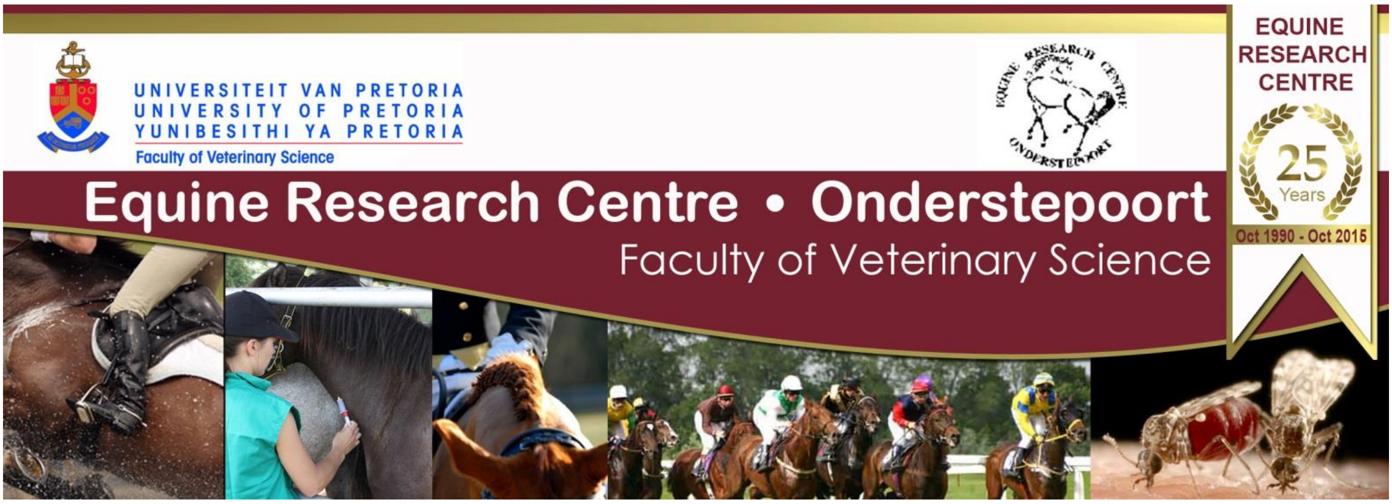

EQUINE RESEARCH … what you need to know

Brought to you by the Equine Research Centre, University of Pretoria

The Equine Research Centre celebrates 25 years of operation in October this year!

During that time the Centre has become internationally renowned, particularly for its work on African horse sickness

What better way to celebrate than with the two ground breaking papers summarised here?

HOT OFF THE PRESS!!

TWO GROUND BREAKING RESEARCH PAPERS FROM ERC

Summarised here for layman readability …

COMPLETE GENOME SEQUENCES OF THE THREE AHS VIRUS STRAINS FROM A COMMERCIAL TRIVALENT LIVE ATTENUATED VACCINE

A study, and subsequent report, of the complete genome sequences of plaque selected isolates of each of the three virus strains included in a South African commercial trivalent African horse sickness attenuated live virus vaccine, was conducted.

African horse sickness (AHS) is caused by AHS Virus (AHSV), the genome of which is comprised of ten segments. In South Africa, a polyvalent AHS attenuated live virus (ALV) vaccine is manufactured by Onderstepoort Biological Products (OBP) Ltd. This vaccine is supplied in two separate vials each of which contains different AHSV serotypes : Bottle I is trivalent and contains serotypes 1, 3 and 4 while Bottle II is tetravalent (x 4) and contains serotypes 2, 6, 7 and 8 (2).

The research team studied the full genome sequences of AHSV1, AHSV3 and AHSV4 which were isolated from Bottle I of the AHS-ALV vaccine. The individual serotypes were independently isolated, and each was passaged been one and three times. AHSV dsRNA was extracted from virus-infected cells and sequencing templates were prepared.

The full genome sequences of the attenuated AHSV serotypes 1, and 4 viruses, which are precursors of the respective AHS-ALV virus strains, are available for public scrutiny from GenBank as accession numbers FJ183364-FJ183373 and KM820849-KM820858 respectively. The pairing nucleotide sequence identity of AHSV1-OBP vaccine and Attenuated serotype 1 was 99.643%, whilst that of AHSV4-OBP vaccine with Attenuated serotype 4 was 99.625%.

The full genome sequences of the AHSV serotype 3 from which AHSV-3-OBP are available for public scrutiny from GenBank as accession numbers KM886354-KM886363. The pairing identity between AHSV3-OBP and AHSV serotype 3, over nine of their ten genome segments was in excess of 99.7% whilst that for the segment carrying the gene encoding the highly conserved VP1 protein was appreciably lower at 94.86%. Further analysis of these sequences clearly showed that AHSV3-OBP is a reassortant comprised of nine genome segments likely derived from an AHSV serotype 3 ancestor closely related to the vaccine.

<u>Comment</u> : Having the sequences of the vaccine viruses means that when we do outbreak investigations, we can categorically determine whether we're dealing with field virus, or a virus in which the vaccine is playing a role. These findings therefore provide the basis for investigations into any future outbreaks, and the potential role of vaccine viruses.

Note also that this addresses Bottle I only of the vaccine. Study is underway on Bottle II, the findings for which will be published in due course.

<u>Publication</u> : Genome Announcements

<u>Research Team</u> :
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DEVELOPMENT OF THREE TRIPLEX REAL-TIME RT-PCR TESTS FOR TYPING OF THE NINE AHSV SEROTYPES

The objective of this study was to assess the AHSV type specific (TS) RT-qPCR tests in a multiplex (3 triplex tests) for the rapid typing of AHSV positive samples. Such tests facilitate in the rapid determination of the virus type in field outbreaks of AHS, thus facilitating implementation of appropriate vaccination and control strategies.

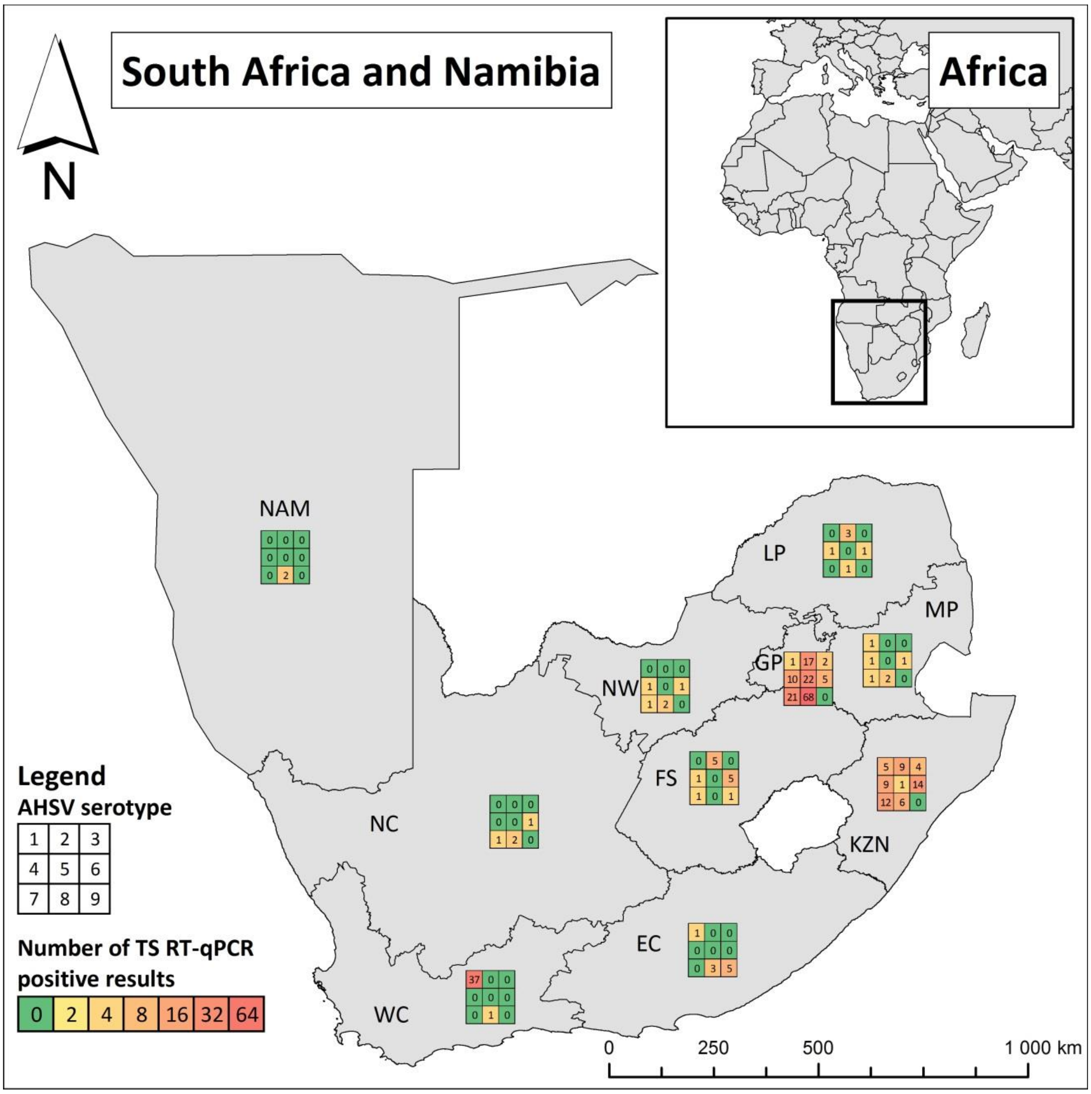
AHSV TS AHSV RT-qPCR tests developed for each of the 9 serotypes of AHSV were evaluated using reference strains of each virus serotype. This analysis confirmed that historic stocks of 3 of the 9 viruses were mixtures of two different AHSV serotypes, specifically the stocks of AHSV-serotypes 3, 7 and 8, also contained AHSV serotypes 1, 3 and 5 respectively. In contrast, with the notable exception of the reference strain of AHSV serotype 3 that included a relatively low level of AHSV serotype 1, the newly propagated stocks of the reference viruses obtained in 2014 were of one single type.

Samples are able to be extracted and evaluated by AHSV RT-qPCR within 4 hours after their arrival at the laboratory. In this study, as the samples had already been extracted the AHSV RT-qPCR was done in 2 hours.

The 291 horse blood samples that tested positive by AHSV RT-qPCR were also evaluated using the AHSV TS RT-qPCR tests. Of these 156 were VI positive. All 9 serotypes of AHSV were isolated from these 156 samples, and there was agreement in the results by the two methods. AHSV type was also determined by AHSV TS RT-qPCR for the 135 samples that were VI negative, but AHSV RT-qPCR positive.

The use of group-specific AHSV RT-qPCR in conjunction with AHSV TS RT-qPCR tests provides an attractive strategy for improved AHS surveillance and outbreak response protocols. Similarly the use of these tests will lead to a better understanding of the epidemiology of AHS, as the applicability and practicability of the AHSV TS RT-qPCR are superior to those of traditional VI and PI tests for virus serotyping. Importantly, virus serotype was readily determined in samples that were positive by AHSV RT-qPCR but negative by VI. Use of both tests in tandem will be invaluable in expediting outbreak responses, such as those that have occurred in the AHS surveillance zone of the AHS Control Area of South Africa.

The geographic distribution of the viruses included in this study can be seen in the map below.



A map of Namibia and South Africa with its provinces. The labels on the map refer to Namibia (NAM) and the nine provinces of South Africa, namely Eastern Cape (EC), Free State (FS), Gauteng (GP), KwaZulu-Natal (KZN), Limpopo (LP), Mpumalanga (MP), North West (NW),Northern Cape (NC) and the Western Cape Province (WC). The total number of positive AHSV TS RT-qPCR samples per type for samples collected between 1 January 2011 and 31 May 2012 is depicted for each area.

Publication : Journal of Virological Methods

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Agricultural Research Council – Otto Koekemoer, Phelix Majiwa, Antoinette van Schalkwyk
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See this link for the full paper : http://dx.doi.org/10.1016/j.jviromet.2015.07.015

ERC CELEBRATES 25 YEARS – 1990-2015 – HOW IT ALL BEGAN

Prof Guthrie mucking out in the early days!

Back in early 1990 a decision was made at Government level to establish an equine research centre through the University of Pretoria. Prof Alan Guthrie was busy with his PhD in Louisiana, USA when he was approached and asked if he would like to apply for the position of Director of the soon to be established research centre. It goes without saying that he took up the invitation, and saw himself on an almost empty Boeing 747 (in the days when it still had to fly around the bulge from Europe) on June 16th for his interview on 17th June 1990.

Having been accepted for the position, Prof Guthrie's first role was to design the facility, which was initially situated in a prefab building on the grounds of Onderstepoort, before the University made the current building available for the Centre. Prof Guthrie was the sole member of staff at the start.

It wasn't until 1995 that the Centre became involved with infectious diseases, which was when the first Export Workshop was held resulting in a relaxation of the stringent export requirements imposed by the OIE as a result of the African horse sickness problem in South Africa.

The Centre's very first research project was to conduct an epizoological study of wastage (inability to train/race due to health or injury) in Thoroughbred racehorses in Gauteng. This study spanned a few years, with the paper being published in 1996 (we've included an edit of this paper in this newsletter for your interest). One of the causes of wastage was respiratory problems, and it was believed at the time that this was due to dust on the stable walkways. Sasol offered a chemical to bind the dust in the walkways, at a considerable cost. It was at this point that the Equine Research Centre was called in to investigate. In a nutshell, what they found was that the problem was not with the dust in the walkway, but the lack of ventilation in the stables. These findings sparked the requirement for a study on stable design, the paper for which was published in 1993 (an edit of which is also included here), and quite possibly started the increased awareness of the importance of good ventilation in stables that most stabled horses enjoy today.

The Centre was initially largely funded by the Highveld Racing Development Fund (HRDF), which was funded by betting levies. With the corporatisation of racing in the late 1990's the HRDF funds were transferred to the Racing

Association. Since ERC's inception the University of Pretoria has provided its infrastructure and all utilities. Operational funding has been provided from donors (54%), competitive grants and self-generated funds (46%).

Following is a list of papers published in the first 5 years of ERC's existence :

YEAR	TITLE
1990	Pharmocokinetics of Gentramicin in newborn to 30 day-old foals By : Cummings, LE; Guthrie, AJ; Harkings, JD; Short, CR (1990) *American Journal of Veterinary Research, 51, 1988-1992.*
1991	Use of the cardiopulmonary flow index to evaluate cardiac function in Thoroughbred horses. By : Guthrie, AJ; Killeen, VM; Mülders, MSG; Grosskope, JFW (1991) *Journal of the South African Veterinary \association 62, pp. 43-47* The effects of endogenously produced carbon monoxide on the oxygen status of dogs infected with ***babesia canis***. By : Taylor, JH; Guthrie, AJ; Leizewitz, A (1991) *Journal of the South African Veterinary Association 62, pp. 153-155* Stimulation frequency-dependent nonadrenergic noncholinergic airway responses of the guinea-pig. By : Venugopalan, CS; Holmes, EP; Fucci, V; Guthrie, AJ (1991) *Journal of Applied Physiology 70, pp. 1006-1010*
1992	Use of a semi-quantitative sweat test in thoroughbred horses. By : Guthrie, AJ; van den Berg, JS; Killeen, VM; Nichas, E (1992) *Journal of South African Veterinary Association. 63, pp. 162-165* Temporal effects of inhaled histamine and methacholine aerosols on the pulmonary mechanics of Thoroughbred horses. By : Guthrie, AJ; Beadle, RE; Bateman, RD; White, CE (1992) *Journal of Veterinary Pharmacology and Therapeutics 15, 317-331* Equine colic By : Guthrie, AJ; van den Berg, JS (1992) *The South African Racehorse November pp. 29-30*
1993	Designing Stables (edit included in this newsletter) By : Guthrie, AJ; Lund, RJ (1993) *The South African Racehorse, August/September, pp. 73-74* Survey of selected design and ventilation, characteristics of racehorse stables in the Pretoria, Witwatersrand, Vereeniging area of South Africa. By : Lund, RJ; Guthrie, AJ; Killeen, VM (1993) *Journal of the South African Veterinary Association. 64, pp. 149-153.*
1994	Detection of Corticosteroids by High-performance Liquid Chromatography/Negative Ion Chemical Ionization Mass Spectrometry using a Particle Beam Interface. By : Stanley, SM; Wilhelmi, BS; Rodgers, JP; Guthrie, AJ *Biological Mass Spectrometry. 23, 1994. Pp. 483-491*
1995	Characterisation of a density-corrected ultrasonic pneumotachometer for horses. By : Guthrie, AJ; Beadle, RE; Kou, AH *Journal of Applied Physiology, 0161, 7567, 1995, pp. 359-367* The arterial oxygen status of clinically healthy dogs at an altitude of 1250 metres. By : Guthrie, AJ; Leizewitz, AL; Berry, W. *Journal of the South African Veterinary Association, 66(4), 1995, pp. 213-218* The effects of three models of airway disease on tidal breathing flow-volume loops of Thoroughbred horses. By : Guthrie, AJ; Beadle, RE; Bateman, RD; White, CE. *Veterinary Research Communications, 19, 1995, pp. 517-527*

	Characterization of normal tidal breathing flow-volume loops for Thoroughbred horses. By : Guthrie, AJ; Beadle, RE; Bateman, RD; White, CE. *Veterinary Research Communications, 19 1995, pp. 331-342* Measurement of maximal oxygen consumption of Thoroughbred horses at an altitude of 1250 metres using open-circuit flow-through calorimetry. By : Lund, RJ; Guthrie, AJ. *Journal of the South African Veterinary Association, 66(4), 1995, pp. 239-243*

TWO OF ERC'S EARLY PUBLICATIONS SUMMARISED

EQUINE RESEARCH CENTRE'S FIRST PROJECT STUDIES WASTAGE IN THOROUGHBRED HORSES IN GAUTENG

This study took place over a few years, with the paper being published in 1996. Here is an edit of this paper.

An epizootical study of wastage in Thoroughbred racehorses in Gauteng, South Africa

Wastage is the term used to describe the loss of racehorses from conception to adulthood due to death or injuries, where they never reach the racetrack, or the days lost by racehorses due to not training or being withdrawn from a race. This study was conducted to investigate wastage in Thoroughbred horses used for flat racing in Gauteng, South Africa.

Similar studies have been performed in Newmarket, United Kingdom, at Canterbury Downs in Minnesota and of the Canadian Standardbreds. However, since no data on the incidence of wastage in South Africa were available, this study was implemented. Data from 6 racing stables were recorded from March 1993 to February 1994. Each trainer was required to complete a daily training record of the horses in his stable. The questionnaire included reasons why a horse failed to train on a specific day, or was withdrawn from a race. During the year 8.1% of the total potential training days were lost by horses in the stables investigated. Of these 72.1% were due to lameness, 8.6% to respiratory problems, and 19.3% to other causes (bad weather, vaccinations, wounds etc). The horses with respiratory conditions showed one or more of the following symptoms : coughing, nasal discharge, pharyngitis, epistaxis and upper respiratory noises. The data on the causes of lameness were not included in this study owing to the absence of veterinary confirmation, or that more than one cause of lameness was cited by the trainer in an extended convalescence period. The lost training days for the individual trainers ranged from 5.4 to 12.6%.

The effect of season (summer, autumn, winter and spring) on the incidence of lameness and respiratory tract conditions were also measured, as was the effect of age of the horse. The training days lost due to lameness were considerably higher in autumn and significantly lower in spring. The number of respiratory problems was significantly higher in winter. Lameness occurred more in 3-year olds than 2-year olds, while respiratory problems were significantly higher than expected in 3- and 4-year olds.

The study was continued in 1994/95, but the numbers of days and horses were not added to the first year's results due to marked decrease in the return of questionnaires from the trainers. However, it was evident that the percentage of training days lost was similar to the previous year, as were the percentages of loss due to lameness (66.9%) and respiratory problems (8.4%) similar to the previous year.

The increased incidence of lameness in autumn months may have been due to relatively low rainfall and temperature, resulting in harder tracks, but there would have to be other causes, as winter also experiences low rainfall, and there was no significant increase in lameness during this season.

The study showed a relatively low level of wastage due to respiratory problems (8.6%) compared with previous studies by Rossdale et al (20.5%) and Herzog & Lindner (22.6%). This could be attributed to the fact that there are lower temperatures prevailing for a longer period of time in the United Kingdom.

The increase of the number of training days lost due to respiratory problems in winter may be caused the lower temperatures or poor stable management. During winter it is common practice to close stables up with the intention of keeping the horses warm, thus affecting ventilation and increasing residual dust.

There could be several causes for the difference in training days lost due to lameness between 2- and 3- year olds, such as that 2- year olds may only start racing from 1 October in the year they turn 2. Trainers may also tend to spare the 2-year olds and push the 3-year olds harder to perform.

The effect of age on the increased incidence of respiratory problems (3- and 4-year olds), may be explained by the fact that 3- and 4-year olds may be travelling more and therefore be more exposed to respiratory pathogens than the other age groups. These 3- and 4-year old horses may also have been stabled with inadequate ventilation, leading to chronic respiratory disease, for relatively longer periods than the 2-year olds.

From the data collected it was concluded that lameness and respiratory conditions were the two most important causes of wastage in Thoroughbred racehorses in Gauteng in 1993/94. Continued research should be conducted to increase our knowledge of the reasons for the occurrence of these conditions in order to prevent them and minimise their effect on Thoroughbred racing in South Africa.

Publication : Journal South African Veterinary Association (1997) 68(4): 125-129

Research Team :
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SURVEY OF SELECTED DESIGN AND VENTILATION CHARACTERISTICS OF STABLES IN THE PRETORIA, WITWATERSRAND AND VERENIGING AREA OF SOUTH AFRICA

The cost of building stables is a major capital expense that faces horse owners today, with the cost of building traditional structures being so prohibitive as to have people resorting to using non-traditional materials and designs. There has also been a trend towards housing several horses in large barn-type buildings, which has made it more difficult to provide a suitable environment for the horses in the buildings, and thus to effectively control disease.

If only considered from the point of view of the horse's health, it would be optimal to leave the animals outside year-round. By providing adequate food and allowing the horse to acclimatise to normal seasonal changes, horses would develop a good coat and there would be no further need for protection from the elements than that provided by windbreaks and trees. However, today there are many factors that require that horses be stabled. Therefore the first essential of stabling is to give the horses as healthy an environment inside the stables as they would have outside, and adequate protection from the elements. Stables should also provide a good environment and facilities for the people involved with the horses.

The essentials that should be provided by stables are :

1. A reasonably uniform temperature;
2. A dry atmosphere with no condensation on the building's surfaces;
3. Good air movement and ventilation without drafts;
4. A sound dry floor;
5. Good drainage;
6. Adequate lighting;
7. Good watering and feeding arrangements.

Basic environmental requirements

The main factors that affect the climatic environment of stables are ambient temperature, relative humidity, ventilation rate and air movement. To provide stabled horses with the correct climatic environment and reduce the likelihood of disease it is important to consider the horse's physiological needs.

- *Ambient temperature :* Unlike humans, acclimatised horses can easily tolerate a wide range of ambient temperature (0°C - 30°C), provided the atmosphere is not damp and drafts are limited. In reality the air flow and ventilation of stables are seriously restricted in what is usually an unnecessary attempt to keep the horses 'warm'.
- *Relative humidity :* The survival of most respiratory viruses and bacteria is greatly enhanced in cold, humid environments. The possibility of developing disease, especially respiratory disease, is much greater in stables that are damp and have condensation on the internal walls. As horses exhale large amounts of moist air, poorly ventilated stables will often have condensation on the walls, roof and windows.
- *Stable ventilation :* Stable ventilation influences environmental temperature, relative humidity and the concentration of noxious gases, dust and microbes. As horses are expected to perform as athletes, lung health is of primary concern. Stable ventilation should thus be provided to maintain 'fresh air' for the horses' respiratory well-being.

The South African situation

In South Africa, the coldest temperatures experienced in stables during winter are well within the horses' range of comfortable temperatures. As such one should not be concerned that a well-ventilated stable will be too cold. In summer, on the other hand, the temperatures often exceed the upper limit of the temperature comfort zone for horses, so measures to maximise ventilation to ensure lower indoor temperatures should be adopted. Incorporating optimal ventilation will also help to provide the 'fresh-air' that is so vital for the stabled horses' respiratory health.

The Equine Research Centre (ERC) completed a survey of the ventilation characteristics of more than 4 700 stables in the then PWV area. Ventilation rates were determined under optimal conditions (i.e. all vents and top stable doors open) and under minimal conditions (i.e. all adjustable vents and top stable doors closed). It was noted that even with all vents and top stable doors open, the ventilation rate of only 33% of the stables was above the recommended value of 8 air changes per hour (ach). It was alarming to note that the ventilation of about 25% of the stables was below the minimum required rate of 4 ach. The effect of closing all adjustable vents and the top stable doors resulted in the ventilation rate being below the minimum value (4 ach) in 35% of the stables, with the ventilation rate of above the recommended 8 ach being evident in only 9% of the stables. These results showed that the vast majority of the stables surveyed were sub-optimally ventilated. The results also showed that older stables (pre-1970) were better ventilated than those built more recently.

Conclusions

It was evident that many design features and management practices were aimed at keeping the horses warm, in spite of the mild South African climate. Due to the wide thermal comfort zone of horses, this is usually totally unnecessary, and is further more detrimental to the health of the horses. The practice of closing top stable doors or shutters on barns, should be avoided year-round.

Excellent computer models are now able to predict the impact of stable design will have on the temperature, humidity and ventilation of the stable, and should be adopted when designing stables. They also allow one to investigate how to improve ventilation in existing buildings. The authors of this paper offered their expertise to assist with building or improving current buildings.

Publication : Journal of the South African Veterinary Association

Research Team : Prof A J Guthrie, Mr R J Lund – Equine Research Centre, Faculty of Veterinary Science, University of Pretoria

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