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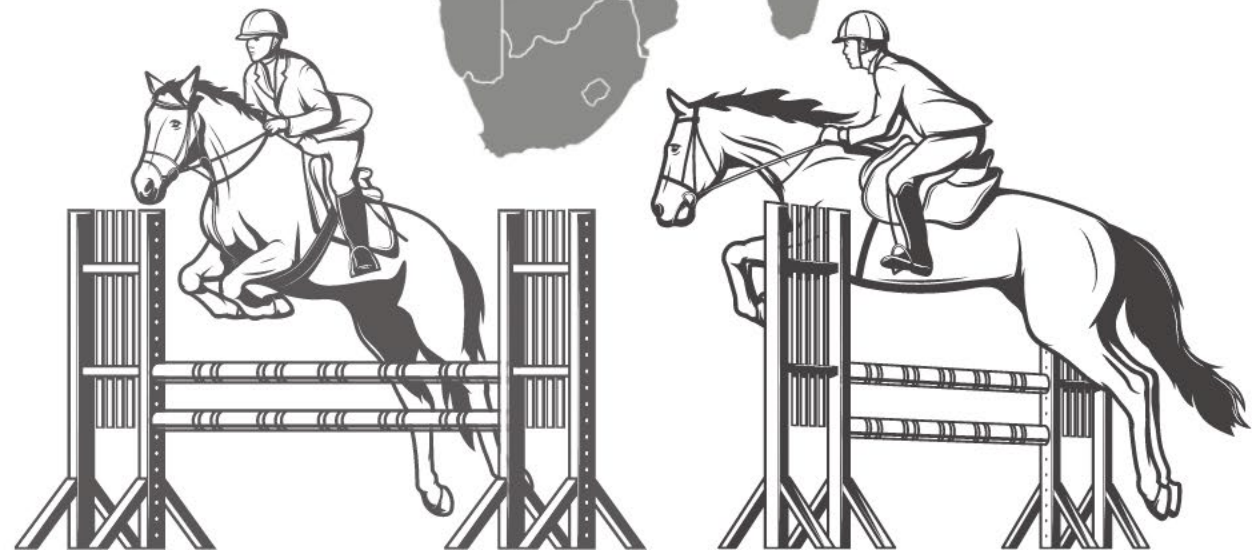
OIE 3rd Regional Webinar on:

**OIE international standards and tools to facilitate international movement of (competition) horses,
procedures supporting the publication of self-declarations of animal health status and the official recognition
of African horse sickness (AHS) free status**

10-11 May 2022 (08:00 AM – 11:30 AM GMT)

New challenges on horses welfare

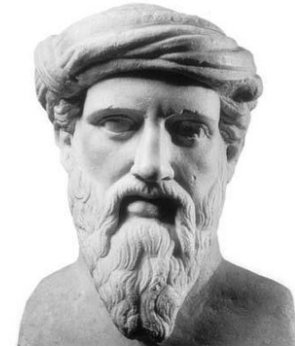
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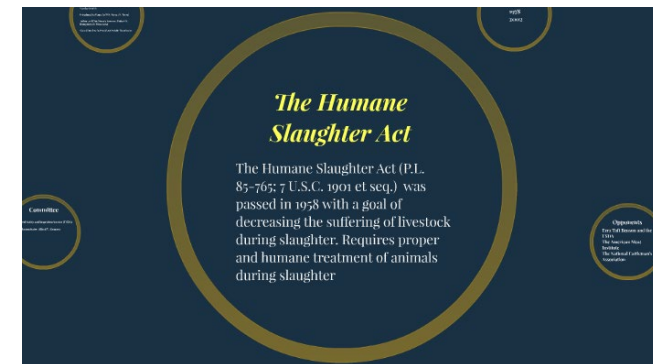
Animal Welfare History.....

Pythagoras is the first in a line of several Greek and Roman Philosophers to teach that animals have souls



The roots of animal welfare began in the UK with the introduction by Richard Martin of «Martin's Act» in 1822

1958...The American Humane Slaughter Act is passed

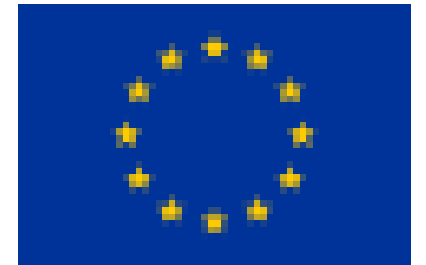


1960...Indian Parliament passes its first national animal welfare legislation, The Prevention of Cruelty to Animals Act



1964 British Parliament Five Freedoms

1974..... The Council of Europe passes a directive requiring that animals be rendered unconscious before slaughter



1992, Switzerland becomes the first country to include protections for animals in its constitution





OIE definition of Animal Welfare

means the physical and mental state of an animal in relation to the conditions in which it lives and dies. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear and distress (OIE, 2008)



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The 5 Freedoms are often used as a framework to assess animal welfare
(Farm Animal Welfare Council, 1992)

“the internationally recognised ‘five freedoms’ (freedom from hunger, thirst and malnutrition; freedom from fear and distress; freedom from physical and thermal discomfort; freedom from pain, injury and disease; and freedom to express normal patterns of behaviour) provide valuable guidance in animal welfare”.

(OIE Terrestrial Animal Health Code, 2016)



Freedom
from pain ,
injury and
disease

Freedom
from thirst,
hunger and
malnutrition

Freedom
from
discomfort
due to
environment

5
freedoms

Freedom
from fear and
distress

Freedom to
express
normal
behavior for
the species



Horse Welfare

Is important to understand the growing interest in horse welfare....

.....the number of published studies that include the key-words "horse welfare" over the time period 2012 - 2021.....

PubMed Timeline Results

Search query: horse welfare	
Year	Count
2022	34
2021	174
2020	163
2019	122
2018	111
2017	90
2016	83
2015	94
2014	72
2013	82
2012	62



**started from 62
....to 174 in 2021**



The main question is.....How we can evaluate and guarantee actually the horse welfare !?



The answers probably will be:

.....through the application of laws.....

.....through the application of the OIE global animal welfare strategy....

.....through the application of horse welfare assessment protocols, for example, AWIN in Europe



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But there is probably a lack of a single, repeatable, reliable and standardizable tool that can provide information on the chronic state of well-being.....

We are working in this direction





Welfare evaluation

Everybody knows that Cortisol is generally considered to be a stress hormone.

Different studies demonstrated that is not so affordable as an indicator of stress-marker if considering blood and stool concentration.

.....instead horsehair concentration seems to be more affordable as evaluation of chronic states (Duran 2017)





Welfare evaluation

On these basis we are conducting studies with the following aims:

- Understand the differences in values in groups of horses with different aptitudes !!
- Understand the reference ranges for different aptitudes!

But especially....

- Standardize the laboratory method that guarantees the best reliability to propose it in the future as stress assessment method !!!





We realized a study to understand values in groups with difference aptitudes and to validate the laboratory test

Forty-seven (47), 18 females and 29 males, clinically healthy horses were enrolled in the study. All horses included in the study were between 5 and 15 years of age; this range of age has been decided as the animal welfare indicators can't be evaluated in individuals under 5 years of age (according to AWIN Protocol) while in those above 15 years the risk of having patients with Cushing disease, that could alter the results, is very high (McGowan et al. 2015)

Three groups of horses with different aptitudes and under different management were selected:

- 1) stabled horses in constant activity
- 2) horses that perform public order service under the Italian state police
- 3) horses kept outdoors in the wild.



We realized a study to understand values in groups with difference aptitudes and to validate the laboratory test

Group 1: ... stabled alone in boxes (4 x 4 metres) with access to the paddock every day. They have been fed with hay and feed, (ration balanced by a veterinarian) and shod every 45-50 days. No pathologies or pharmacological treatments have been recorded in the last 5 months. The horses included in this group carry out training activities and level work 3-4 times a week and are in forces to the Italian State Police





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Group 2:stabled alone in boxes (4 x 4 metres), with no access to the paddock. They have been fed with hay and feed (ration balanced by a veterinarian) and regularly shod every 45-50 days. The horses of this group have carried out every day public order activities in forces to the Italian State Police. Anyway, also when horses do not carry out service, they are moved daily from the box.





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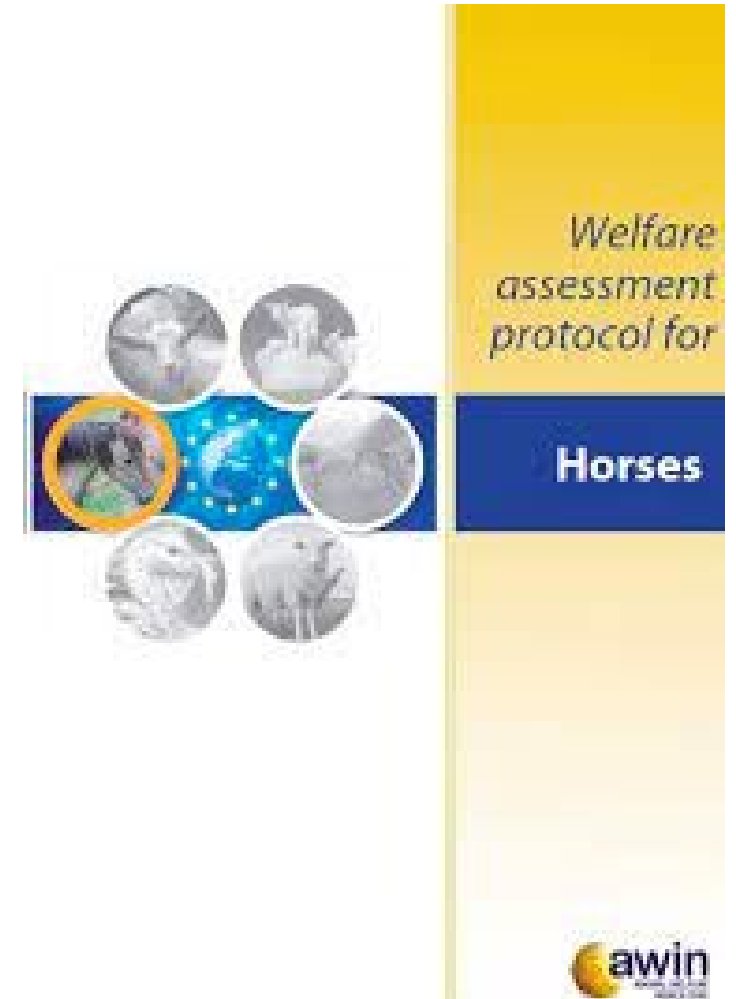
Group 3: feral horses , in the mountains at an altitude of about 600 meters above sea level (Anversa degli Abruzzi - AQ) with access to natural food and hay, while drinking water was in water tanks. Animals, owned by a local farmer, were not managed everyday and spent most of the daily time alone.





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To select suitable horses to be included in the study, the AWIN welfare assessment protocol was compiled. The aim was to enroll subjects with a good state of welfare based on the parameters of the AWIN protocol applied to categories of horses included in the study.....in this way we had no difference between the selected horses





We realized a study to understand values in groups with difference aptitudes and to validate the laboratory test

The protocol identifies 4 principles (good feeding, good housing, good health, appropriate behavior), and 12 criteria. Each criterion is assessed based on specific welfare indicators.

For this study, 10 welfare indicators were considered adequate since they could be assessed in all the three management conditions evaluated. These indicators, always evaluated by the same operator, were:

1. Body condition score (Principle: good feeding, criterion: appropriate nutrition)
2. Body/skin lesions (Principle: good health, criterion: absence of injuries)
3. Lameness (Principle: good health, criterion: absence of injuries)
4. Nasal and ocular discharge (Principle: good health, criterion: absence of disease)
5. Stereotypies and abnormal behaviors (Principle: appropriate behavior, criterion: expression of other behaviors)
6. Apathy (Principle: good housing, criterion: thermal comfort)
7. Vulvar or penis discharge (Principle: good health, criterion: absence of disease)
8. Prolapses (Principle: good health, criterion: absence of injuries)
9. Joint swellings (Principle: good health, criterion: absence of injuries)
10. Availability of water (Principle: good feeding, criterion: absence of prolonged thirst)





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Sampling.....

..from each animal, horsehairs including hair bulb were sampled at the mid-neck region and collected in a sterile box. The quantity taken was about 1 gr/animal. Sampling was carried out by tearing the horsehair by hand. The sterile boxes containing horsehair samples were kept at room temperature and delivered to the laboratory as quickly as possible always within 24 hours after the sampling.

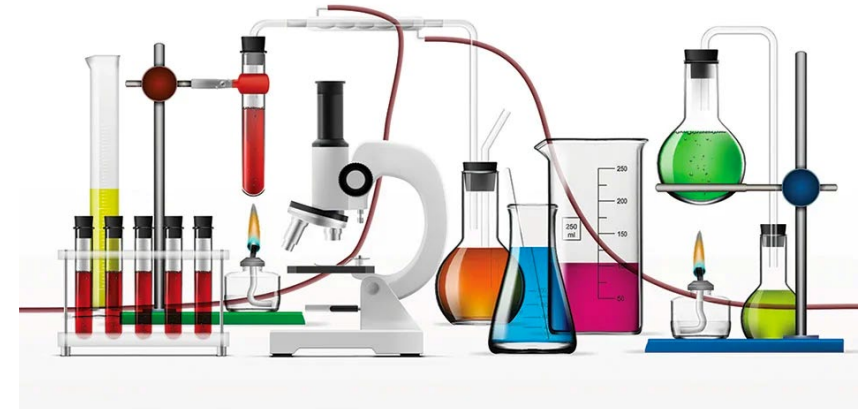
All the samples of this study were collected in the first week of June. Previous studies have shown that seasons do not affect hairhorse cortisol levels (Mazzola, 2021)





We realized a study to understand values in groups with difference aptitudes and to validate the laboratory test

Laboratory test, validation of the method



The cortisol present in the horsehair was determined by using the **liquid chromatography coupled to hybrid Orbitrap high-resolution mass spectrometry**

It's the first study using this technique! We hope to continue this way.....



We realized a study to understand values in groups with difference aptitudes and to validate the laboratory test

Results.....

Statistic	Group 1	Group 2	Group 3
N. of observations	16	16	15
	Detected concentration (pg mg ⁻¹)		
Minimum	1.32	1.36	3.67
Maximum	3.64	5.60	8.822
1st Quartile	1.931	2.807	4.315
Median	2.600	3.449	4.850
3rd Quartile	2.832	4.287	5.857
Mean	2.475	3.546	5.224
Variance (n-1)*	0.450	1.744	2.027
Standard deviation (n-1)	0.671	1.320	1.424
Lower bound on mean (95%)	2.117	2.843	4.435
Upper bound on mean (95%)	2.832	4.250	6.012



We realized a study to understand values in groups with difference aptitudes and to validate the laboratory test

Results.....

Mean values detected

Group 1: 2,4 pg /mg⁻¹

Group 2 : 3,5 pg /mg⁻¹

Group 3: 5,2 pg /mg⁻¹





We realized a study to understand values in groups with difference aptitudes and to validate the laboratory test

we concluded

....As expected, the cortisol levels found in horsehair of the three groups of animals managed in this study were significantly different. Unexpectedly, the higher levels were detected in the group of horses raised in the mountain (wild horses).....

.....in this study even though all enrolled horses reflected clinical and behavioral parameters of good welfare status and good management has been ensured to these animals, **it seems that horses stabled individually with access to paddock can experience better animal welfare compared to feral.....!!!**

.....in particular, adequate training, good management including regular exercise, paddocking, good feeding, good litter, watering, well-established work routine allow a satisfactory degree of animal welfare despite the constraining to which they are subjected living indoors for most of their life.....



we concluded

.....It is also noted that also group 2, compared with 1, had higher values of cortisol. This is likely due to the type of activity carried out (public order service) and to the lack of access to the paddock; these results are in line with the initial expectations. On the other side, feral horses included in group 3 experienced lower animal welfare even if they could express social behaviour, and despite showing, at least apparently, very good health.

This study also reveals how good human management of the horse, even if subjected to work and training, can provide greater levels of well-being than horses in the wild that in the common imagination live in the most natural and less stressful conditions





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But especially.....

...in addition to providing scientific data, a reliable laboratory method was standardized.....

Liquid chromatography coupled to hybrid Orbitrap high-resolution mass spectrometry (HPLC-Q-Orbitrap)



What we wish for the future....

We hope in the future to obtain reference values (range) on large numbers of horses, so as to provide the scientific community with consolidated data

and above all

to standardize the laboratory and sampling techniques *to provide an objective tool for the chronic assessment of well-being in horses*



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Thank You