Case Report
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Sweat Hypersensitivity-Induced Urticaria and Sebaceous Adenitis in an American Saddlebred

G. Lorch, M.B. Calderwood Mays, H.A. Roberts, and K.K. Isler

Key words: Allergy; Epitrichial sweat glands; Horse; Immune-mediated.

A 5-year-old Saddlebred gelding was presented for the evaluation of nonseasonal chronic progressive dermatitis that began at 1 year of age. The dermatitis started as a linear area of crusting and scaling on the lateral aspect of the left shoulder that would resolve in the spring and summer months. However, for the previous 3 years the lesions progressed and were present year round. Many topical and systemic treatments had been used over the 3-year period with no clinically appreciable response, including penicillin injections (dosage unknown), antifungal topical agents, iodine baths, and prednisolone (1 mg/kg PO q24h for 14 days then tapered over 30 days).

For the previous 2 years, nonseasonal pruritic urticaria occurred when the horse would sweat. The pruritus was graded as 9/10 with 10 representing severe pruritus. The intensity of the pruritus limited the horse's ability to work and had increased each year. The pruritus was manifested as stomping, biting at the sides, forceful tail whipping, refusing to stand in place, extreme anxiousness, and rubbing. The urticaria subsided within 20 minutes of working, as did the pruritus. Baths with lukewarm water immediately after working controlled the pruritus. Preemptive 14-day courses of hydroxyzine pamonate (2 mg/kg q8h) and chlorpheniramine (0.5 mg/kg q12h) were ineffective.

On physical examination, generalized distribution of circular to annular areas that had varying degrees of crusting and alopecia, moderate-to-severe scaling, and mild leukoderma was present. Although the lesions were generalized, the most severely affected regions included the face, neck, chest, lateral thorax, and the proximal aspect of all 4 limbs. Two smaller focal areas of thick crusts were removed, disclosing circular erosions. Focal areas of severe scale and crusting and partial alopecia were present at the junction of the pelage and mane, causing loss of the mane (Fig 1A, B). Differential diagnoses for the dermatitis included sebaceous adenitis, discoid lupus erythematosus, and sarcoidosis. Differential diagnoses for the nonseasonal urticaria included exercise-induced cholinergic urticaria (CU), as well as heat, stress-induced, or idiopathic urticaria.

Abbreviations:

CBC	complete blood count
CU	cholinergic urticaria
DTM	dermatophyte test medium
IgE	immunoglobulin E
PCA	passive cutaneous anaphylaxis
RBCs	red blood cells

Initial diagnostic tests consisted of a complete blood count (CBC), serum biochemical profile, cutaneous surface cytology, superficial and deep skin scrapings, a tissue punch biopsy for fungal and bacterial cultures, and seven 6–8 mm skin punch biopsy specimens from multiple affected sites for histopathology. All tests were performed in a routine manner.[1] Impression smear cytology from an erosive lesion on the dorsolumbar trunk showed many neutrophils, occasional red blood cells (RBCs) and rare extracellular cocci per oil immersion field. All superficial and deep skin scrapings were negative for parasites. All CBC and serum biochemical results were within normal limits. Bacterial and fungal tissue cultures were negative for growth of organisms. Histopathology from an alopecic and crusted lesion identified hyperplastic crusting, suppurative lymphohistiocytic and plasma cellular perivascular and periadnexal dermatitis, with compact orthokeratotic hyperkeratosis, follicular keratosis, sebaceous adenitis, and mural folliculitis at the level of the isthmus, and occasional apoptotic epidermal cells (Fig 2A, B). The cells infiltrating the hair follicle walls and sebaceous glands were mostly small lymphocytes (Fig 2C,D). Physical urticaria was ruled out by a negative response to the application of heat, cold, pressure, and water to the skin.

Exercise provocation was used to induce urticaria from sweating. Papular urticaria was observed in areas that had evidence of sweat, which included the trunk, neck, and shoulders (Fig 1C,D). Intense pruritus ensued when the horse was made to stand and included tail swishing, foot stomping, skin twitching,

From the Department of Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH (Lorch, Roberts); Veterinary Pathology, Florida Vet Path, Inc, Bushnell, FL (Calderwood Mays); and Granville, OH (Isler).
This report is from a patient who presented at The Ohio State University College of Veterinary Medicine's Dermatology Service. The reagents used in the intradermal tests were purified and prepared in the corresponding author's laboratory. This case has not been presented at any scientific meetings.

Corresponding author: G. Lorch, DVM, PhD, DACVD, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, 325 Goss Labs, 1925 Coffey Road, Columbus, OH 43210; e-mail: gwendolen.lorch@cvm.osu.edu.

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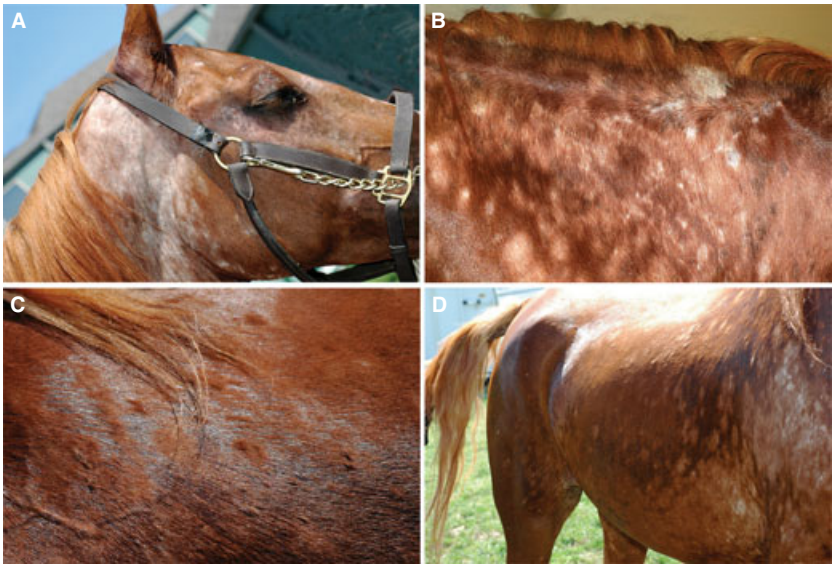

Fig 1. Sebaceous adenitis in a horse. **(A)** Lateral view of head and throatlatch exhibits diffuse areas of alopecia to annular areas of partial alopecia on the jaw, multifocal areas of accumulated fine white adherent scale, and patchy leukoderma. **(B)** Lateral view of neck and mane showing scale at the base of the mane with mane loss. **(C)** Lateral view of the withers demonstrating sweat with fine papular urticaria. **(D)** Lateral aspect of trunk during exercise and sweating displaying fine papular urticaria over the dorsum, multifocal areas of alopecia most severe on the right shoulder and girth region and evidence of tail swishing.

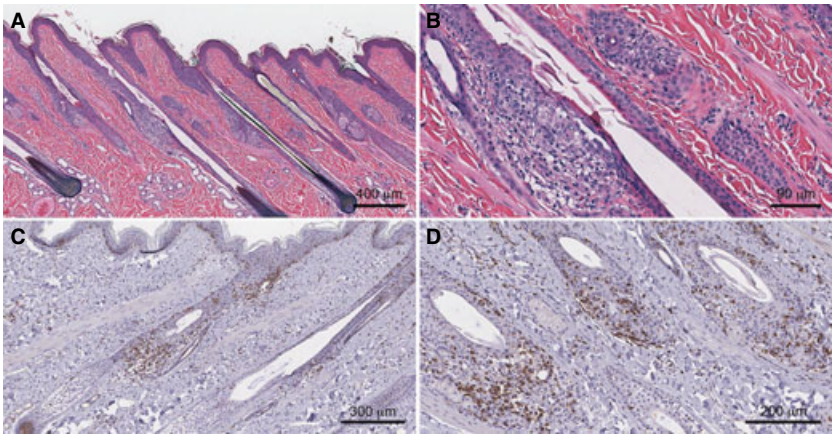

Fig 2. Representative photomicrographs of skin biopsies from the horse with sebaceous adenitis. **(A)** Overview of the sebaceous adenitis and mural folliculitis. There is a predominately lymphocytic infiltrate at the level of the sebaceous glands and the isthmus. Stained with hematoxylin and eosin. **(B)** Higher power view of the sebaceous glands infiltrated by small lymphocytes. Stained with hematoxylin and eosin. **(C)** Overview of the immunohistochemistry. Numerous CD3-positive lymphocytes infiltrate the sebaceous glands and the follicular epithelium at the levels of the isthmus and the infundibulum. Stained with anti-human CD3 and counterstained with hematoxylin. **(D)** Higher power view of the immunohistochemistry. The small lymphocytes infiltrating the sebaceous glands are CD3 positive, and thus are T lymphocytes. Stained with anti-human CD3 and counterstained with hematoxylin.

and generalized agitation. The urticaria subsided within 10–15 minutes after the horse cooled. Pharmacologic induction of papular urticaria occurred with IV administration of detomidine hydrochloride, a synthetic α_2-adrenoreceptor agonist. Five minutes after the horse was sedated, a light transient discharge of sweat was produced on the lateral aspects of the neck in conjunction with papular urticaria. No visible evidence of pruritus was present, presumably because of the immobilization effects of the sedation.

Sera and sweat were collected to prepare autologous extracts for the intradermal test. Serum was prepared as previously described.[2] The horse was trotted on a longe line until there was evidence of copious sweat. Sweat was collected using tongue depressors to scrape surface moisture from the neck, lateral thorax, and abdomen directly into 50 mL sterile conical tubes. The sweat was centrifuged at 4°C at 500 × *g* for 5 minutes to pellet excess dander and dirt. The supernatant was removed and filtered using a sterile 0.45-µm membrane filter.

Table 1. Summary of intradermal test results at 15 minutes.

Allergen (Volume)	Objective Wheal Diameter (mm)	Subjective Wheal Score
Phenolated saline (0.1 mL) Negative control	84.5	0
Phenolated saline (0.1 mL)	72	0
Autologous purified serum (0.1 mL)	66	1+
Autologous purified serum (0.1 mL)	55	0
Autologous purified sweat 1:10 (0.1 mL)	112.5	3+
Autologous purified sweat 1:10 (0.1 mL)	120	3+
Autologous purified sweat 1:100 (0.1 mL)	72	2+
Autologous purified sweat 1:100 (0.1 mL)	66	2+
Autologous purified sweat 1:1,000 (0.1 mL)	60.5	0
Autologous purified sweat 1:1,000 (0.1 mL)	55	0
Histamine 1:100,000 (wt:vol) Positive control	136	4+
2% Pilocarpine (0.1 mL)	84	2+
2% Pilocarpine (0.1 mL)	84	2+

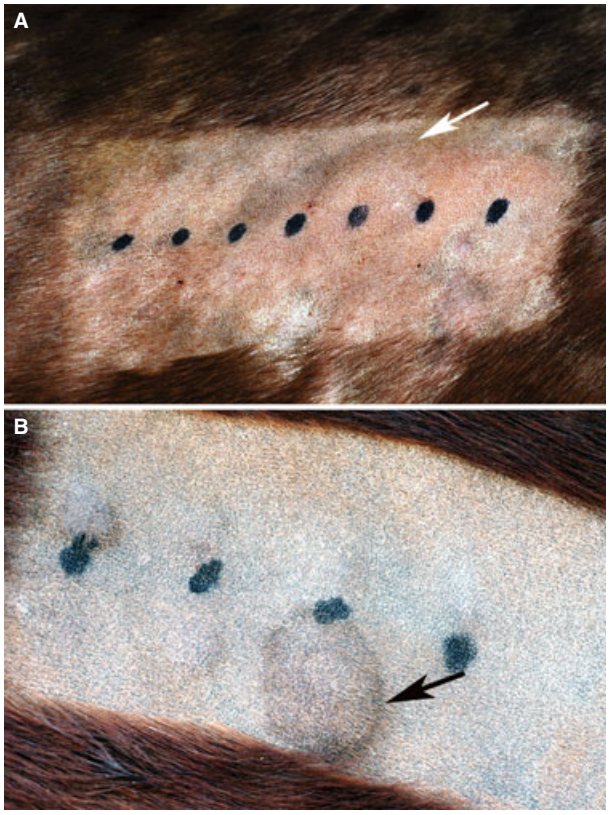

Fig 3. Intradermal tests. **(A)** The intradermal injections of 1:10 dilutions of autologous sweat (white arrow) in the horse with sebaceous adenitis initially formed discrete turgid erythematous wheals at 15 minutes but later coalesced to form a large wheal at 30 minutes that resolved within 1 hour. **(B)** The normal recipient's passive cutaneous anaphylaxis skin test developed a large wheal with a reddish brown hue and distinct dark perspiration ring around the circumference (black arrow) from the intradermal injection of the 1 : 100 dilution of the affected horse's sweat 15 minutes after provocation.

Both the sera and sweat were frozen at −80°C until used.

Before the intradermal test, the horse had not received systemic medications, injections, or topical therapy for the last 20 weeks. An intradermal test was performed as previously described.[3] Intradermal wheals representative of potential putative type I hypersensitivities against serum and sweat antigens were evaluated at 15 minutes (Table 1). Pilocarpine was used as a nonselective muscarinic receptor agonist that would induce a localized sweat response and resultant wheal if the sweat response was cholinergic. Positive reactions as determined by both objective and subjective measurements were present to histamine and to both of the 1:10 dilutions of purified autologous sweat (Fig 3A). When considering subjective scoring alone, autologous sweat dilutions of 1:10, 1:100 and 2% pilocarpine were considered positive.

A 12-year-old Saddlebred gelding was used as a normal recipient to evaluate if the presumed sweat hypersensitivity was transferable through passive cutaneous anaphylaxis (PCA). The recipient horse was clinically normal had not received any medications or sedation for approximately 1 year and had no history of skin disease. The patient's and clinically normal horse's sera were thawed at room temperature and heat inactivated in a water bath at 56°C for 30 minutes to destroy IgE.

An intradermal PCA test was performed in the normal recipient horse (Table 2). Baseline wheal diameters were recorded 15 minutes after injection. Three hours after the initial intradermal injections, the recipient horse received an intradermal injection of histamine just before provocation. For provocation, the horse was removed from the pasture and placed in a stall for 15 minutes away from the view of its pasture mates, which was known to induce severe anxiousness and unremitting pacing. The horse was removed from the stall and the intradermal injection site wheals were evaluated. A large wheal formed within 15 minutes of the intradermal injection of 1:100 sweat dilution, indicating an immediate hypersensitivity. After provocation, the affected horse's serum and sweat elicited wheals that were considered positive reactions when compared to the average of the wheal diameter from the positive and negative control (Table 2). Over a period of 3 hours and 30 minutes, the wheal formed from the affected horse's sweat increased in diameter by 90 mm. After the incitation period, the recipient did not have clinically relevant evidence of sweat on the hair coat or on the skin where the hair had been clipped for the test site. However, the site of intradermal injection of diluted sweat was "sweating" as

Table 2. Summary of the normal recipient horse's intradermal passive cutaneous anaphylaxis objective wheal diameter measurements in millimeters.

Allergen (Volume)	Initial 15 Minutes Objective Wheal Diameter (mm)	3-Hour Wait Period	Objective Wheal Diameter (mm) after Provocation
Histamine (0.1 mL)	Not done		50
Phenolated saline (0.1 mL)	8		6
Normal horse's purified serum (0.1 mL)	8		8
Normal horse's heat inactivated purified serum (0.1 mL)	10		8
Affected horse's heat inactivated purified serum (0.1 mL)	8		8
Affected horse's purified serum (0.1 mL)	50		112.5
Affected horse's purified sweat 1 : 100 (0.1 mL)	375		465

evidenced by the dark rim of perspiration around the circumference of the large wheal (Fig 3B).

A rapid desensitization protocol was developed and used to initiate rush immunotherapy with autologous sweat. The desensitization protocol was carried out over an 8-hour period and involved the following steps. An autologous sweat dilution of 1 : 100 was prepared in phenolated saline. Subcutaneous injections were started at a 1:100 dilution concentration, which was 1 dilution factor weaker than the dilution that elicited both subjective and objective positive reactions. The amount of diluted sweat injected was 0.1 mL every hour for the first 6 consecutive injections. The volume was increased by 0.2 mL for the last 2 injections until a final total volume of 1.0 mL was given. The SC injections were administered in alternating sides of the neck. The horse was monitored each hour for the development of wheals at the site of injection as well as for other systemic signs indicative of anaphylaxis. Maintenance sweat immunotherapy was 1 mL of the 1 : 100 dilution of sweat given SC every 7 days.

Finally, the owners elected not to invest in treatment for the sebaceous adenitis of the horse. The efficacy of the horse's sweat immunotherapy is unknown, because the horse was euthanized 3 months after starting treatment because of its inability to maintain a hair coat acceptable for a show horse.

Cholinergic urticaria with sweat hypersensitivity is a well-documented pruritic condition in humans, but its pathogenesis still is not completely understood.[4–7] In humans with CU with sweat hypersensitivity, an intradermal injection of acetylcholine is known to induce both sweating and wheals,[8,9] and these reactions are further enhanced when cholinergic nerve fibers are stimulated during exercise. In species other than human, sweat hypersensitivity previously has not been documented. This report describes concurrent sweat hypersensitivity and progressive sebaceous adenitis in a horse documented by histopathology, and PCA.

In the horse of this report, sweating in either the presence or absence of exercise was essential for the development of papular urticaria. The first diagnostic step to determine the etiology of this horse's urticaria was to discern if the "hypersensitivity" reagin-like substance could be localized to the patient's serum, sweat, or both. Whereas the autologous intradermal injections of serum did not cause wheal formation in the patient, the intradermal injection of sweat not only caused large wheals in the patient but also an increase in wheal diameter and sweating that was transferable to a normal horse. These reactions suggested that the sweat itself (or substances related to sweating) might act as an antigen in this urticaria. Although 2% pilocarpine provoked wheal formation in this patient, the intradermal response did not elicit localized sweating or pruritus, which would be suggestive of CU in humans.[10] Currently, there is no direct evidence that cholinergic receptors are present on the equine sweat gland and there is little indication for the role of cholinergic agents in the control of sweating in horses, making a cholinergic mechanism for the induction of pruritic papular urticaria in our patient unlikely.[11]

When considering the pathogenesis of equine sweat hypersensitivity, it is known that equine sweat glands are not directly innervated but rather are activated in response to heat stress, exercise, neural, humoral, and paracrine factors. Sweat is produced in the epitrichial glandular fundus epithelium and stored in cytoplasmic vesicles that are extruded, in part, by a microapocrine mechanism. The expelled fluid travels through the lumen of a duct into the piliary canal in the infundibulum of the hair follicle just above the sebaceous duct opening.[12] There is convincing evidence that main receptors on the equine sweat gland are β_2 adrenoceptors, although horse sweat glands have been found to discharge in response to α_2 adrenergic stimulation.[13–17] Evidence for the role of α_2 adrenergic stimulation of sweat-induced urticaria in this horse was seen with the use of an α_2 adrenergic agonist.

Assessment of sweat hypersensitivity in humans is confirmed with positive intradermal wheal and flare reactions to diluted autologous sweat but not autologous serum.[7,18] Sweat constituents are known to be irritating when used undiluted for intradermal injections as a result of contamination by substances from the epidermal surface. Surface contamination can increase the concentrations of protein, urea, and calcium.[11] The irritant nature of sweat can be diminished if the sweat is diluted, which increases the reliably of the skin test result. The acknowledged limitation to

this report is the need to further characterize intradermal responses in normal horses to the selected sweat dilutions obtained from apparently healthy individuals, as well as 2% pilocarpine solution. Intradermal sweat threshold concentrations then can be defined as the highest concentration of diluted sweat extract injected intradermally for which ≤10% of normal horses have a "positive" reaction. These findings would help further clarify whether the sweat wheal responses documented in both the patient and the normal recipient are contact irritant reactions or true hypersensitivity reactions.

The identities of equine sweat antigens are unknown. However, evaluation of latherin as a candidate allergen is warranted, because the equine serum protein allergen, Equ c 4 is a direct cleavage or degradation product of latherin, and is known to cause an IgE-mediated hypersensitivity reaction in humans.[19]

Rapid desensitization and immunotherapy with autologous sweat have been shown to control urticaria in 5 of 6 humans diagnosed with CU with sweat hypersensitivity that had been refractory to conventional treatment with H1-receptor antagonists.[2] All of these patients had negative skin test results using autologous serum, but positive intradermal reactions to autologous sweat. Severe CU with sweat hypersensitivity also has been successfully treated with an anti-IgE antibody, omalizumab, suggesting that an IgE-mediated response is involved in sweat hypersensitivity in humans.[20] A similar lack of efficacy with administration of several types of antihistamines to control the pruritic urticaria was seen in this horse. Whether or not prednisolone would have controlled the pruritic urticaria is unknown, because the trainer did not work or sedate the horse when the horse was receiving steroids.

This horse's initial sebaceous adenitis remained localized to the shoulder over the first 2 years of life and spontaneously resolved in the spring and summer. This spontaneous regression of the dermatitis is consistent with a single published case report, in which the horse's generalized sebaceous adenitis resolved without treatment.[21] However, in the patient of the present report, spontaneous remission ceased and the sebaceous adenitis progressed to involve multiple body sites which have not been described previously. The onset of sweat hypersensitivity 2 years after the sebaceous adenitis may be unrelated, or alternatively, could support a proposed mechanism for the development of sweat hypersensitivity in humans. The "sweat leakage theory" assumes that sweat ducts are obstructed by lymphocytic inflammation around the ducts, and the resultant retention and subsequent leakage of sweat from the damaged ducts induce wheals because of sweat hypersensitivity.[22] In the horse of this report, lymphocytic mural folliculitis at the level of the isthmus was a common finding, in addition to lymphocytic folliculitis of the infundibulum and lymphocytic sebaceous adenitis. Because sweat is deposited into the hair follicle infundibulum, leakage could occur through the damaged follicular epithelium and eventually result in mast cell activation and development of a type I hypersensitivity. Whereas the normal recipient horse's PCA positive reaction to the patient's serum suggests immediate hypersensitivity, the combination of both an idiopathic disease that could be immune-mediated with the production of autoantibodies and sweat hypersensitivity makes it impossible to determine which disease process was responsible for this intradermal response.

In conclusion, to the authors' knowledge, this is the first case report of sweat hypersensitivity and progressive sebaceous adenitis in the horse. Sweat hypersensitivity-induced urticaria should be considered as a differential diagnosis for a horse that develops pruritic urticaria when working or when sedated with an α_2-adrenoreceptor agonist.

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