount of hair between different parts of the body. Differences were noted between the forehead and neck (P=0.00005), forehead and flank (P=0.014), neck and breast (P=0.023), neck and mane (P=0.00006), neck and tail (P=0.006), flank and mane (P=0.02), and rump and mane (P=0.003).

Clinical results of the videodermoscopic evaluation

In most body regions, the hair was short, pointed, and fully visible in the field of view of the videodermoscope. The hair tightly covered the surface of the skin, making it difficult to visualise. In some horses, both grey and bay horses, the skin was unevenly pigmented. Grey horses were the largest group in terms of coat colour (n=11). A characteristic feature of Arabian horses is a large individual variation in pigmentation in those with a grey coat. Grey horses have lighter hair on the head in comparison to the much darker hair on the rump area. A characteristic feature of the other coats (bay, seal brown, and chestnut) was the lighter colour of the hair on the chest and flanks compared with other regions.

Table 1. Measurements and statistical differences for hair diameter in different areas of the skin in Arabian horses

	Forehead	Neck	Breast	Flank	Croup	Mane	Tail
Mean average (μm) ± SD	55.387 ± 15.830	44.821 ± 18.315	49.776 ± 9.617	52.996 ± 8.034	51.453 ± 15.158	75.537 ± 16.798	52.291 ± 37.844
Forehead	-	0.007101	0.010292	0.052747	0.068185	0.000169	0.724102
Neck	0.007101	-	0.715270	0.166475	0.174265	0.000008	0.129898
Breast	0.010292	0.715270	-	0.166492	0.351977	0.000021	0.198875
Flank	0.052747	0.166475	0.166492	-	0.959874	0.000037	0.301674
Croup	0.068185	0.174265	0.351977	0.959874	-	0.000067	0.449416
Mane	0.000169	0.000008	0.000021	0.000037	0.000067	-	0.038857
Tail	0.724102	0.129898	0.198875	0.301674	0.449416	0.038857	-

Table 2. Density and statistical differences for hair in the dermoscope field of view (9.62 cm^2) in different areas of the skin in Arabian horses

	Forehead	Neck	Breast	Flank	Croup	Mane	Tail
Mean average ± SD	1020.055 ± 666.934	3004.667 ± 2602.037	1816.150 ± 1824.358	1873.714 ± 1247.306	1996.660 ± 1348.527	990.210 ± 490.564	1326.210 ± 1066.615
Forehead	-	0.000045	0.101730	0.014742	0.002768	0.855085	0.605389
Neck	0.000045	-	0.023468	0.139136	0.207290	0.000570	0.006339
Breast	0,101730	0.023468	-	0.592870	0.255141	0.160037	0.325444
Flank	0.014742	0.139136	0.592870	-	0.745176	0.022903	0.115886
Croup	0.002768	0.207290	0.255141	0.745176	-	0.002638	0.068597
Mane	0.855085	0.000570	0.160037	0.022903	0.002638	-	0.754919
Tail	0.605389	0.006339	0.325444	0.115886	0.068597	0.754919	-

Forehead

Short, pointed hair was observed and was fully visible in the tested field.

Pigmented skin was present in eight of the bay horses and the one seal brown horse. A characteristic darker keratin rim was observed around the follicle ostium (light or darkening around the mouth of the hair follicle; Fig. 1). In horses with a lighter bay colour, the core and hair cortex were clearly visible. In darker-coloured horses, the hair was uniform (the core could not be distinguished from the cortex). The grey horses comprised 11 animals. The skin of one of the horses was unpigmented (Fig. 2), whereas the others had pigmented skin containing spots of unpigmented skin (Fig. 3). The level of skin pigmentation increased with age. Only two horses had a characteristic darker keratin rim around the hair follicle ostium. It was not observed in the rest of the horses. On the forehead, the hair was short, pointed, and fully visible in the field of view of the videodermoscope (Fig. 4). The skin of all of the flea-bitten grey horses was pigmented.

Only one horse of chestnut colour took part in the study. His skin was unpigmented, with no visible follicles or vessels. His hair was short and pointed.

Mane

Mane hair was thicker and less dense compared with hair elsewhere on the body. It was difficult to differentiate between the cortex and medulla, especially with dark hair. Empty hair follicles and newly growing short, thin hairs were visible (Fig. 5). Blood vessels were visible on unpig-

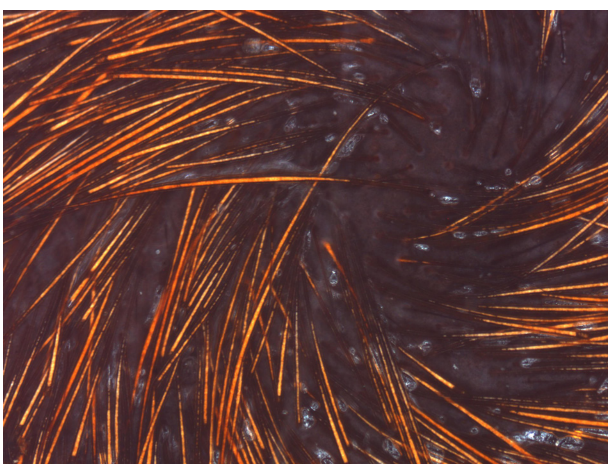

Fig. 1. Characteristic darker keratin around the mouth of the hair follicles.

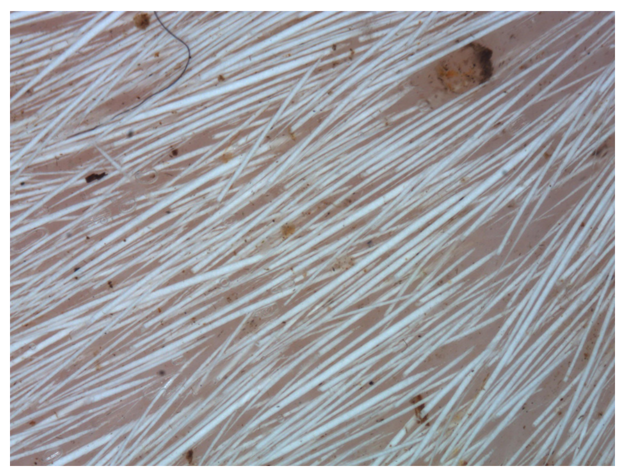

Fig. 2. Unpigmented skin.

Fig. 3. Pigmented skin.

Fig. 4. Short, pointed, and fully visible hair in the field of view of the videodermoscope.

mented skin (chestnut and grey; Fig. 6). In some grey and bay horses, the skin was unevenly pigmented. Furthermore, some grey and bay horses had a visible concentration of pigment at the mouth of the hair with the colour lightening with distance from there.

Neck

On the neck, the hair was much denser and longer compared with the hair on the other parts of the body. It was so short that it was difficult to part the hair to reveal the skin for testing. However, in the videodermoscope image, the hair is so long that you cannot see its full length in the field of view. In the bay, seal brown, and chestnut horses, the lighter core of the hair and darker cortex could be distinguished in most cases.

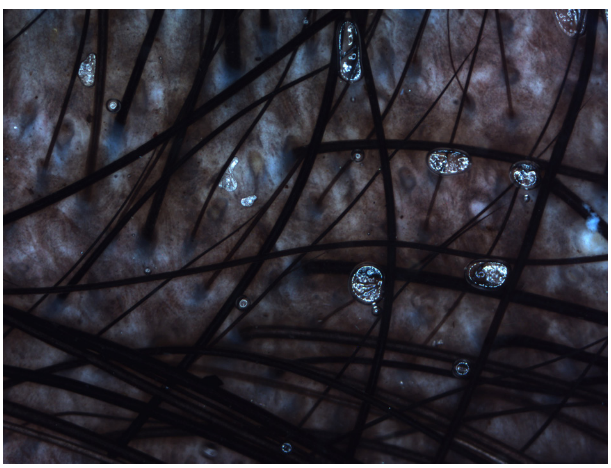

Fig. 5. Empty hair follicles and newly growing short, thin hairs are visible.

Breast

The lowest number of hairs was found on the breast. The hair on the chest was lighter in comparison with the hair on the neck and forehead in the bay, seal brown, and chestnut horses. In the bay and chestnut horses, the hair had a lighter core and darker cortex. The skin was more visible. The hair was shorter compared with the hair elsewhere. In the images, we could see them in their entirety. The skin was pigmented in much the same way as on the neck. Capillary vessels were not visible on pigmented skin.

Flank

In the area of the flank, the colour of the hair of bay, seal brown, and chestnut horses was clearly lighter when compared with the hair on the forehead, neck, and rump (Fig. 7). The hair was short and fully visible in the test field. The cortex and core were easily distinguishable, with the core lighter and the cortex darker. The skin was similarly pigmented, and capillary vessels were not visible.

Rump

The hair on the rump was longer than on the side in most cases. The full hairline was not visible in images. The colour of the hair was much darker in comparison with other parts of the body in bay, chestnut, seal brown, and grey horses in which the greying process had not yet completed. In this area, newly growing thin hairs were visible. The skin was pigmented in some cases and not pigmented in others.

Capillary vessels were not visible on pigmented skin.

Tail

The hair on the tail was thinner compared with the hair on

Fig. 6. Blood vessels are visible.

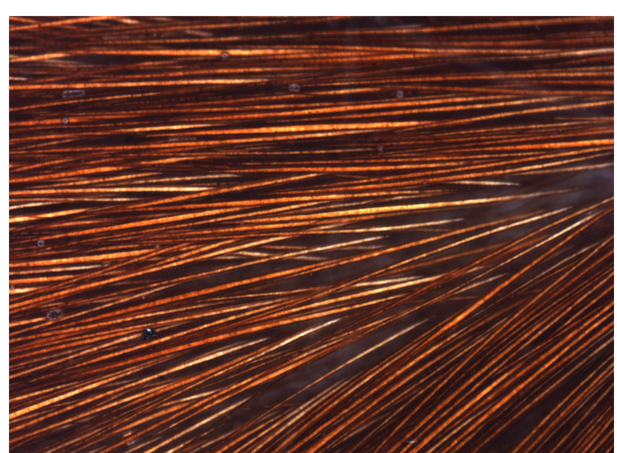

Fig. 7. In the area of the flank, the colour of the hair of bay, seal brown, and chestnut horses is clearly lighter when compared with the hair on the forehead, neck, and rump.

the other areas, with the exception of the mane, and denser than in most of the other areas examined. The core and cortex were easy to distinguish. Hair follicles were visible in thicker hair. Pigmented skin was slightly lighter compared with other areas of the body in most cases. Short and thin growing hair was visible.

Discussion

In mammals, several types of hair can be distinguished. Equines have cover hair, sensory hair, and long hair. Cover hair is further subdivided into two types, the first of which is overhair. This is topcoat hair with a protective function, which is referred to as the coat. The second type is underhair, which is also referred to as down and plays a role in thermoregulatory function. Sensory hair (vibrissae), on the other hand, is located around the upper and lower lips and eyelids.

In equines, we can also distinguish long hair forming the hocks, mane, and tail [4]. The appearance of the hair and its physical properties depend on the proportion between the hair core and cortex. The thicker the core, the more brittle and stiffer the hair. The thickness of the cortex determines the elasticity of hair [4].

Research conducted on the Konik (Polish primitive horses) and (Hucul/Carpathian ponies) has shown that 70% of their hair is underhair and that the other 30% is overhair. In the Polish Koniki, overhair was 50% longer than in the Hucul. On the other hand, the underhair did not differ in length between the two breeds. Overhair and underhair were 25% thicker in the Polish Koniki than in the Hucul [11]. In our study of Arabian horses, longer overhair predominated, and underhair was practically absent. It is possible that this is a typical feature of the breed and that the Hucul and Polish Koniki, being more primitive breeds, differ from riding horses in terms of the type of hair.

The hair on the forehead, breast, and flank was shorter compared with that on the other areas examined. The full lengths of the hair in these areas were visible in the field of view of the videodermoscope. In addition, we found the lowest number of hairs on the breast, which significantly facilitated the videodermoscopic examination of the skin. Longer hair, which was not entirely visible within the field of view, was located on the neck and rump. Research carried out by other authors shows that the weather has a direct influence on the quality of the coat in a given area of a horse's body. The authors showed that the shoulders, back, and loins are much more exposed to rainfall and snowfall residues. Horses kept permanently outdoors have longer hair in these areas [17].

In our research, we showed that longer hair was present on the neck and rump. We did not take into account the shoulders, back, lumbar region, or other areas, so we cannot compare the results of our observations with the above-mentioned observations by Stachurska *et al.* [17]; however, it can be assumed that the hair around the neck and rump is similar to that in the adjacent areas of the back or lumbar region. Short and thin hair (underhair) was scarce in the areas of the rump and mane.

In most of the horses, the hair on the forehead was much lighter compared with the colour of the hair on the rump. In the grey horses, this was related to the gradual decrease in pigmentation from the head to the rump. In all horses (bay, seal brown, grey, and chestnut), a lighter colour of hair was observed in the area of the flank. In the vicinity of the so-called thurl (fossa paralumbalis) in the summer months, you can easily observe the lightening of the hair, the so-called black-and-tan coat colour. Seal brown horses are characterised by the colour of the hair on their entire bodies being uniformly dark in the winter months, while in the summer months, the hair is lighter in colour around the nostrils, flanks, and the inside of the thighs. With the help of a videodermoscope, we were also able to observe this tendency in horses of different colours. In the rump area, the hair was much darker than in the rest of the body.

The skin was pigmented in all of the bay horses, one seal brown horse, and the 10 grey horses. Only one grey horse and one chestnut horse had unpigmented skin. The skin was pigmented in all the flea-bitten grey horses.

In studies conducted by other authors, no visible blood vessels were found on the skin. Research by other authors was conducted at a magnification of 20× [6]. Our study was conducted at 30× magnification, and blood vessels were visible on unpigmented skin in various parts of the body. Similar observations were made in purebred Arabian horses in studies carried out in the winter months [10].

With regard to the hair thickness, our results are consistent with those obtained by other authors [6]. Legnani *et al.* rated the thickness of large primary hair as 50 to 90 μm, Roman *et al.* rated it as 64 to 80 μm, and Pomorska *et al.* rated it as 55 to 87 μm.

We also showed statistically significant differences in hair thickness depending on the studied area. The differentiation of hair thickness depending on the area examined was first identified by Legnani *et al.* These authors found that the thickness of hair was greatest on the mane and was statistically different from the thicknesses of hair on most of the areas examined. Our results coincide with the results obtained in the studies of other authors.

The statistically significant difference in hair thickness between the mane and individual body parts was easy to predict, but we also found statistically significant differences in hair thickness between other body parts. We observed statistically significant differences in hair thickness between the forehead and neck (P=0.007), forehead and breast (P=0.01), and flank and rump (P=0.0004). In these cases, our results do not coincide with those of other authors. Legnani *et al.* did not indicate any difference in hair thickness depending on the area assessed.

The differences between our results and results of other authors are due to our study being conducted on a homogeneous group of animals.

The hair density values we obtained are lower than those described by Legnani *et al.*, who estimated the hair density to be 400–920 cm^2. It is possible that this difference is related to breed-dependent characteristics. In our study, we focused on a homogeneous group in terms of the breed, while this was not the case in the study by Legnani *et al.*

The differences in individual body areas observed by us indicate that both the breed of horse and anatomical area should be considered when making comparisons between individuals and breeds.

There are many differences between summer and winter hair, so it is necessary to limit the conduct of research to specific seasons. Regardless of the assessed area of the body, the skin in the summer was clearly darker compared with the result obtained in winter [10]. Hair thickness during the summer ranged from 44.82 μm (neck) to 75 μm (mane), while in the winter, it ranged from 52.70 (chest) to 87.45 μm (tail) [10]. Regarding the amount of hair in the field of view in summer, the highest and lowest numbers of hairs were found on the neck (3004) and mane (990), respectively, while the amounts ranged from 1,458 (croup) to 3,680 (head) hairs [10]. Our research showed that the season of the year influences the amount of hair without significantly affecting hair thickness.

A limitation of our research was the necessity of performing it only within one (summer) season. Further research is needed to compare the values for hair density and thickness between different times of the year.

A valuable insight obtained from our research is that it is possible to use digital image analysis for precise quantitative and qualitative evaluations of the skin and its products in a standardized study group during a single season. We hope that videodermoscopic examination will be helpful in the diagnosis of alopecia, blood supply, and pigmentation disorders in horses in the future. It will be useful in monitoring the progression and treatment of skin diseases.

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