Effect of an ear cleaner instillation containing lipacids in a model of re-acidification of the external auditory canal in dogs

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Funding information
This study was funded by Vetoquinol.

Abstract

Background: In humans, the acidic pH of the ear canal plays a protective role against infection and a change towards alkalinity of the external auditory canal (EAC) is a local factor in the progression of acute to chronic otitis externa (OE). The use of acidic preparations alone for treatment of OE without concurrent antibiotic use is well-documented in humans. In dogs, only one study has investigated the EAC pH in healthy dogs and in dogs with OE, and investigations to understand the role of EAC pH in the pathogenesis of canine OE are lacking.

Hypothesis/objectives: To obtain physiological EAC pH values in beagle dogs. To develop a model of re-acidification of the EAC in dogs and to investigate how an acidic solution may accelerate the return to a physiological pH.

Animals: Ten healthy beagle dogs in a laboratory setting.

Materials and Methods: A model of re-acidification of the EAC was developed by instillation of a pH 10.1 phosphate-buffered saline (PBS) solution and the subsequent acidic effect of an ear cleaner containing lipacids was evaluated in this model.

Results: Mean physiological EAC pH was 6.12 (± 0.36). EAC re-acidification took up to 9 h in this model. Mean pH values dropped immediately to 6.38 (± 0.27) on ears treated with an acidic ear cleaner. No abrupt drop was observed of the mean pH values for the control ears.

Conclusion and clinical importance: This study confirms that physiological EAC pH in dogs is acidic. This model of re-acidification of the EAC pH allows investigations on acidic properties of topical ear products in healthy ears.
INTRODUCTION

The pH, defined as the negative logarithm (base ten) of the concentration of free hydrogen ions in aqueous solutions, is important in the maintenance of healthy skin. A pH of <7.0 is considered acidic, whereas >7.0 is alkaline. The pH of the skin results from numerous substances such as filaggrin breakdown products, amino acids produced by epidermal cells and glands (sebaceous, apocrine and eccrine) and also from fatty acids and cholesterol sulfate found at the surface of the skin. Human skin pH varies from an acidic pH of 3 to almost neutral pH of 6.5.1,2

In humans, skin pH presents an antibacterial barrier preventing colonisation (e.g. Staphylococcus aureus and Malassezia), and also influences skin barrier function, lipid synthesis, epidermal differentiation and skin inflammation.1,2,3,4,5 Canine skin pH differs from human skin and ranges from 4.84 to 9.95.2 Cutaneous pH varies among individuals and particularly among anatomic locations, age, sex, environmental temperature and relative humidity.2 The human external auditory canal (EAC) has an acidic pH ranging from 4.2 to 5.6, whereas canine EAC pH ranges from 4.6 to 72 with a mean around 6.1,6,7

Skin pH is altered in many inflammatory skin conditions, such as human3 and canine4 atopic dermatitis (AD), and also in otitis externa (OE), in which an increased pH has been demonstrated. The human EAC changes to a more neutral or alkaline state in OE and this change has been shown to be very important in the pathogenesis of OE.5 Indeed, the acidity of cerumen has bactericidal effects, so it has been theorised that if the pH of the EAC increases (e.g. because of high humidity, bathing, swimming or frequent ear cleansing), then the risk of infection also would increase.6 Following infection, the seruminous glands, which produce acidic cerumen, may be damaged and produce less, or an altered, cerumen leading to a more alkaline EAC. This state could worsen infection in a vicious circle.6 Thus, the restoration of acidity instead of using acidic preparations alone,4,6

Low pH ear cleaners also have been reported to inhibit bacterial growth.6 Another advantage of maintaining a low pH is its impact on biofilm formation. An in vitro study revealed a positive correlation between biofilm production and an increase in pH. At pH 8.5, all strains of Pseudomonas aeruginosa displayed an elevated biofilm production.8 In canine OE, the Gram-negative P. aeruginosa is commonly isolated in OE and accounts for ≤30% of cases.9 Treatment of P. aeruginosa-associated OE is challenging as a consequence of the severe inflammation that commonly accompanies it, bacterial resistance and biofilm production.10,11 The ear cleaner used in the present study also has been shown to achieve 100% bactericidal activity against P. aeruginosa in vitro.12 All of these considerations should lead to more studies in the role of acidic ear cleaners in veterinary otology, especially in P. aeruginosa-associated OE management.

Although OE is very common in dogs, with a reported prevalence of ≤20%,13,14 there is a lack of knowledge regarding the physiological values of EAC pH and the role of EAC pH in canine OE. A pH >6 has been suggested to be associated with otic infection.15 Only one study from 1970 has investigated the EAC pH in dogs with OE and found elevated EAC pH levels.7

The aim of this study was: (i) to obtain physiological EAC pH values in beagle dogs; and (ii) to develop a model of re-acidification of the EAC in order to (iii) investigate how an acidic solution may accelerate the return to a baseline pH.

MATERIALS AND METHODS

All of the procedures were approved by the Institutional Animal Care and Use Committee of our institute (ethics committee reference 1812 following the 63/2010 directives).

Animals

Ten healthy, male 2-year-old beagle dogs were used. None of them had any history or evidence of OE. They did not receive any systemic or topical therapies for three months before the study. The dogs belonged to a research colony housed outdoors when not part of any research. One week before the beginning of the study, the dogs were acclimatised to an indoor environment in a temperature- and humidity-controlled facility (25–28°C, relative humidity 40–60%). The dogs were housed in individual cement runs cleaned twice daily. Twelve weeks before the start of the study and over the course of the study, they were fed with the same maintenance dry food regimen and tap water ad libitum.

Model of re-acidification of the external auditory canal and measurement of the EAC pH.

Five mL of a pH 10.1 phosphate-buffered saline (PBS) solution was instilled into one ear in five dogs. A gentle massage of the ear was performed, and the dogs were then left free in order to shake their head. Using a skin pH probe (Hannah Instruments; Tanneries, France) applied on the cavum conchae, EAC pH measurements were performed before the pH 10.1 PBS solution instillation in order to measure the basal pH, immediately after and then 1h, 2h, 3h, 4h, 9h, 24h and 48h post-instillation.

Acidic effect of an ear cleaner containing lipids in a model of re-acidification of the EAC in dogs

Five mL of a pH 10.1 phosphate-buffered saline (PBS) solution were instilled into both ears in 10 dogs. A gentle massage of the ears was performed for 30 seconds, and the dogs then were left free in order to shake their head. After 2 min, 2 mL of an ear cleaner (Sonotix®, Vétoquinol; Lure, France) was instilled into
the right ears and the left ears were kept as a control. Ear cleaner pH was slightly acidic (6.8). Gentle massage of the ears for 30 s was performed, and the dogs then were left free in order to shake their head. Using a skin pH probe (Hannah Instruments) applied on the cavum conchae, EAC pH measurements were performed before ear cleaner instillation, immediately after, and then 1 h, 2 h, 3 h, 4 h, 9 h, 24 h and 48 h post-instillation.

**Statistical analysis**

For the model of alkalisation of the EAC, a one-sided Wilcoxon signed-rank test was used to test for how long basal mean EAC pH was higher than mean EAC pH after alkalisation at different times in the model of re-acidification.

In order to test the effect of the ear cleaner on pH, we implemented a generalised additive mixed model (GAMM) using the gamm() function of the mgcv package. We used a mixed model to account for the nonindependence between measures made on the same dog and we used a GAMM because we expected one explanatory variable (time) to have a nonlinear fixed effect on the response variable (pH) as we expected the pH to decrease faster during the first hours post-instillation.

In our GAMM, pH was used as the response variable with a normal distribution because pH data measured at the sites treated with the ear cleaner were normally distributed (Shapiro–Wilk normality test, P-value = 0.1715). The treatment (ear cleaner or control) was used as an explanatory variable with a fixed effect in order to test the impact of the ear cleaner on the pH. Dog ID was used as an explanatory variable with a random effect to take into account the nonindependence between measures made on a same dog.

In order to test for how long the pH on the ear cleaner side was significantly different from the control side, the normal distribution of the data was verified using the Shapiro–Wilk normality test and then paired-matched Student’s t-test was used to compare mean EAC pH of the control ear to mean EAC pH on the treated ear at different times.

All analyses were performed using R Development Core Team (2008) statistical software (R: a Language and Environment for Statistical Computing. R Foundation Core Team. Vienna, Austria).

**RESULTS**

**Phase 1: Model of re-acidification of the EAC**

The mean normal EAC pH was 6.12 (± 0.36). Immediately following administration of the PBS solution, mean EAC pH increased to 9.06 (± 0.21) and this difference was highly statistically significant (one-sided Wilcoxon signed-rank test, P = 0.03125). Mean pH decreased slowly over time yet remained statistically significantly different from mean baseline values up to 9 h post-installation (paired-matched Student’s t-test, P = 0.046). At 24 h post-installation, mean EAC pH was returned to normal (6.6 ± 0.48) as no statistically significant difference was found compared to normal EAC pH. Result analysis showed that significant alkalislation of the EAC lasts ≥9 h. Therefore, our model appeared well-suited to study the acidic effect of ear cleaners over the course of 9 h (Figure 1).

**Phase 2: Acidic effect of an ear cleaner containing lipacids in a model of re-acidification of the EAC in dogs**

Mean pH values pre-alkalisation were homogenous in both sides and were 5.81 (± 0.36) and 5.72 (± 0.37), respectively, for ear cleaner-treated and control ears, and this difference was not significant (paired-matched Student’s t-test, P = 0.34). Mean pH values immediately post-alkalisation were similar on both sides, and were 9.39 (± 0.16) and 9.41 (± 0.13) for the ear cleaner-treated and the control ears, respectively.

Immediately after ear cleaner instillation, mean pH values markedly dropped to 6.38 (±0.27) in treated ears and then slowly decreased over the next 48 h before reaching pre-alkalisation values of 5.87 (± 0.66). By comparison, no abrupt reduction in mean pH values was observed for the control ears, yet a moderate decrease was observed ≤2 h after the EAC alkalisation, and then a slower decrease was noticed until EAC pH reached the pre-alkalisation level of 5.89 (± 0.41) (Figure 2).

Overall, pH values were lower for ear cleaner-treated ears versus control ears. Results for the GAMM showed that these differences were significant for the quantitative variable tested (pH) (Table 1).

However, mean pH values were significantly different between ear cleaner-treated and control in only the first 4 h following the instillation (paired-matched Student’s t-test, P ≤ 0.05).

**FIGURE 1** External auditory ear canal pH values over time post-alkalisation with a pH 10.1 phosphate-buffered saline solution. Data represent the mean values ± SD from five dogs. ***, P ≤ 0.001; **, P ≤ 0.01; *, P ≤ 0.05; n.s., P ≥ 0.05**
We developed a model of re-acidification of the ear canal in order to study an ear cleaner’s acidic properties. Using this model, we demonstrated the buffering effect of this ear cleaner, which allowed a quick re-acidification of the EAC, as the EAC pH immediately dropped from 9.06 (± 0.21) to 6.38 (± 0.27). These results could be explained by the lipacids contained in this ear cleaner, which act as a buffer and help to restore and maintain the natural acidic pH of healthy skin. However, a mechanical removal of the alkaline solution by the ear cleaner could have led to these results.

We also were able to collect physiological values of EAC in beagle dogs. Only one study from 1970 has investigated EAC pH in healthy dogs. Canine EAC ranged from 4.6 to 7.4, with a mean of 6.1 for males and 6.2 for females. In our study, the mean physiological EAC pH was 5.9 and then dropped immediately to 4.6 after the instillation of the acidic solution. Return to physiological values was rapid and complete in 1 h. In our study, the ear cleaner has a pH of 6.8, which is slightly acidic. However, the ear cleaner pH was higher than physiological EAC pH. Moreover, a pH of >6 has been associated with otic infection in dogs and a pH of 7 has been associated with pathological changes in humans. One could speculate whether an ear cleaner with a pH slightly higher than physiological level could impact upon the homeostasis of a healthy ear.

The limitations of this study include the location of pH measurement, the impact of individual head shaking, and the use of healthy dogs. Measurements were performed on the cavum conchae yet EAC pH may differ depending on the depth in the EAC. However, the cavum conchae was the area closest to the EAC where the probe could be applied easily. The method of “head-shaking” used to remove the excess ear cleaner could have led to discrepancies in our results, as the intensity may have differed between dogs leading to different levels of residual cleaner. Additionally, our model has been developed in healthy dogs without any history of OE; therefore, pH returned innately to baseline values without any cleaner. It is difficult to say how long the buffering effect of the cleaner would last in a diseased ear in which the pH consistently tends toward alkalinity as a result of inflammation. It will be important to repeat the present study by evaluating the acidifying role of this ear cleaner in dogs with spontaneous OE and couple this with determining the clinical, cytological and otoscopic responses. Finally, pH is known to affect the performance of antibiotics, so the simultaneous use of an acidic ear cleaner and a topical antibiotic must be done with full knowledge that some antibiotics, such as macrolides, genaminicin and some fluoroquinolones may have their activity reduced in an acidic environment. It has been suspected that pH affects antibiotic efficacy by

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**TABLE 1** Results from the generalised additive mixed model to evaluate the effect on pH of an ear cleaner

| Explanatory variable | Model coefficient | Model coefficient 95%CI | P-value |
|----------------------|-------------------|--------------------------|---------|
| Treatment = Sonotix  | −0.75535          | [−0.8707; −0.6400]       | <2 × 10⁻¹⁶ |
| s(Time)              | n.a.              | n.a.                     | <2 × 10⁻¹⁶ |

*Approximate significance of smooth terms given by the gamm() function.

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**FIGURE 2** External auditory ear canal pH values over time post-alkalisation for control ears and ears treated with an acidic ear cleaner. Data represent the mean values ± SD from 10 dogs. ***, P≤0.001; **, P≤0.01; *, P≤0.05; n.s., P≥0.05

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**DISCUSSION**

It has been shown that cutaneous pH varies among individuals and that excitement could lead to elevations in cutaneous pH levels in dogs. This could explain the difference in EAC pH levels between phases 1 and 2 as five new individuals were used in addition to the five dogs from the phase 1. This underlines the importance of using standardised protocols in studies involving skin pH measurement. In the only previous study that investigated EAC pH in dogs in normal and diseased ears, the authors also demonstrated the buffering effect of a 10% acetic acid in propylene glycol solution (pH4). In that study, the mean physiological EAC pH was 5.9 and then dropped immediately to 4.6 after the instillation of the acidic solution. Return to physiological values was rapid and complete in 1 h. In our study, the ear cleaner has a pH of 6.8, which is slightly acidic. However, the ear cleaner pH was higher than physiological EAC pH. Moreover, a pH of >6 has been associated with otic infection in dogs and a pH of 7 has been associated with pathological changes in humans. One could speculate whether an ear cleaner with a pH slightly higher than physiological level could impact upon the homeostasis of a healthy ear.
modulating the binding sites and/or target sites for certain antibiotics and the transport of antibiotics such as gentamicin into bacteria could be inhibited in an acidic environment.  

In conclusion, this study is, to the best of the authors’ knowledge, the first establishment of a model of EAC re-acidification allowing investigation of the acidic properties of topical ear products such as an ear cleaner containing lipacids. Although there is a lack of knowledge regarding the role of EAC pH in canine OE, knowledge emerging from human medicine supports its importance. Further studies using standardised protocols should be conducted on the role of lipacids in canine OE to answer these open questions, bearing in mind that cutaneous pH can be influenced by various factors.

AUTHOR CONTRIBUTIONS
Pauline Panzuti: Investigation; writing – original draft; writing – review and editing. Marion Mosca: Investigation; writing – original draft; writing – review and editing. Oscar Fantini: Conceptualization; funding acquisition. Guillaume Noel: Project administration. Julien Cappelle: Formal analysis; software. Didier Pin: Conceptualization; supervision; validation.

ACKNOWLEDGEMENT
Open access funding enabled and organized by ProjektDEAL.

CONFLICTS OF INTEREST
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SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Panzuti P, Mosca M, Fantini O, Noel G, Cappelle J, Pin D. Effect of an ear cleaner instillation containing lipacids in a model of re-acidification of the external auditory canal in dogs. Vet Dermatol. 2022;33:402–406. https://doi.org/10.1111/vde.13101
Résumé

Contexte – Chez l’homme, le pH acide du conduit auditif joue un rôle protecteur contre l’infection et l’évolution vers l’alcalinité du conduit auditif externe (CAE) et est un facteur local de progression de l’otite externe (OE) aiguë à chronique. L’utilisation de préparations acides seules pour le traitement de l’OE sans utilisation concomitante d’antibiotiques est bien documentée chez l’homme. Chez les chiens, une seule étude a étudié le pH du CAE chez les chiens en bonne santé et chez les chiens atteints d’OE, et les recherches pour comprendre le rôle du pH du CAE dans la pathogénèse de l’OE canine font défaut.

Hypothèses/objectifs – Obtenir des valeurs physiologiques de pH du CAE chez des chiens beagle. Développer un modèle de réacidification du CAE chez le chien et étudier comment une solution acide peut accélérer le retour à un pH physiologique.

Animaux - Dix chiens beagle de laboratoire en bonne santé.

Matériels et méthodes - Un modèle de réacidification du CAE a été développé par instillation d’une solution saline tamponnée (PBS) à pH 10,1 et l’effet acide ultérieur d’un nettoyant pour oreilles contenant des lipacides a été évalué dans ce modèle.

Résultats – Le pH physiologique moyen du CAE était de 6,12 (± 0,36). La réacidification du CAE a pris jusqu’à 9 h dans ce modèle. Les valeurs moyennes du pH chutent immédiatement à 6,38 (± 0,27) sur les oreilles traitées avec un nettoyant auriculaire acide. Aucune chute brutale n’a été observée des valeurs moyennes de pH pour les oreilles témoins.

Conclusion et importance clinique - Cette étude confirme que le pH physiologique du CAE chez le chien est acide. Ce modèle de réacidification du pH du CAE permet des investigations sur les propriétés acides des produits topiques auriculaires dans des oreilles saines.

Resumen

Introducción; en humanos, el pH ácido del canal auditivo juega un papel protector contra la infección y un cambio hacia la alcalinidad del canal auditivo externo (EAC) es un factor local en la progresión de la otitis externa (OE) aguda a crónica. El uso de preparaciones ácidas solas para el tratamiento de la OE sin el uso concomitante de antibióticos está bien documentado en humanos. En perros, solo un estudio ha investigado el pH de EAC en perros sanos y en perros con OE, y faltan investigaciones para comprender el papel del pH de EAC en la patogenia de la OE canina.

Hipótesis/objetivos – Obtener valores de pH fisiológico de EAC en perros beagle. Desarrollar un modelo de reacidificación del EAC en perros e investigar cómo una solución ácida puede acelerar el retorno a un pH fisiológico.

Animales- diez perros beagle sanos en un laboratorio.

Materiales y métodos- se desarrolló un modelo de reacidificación del EAC mediante la instilación de una solución salina tamponada con fosfato (PBS) a pH 10,1 y se evaluó en este modelo el efecto ácido subsiguiente de un limpiador de oídos que contenía lípidos.

Resultados – El pH fisiológico medio del EAC fue de 6,12 (±0,36). La reacidificación del EAC tomó hasta 9 h en este modelo. Los valores medios de pH cayeron inmediatamente a 6,38 (±0,27) en los oídos tratados con un limpiador de oídos ácido. No se observó una caída abrupta de los valores medios de pH para los oíds de control.

Conclusión e importancia clínica- este estudio confirma que el pH fisiológico de EAC en perros es ácido. Este modelo de reacidificación del pH de EAC permite realizar investigaciones sobre las propiedades ácidas de los productos tópicos para el oído en oídos sanos.

Zusammenfassung

Hintergrund – Beim Menschen spielt der saure pH-Wert des Ohrkanals eine schützende Rolle gegenüber Infektionen und eine Veränderung des externen Gehörkanals (EAC) in Richtung Alkalinität ist ein lokaler Faktor beim Fortschreiten von einer akuten zu einer chronischen Otitis externa (OE). Beim Menschen ist der Einsatz säurehaltiger Präparate alleine zur Behandlung von OE ohne gleichzeitige Verabreichung von Antibiotika gut dokumentiert. Bei Hunden hat nur eine Studie den pH des EAC gesunder Hunde mit OE untersucht und es fehlen Untersuchungen, um das Verständnis der Rolle des pH des EAC bei der Pathogenese der caninen OE zu verbessern.
Hypothese/Ziele – Das Ziel war es, physiologische pH-Werte des EAC bei Beagles zu erfassen. Die Entwicklung eines Modells einer Wieder-Ansäuerung des EAC bei Hunden, um zu untersuchen, wie weit eine säurehaltige Lösung die Rückkehr zum physiologischen pH beschleunigen könnte.

Tiere – Zehn gesunde Beagles in einem Labor-Setting.

Materialien und Methoden – Es wurde ein Modell einer Wieder-Ansäuerung des EAC entwickelt, indem eine physiologische Kochsalzlösung (PBS Lösung) mit pH 10,1 ins Ohr eingebracht und in der Folge die ansäuernde Wirkung eines Ohrenreinigers mit Fettsäuren in diesem Modell evaluiert wurde.

Ergebnisse – Der durchschnittliche pH des EAC lag bei 6,12 (± 0,36). Die Wiederansäuerung des EAC dauerte in diesem Modell bis zu 9 h. Die durchschnittlichen pH-Werte sanken sofort auf 6,38 (± 0,27) bei den Ohren, die mit einem sauren Ohrenreiniger behandelt worden waren. Bei den Kontrollohren wurde keine abrupte Absenkung des durchschnittlichen pH-Werts beobachtet.

Schlussfolgerungen und klinische Bedeutung – Diese Studie bestätigt, dass der physiologische pH-Wert des EAC bei Hunden sauer ist. Dieses Modell der Wiederansäuerung des pH-Werts im EAC ermöglicht Untersuchungen über die sauren Eigenschaften topischer Produkte in gesunden Ohren.
**Hipótese/objetivos** – Se obter os valores fisiológicos do pH dos CAEs de cães Beagle. Desenvolver um modelo de re-acidificação do CAE em cães e investigar como uma solução ácida pode acelerar o retorno ao pH fisiológico original.

**Animais** – Dez cães Beagle saudáveis de laboratório.

**Materiais e Métodos** – Um modelo de re-acidificação do CAE foi desenvolvido por instilação de solução salina tamponada com fosfato (PBS) com um pH 10,1 e o efeito acidificante subsequente de um limpador de ouvido contendo ácidos lipídicos foi avaliado neste modelo.

**Resultados** – O pH fisiológico médio do CAE foi de 6,12 (± 0,36). A re-acidificação do CAE levou até 9h neste modelo. Os valores médios de pH caíram imediatamente para 6,38 (± 0,27) nas orelhas tratadas com um limpador otológico ácido. Não foi observada queda abrupta dos valores médios de pH para as orelhas controle.

**Conclusão e importância clínica** – Este estudo confirmou que o pH fisiológico do CAE de cães é ácido. Este modelo de re-acidificação do pH do CAE permite investigações sobre as propriedades acidificantes de produtos otológicos tópicos para orelhas saudáveis.