

Research internship

safety of horse milk to humans

and

the effects of milking on the welfare of horses

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FOREWORD

This study is about horse milk. I have visited 12 horse dairy farms and made an inventarisation of the HACCP practices. Afterwards I made a comparison between the microbiological results and HACCP practices in place at the farms to assess the influence of the use of and the effectiveness of the introduced HACCP practice.

The welfare of the horses in milking dairies was also assessed in this research. The research has been conducted in cooperating with the University of Utrecht and the cooperative association horse dairies Netherlands-Flanders (SPaN-V).

With this study I hope to be able to provide a contribution to improving the quality of horse milk. Further I hope that with regards to legislation that is becomes clearer, so that this can be applied correctly. By explaining the term HACCP in this report, I hope that the system is better understood and will be applied correctly.

I want to thank all the horse dairy farms who helped with this research, for their commitment and providing the milk.

Moreover I want to thank my supervisor Liny Keessen for all her commitment, support and answering all my questions. Furthermore there are also the analysts of the laboratory of the University of Utrecht, Ali and Angele, who I also want to thanks for all their commitment.

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Review: Legislation concerning food safety of horse milk

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ABSTRACT

In 2006 the legislation for raw horse milk changed, because regulation (EC) No. 853/2004 became effective and changed the definition for raw milk. Together with Regulation (EC) No. 853/2004 became Regulation (EC) No. 852/2004, Regulation (EC) No. 854/2004 and Regulation 882/2004 effective. All these regulations are complements of the general food law (Regulation (EC) No. 178/2002) which already was effective in 2004.

The above mentioned regulations must ensure the safety of horse milk. To ensure safe milk, farms have to use HACCP, which can be implemented by using hygienecodes.

The horses at the horse dairy farms must be healthy. Hygiene standards must be high, like washing hands before contact with raw milk. The nipples and surrounding area must be clean. The milk must be checked on the presence of abnormalities. The milk must be cooled immediately to six degrees and be frozen at a temperature of -18 degrees.

Horse milk and products made of horse milk must be traceable. Traceability can be ensured with the required identification mark.

Keywords: Horse dairy farms, legislation, food safety

1. INTRODUCTION

In 2002, there were only 10 horse dairy farms in the Netherlands. In 2007, the number of horse dairy farms in the Netherlands had already doubled. Legislation especially for horse milk does not exist.

Horse milk was considered according to EU-guideline No. 92/46/EG as a food product. This changed in 2006 with Regulation (EC) No. 853/2004 where the definition of raw milk was broadened.¹ The definition of raw milk has been determined as: "Milk separated by the mammary gland of one or more farm animals, which has not been heated to over 40°C or any treatment with an equivalent effect has undergone."²

In 2008 it is still not clear to which regulations horse dairy farms have to comply. The Dutch government is not yet very much involved in the regulations of horse milk in clear contrast to the Belgian government. The Belgian horse dairy farms have already received an identification number and the horse milk is checked by the Belgian Food Safety Authority. The horse dairy farms in the Netherlands have at this moment no official identification number and the control of the horse milk is not yet obligatory.

Due to the change in legislation for horse milk it is still rather unclear for the horse milkers to which regulations they have to comply. With this article it is the intention to clarify this.



2. RESULTS

2.1. European legislation

2.1.1. Regulation (EC) No. 178/2002, General Food Law (GFL)⁴

This regulation aims at a high level of protection of public health and consumers' interests in relation to food supply. To ensure safety, all aspects of the food production chain must be considered as a whole. Therefore, the regulation applies to all stages of production, processing and distribution of food (and animal foods). This regulation applies to horse dairy farms, because they are producing, processing and distributing food.

A high level of protection can be obtained by food safety rules, which are based on risk analysis. These food safety rules are set out in Article 14 of the GFL. This article states that food is considered unsafe when it is unfit for human consumption.

Food business operators are the most suitable industry to develop a safe system for supplying safe food. Therefore food business operators are responsible for food safety. This responsibility is given in article 14 and 17 to 19 of the GFL. Article 17 requires operators of food companies to adhere to the requirements, which are set out in the food law. The general rules for food safety have been linked with other binding rules which have been determined in specific legislation such as Regulation (EC) No. 852/2004 and Regulation (EC) No. 853/2004 (for example the application of HACCP in the field of food hygiene). The purpose of article 19 is to reduce, limit or eliminate the risk associated with the marketing of unsafe foods. Operators must warn the competent authorities when they suspect that the supplied food does not meet the requirements of a safe product.

Food (also horse milk), animal feed and food-producing animals must be traceable at all stages. Traceability is important for risk management, which should help to control food safety. In order to be able to adhere to article 18, information must be registered.

The following information could be registered by horse dairy farms:

- Date of transaction/delivery
- Volume or quantity
- Charge number

2.1.2. Regulation (EC) No. 852/2004^{5;6}

This regulation contains the general hygiene regulations which food companies at all stages of the food chain must comply to.

Article 1 indicates that account should be taken on food hygiene. The following principles, among others, must be taken into account:

- Responsibility for food safety rests at the operator of the food.
- Food which can not be kept in a safe manner at ambient temperature, particularly frozen products must be kept at a cold chain.
- There must be microbiological criteria and rules concerning temperature control, based on scientific risk assessment.

This regulation stated that points must be taken to comply with microbiological criteria of food and temperature control. To comply with this regulation HACCP must be used. In this regulation guidelines are set out in article 7 to 9. These guidelines ensure that small businesses can meet the HACCP standards with so-called hygienecodes. Guidelines for primary products must meet annex I of Regulation (EC) No. 852/2004 as well. This appendix determines general and specific hygiene rules



for raw milk. Operators of raw milk must adhere to the general hygiene of this annex, as well as the other specific requirements of Regulation (EC) No. 853/2004.

Annex I, part A of Regulation (EC) No. 852/2004 is about transport, storage and handling of primary products, hygiene regulations (to prevent contamination) and registration. Measures must be taken to prevent contamination by air, water, soil and by medicine. To prevent these contaminations zoonoses must be prevented by controlling the agents. Operators must also register which type of feed, medicines and diseases the horses have had and the results of researches/analysis must be kept.

Annex I, part B of Regulation (EC) No. 852/2004 contains recommendations for good hygiene practices. This annex gives examples of dangers and measures to be taken in order to reduce risks, such as:

- the proper disposal of dead animals and litter
- measures to ensure that slaughter and production animals are clean
- measures regarding the keeping of registers
- measures to prevent the introduction of contagious diseases through food to humans
- Procedures and methods to ensure that food is produced, handled, packed, stored and transported under appropriate hygienic conditions

Annex II of Regulation (EC) No. 852/2004 contains hygiene regulations for all operators of food companies (unless annex I applies). This annex states that the production area must be well maintained. And that the area is easy to clean and disinfect. Lavatories should not be connected to the area where food is handled. There must be sufficient washbasins for cleaning hands, which are provided with hot and cold running water, in the area. Furthermore, there has to be sufficient ventilation and light. Cleaning and decontamination materials cannot be stored in the area, where food is handled.

2.1.3. Regulation (EC) No. 853/2004^{2;7}

This regulation contains specific hygiene regulations concerning food of animal origin. Regulation (EC) No. 853/2004 applies only to unprocessed and processed food of animal origin. The regulation concerns mainly animal health, hygiene on the production companies and criteria for foodstuffs, including raw milk.

The definition of raw milk has been determined in this regulation as: "milk separated by the mammary gland of one or more farm animals, which has not been heated to over 40°C or any treatment with an equivalent effect has undergone."

Annex III of Regulation (EC) No. 853/2004 contains the following information: Milk must come from animals which show no symptoms of zoonoses, digestive problems, reproduction problems or udder problems. The animals must be free from Brucella and Tuberculosis.

Milk must be stored so that it reduces the risk of contamination. Vermin is not allowed in milk storage rooms. The area where the milk is stored has to be separated from the area of housing of the animals. Materials that come into contact with the milk must be easy to clean and be disinfected if necessary. That means that the material should be smooth, washable and non-toxic. All equipment must be cleaned after use and disinfected if necessary.

Hygiene during milking: The nipples and surrounding area should be clean and milk must be checked on the presence of abnormal properties. This can be done by



performing a visual inspection of the milk from each animal. Furthermore, the milk from sick or treated animals may not enter the tank for human consumption. After milking, the milk must directly be stored cold (at least 6 degrees) in a clean space. Suitable facilities must be available near the place of milking to enable persons performing milking and handling raw milk to wash their hands and arms. The person in charge of the raw milk must wear clean clothing.

In this regulation a criterion for total viable count in the raw milk is determined, this in anticipation on more specific guidelines for raw horse milk. At this moment the guidelines which apply to horse milk are: raw milk from animals other than cows.

- Raw milk from animals other than cows which did not undergo a heat treatment. Plate count at 30°C (per ml) $\leq 500\,000$ (*)

(*) advancing geometric average over a period of two months, with at least two samples per month.

Operators of food must ensure that milk containing more antibiotics than permitted according to Regulation (EC) No. 2377/90, is not used for human consumption.

When the milk does not meet the guidelines with respect to total viable count or antibiotics, the competent authority must be informed and corrective measures must be taken.

Raw milk and products which have been prepared with raw milk must, as stated in Annex III, Section IX, Chapter IV of Regulation (EC) No. 853/2004, be labelled according to labelling directive 2000/13/EC. Besides labelling there must also be an identification mark on the packaging

The identification mark contains the code of the country of origin, existing of two characters in conformity with ISO-norm, the recognition number of the horse dairy farm and when the horse dairy farm is confirmed in the community, the identification mark must be oval and must contain the abbreviations CE, EC, EF, EC, EK, EY, ASH, EU, EK, EB or WE.

2.1.4. Regulation (EC) No. 854/2004⁸

This regulation contains specific rules concerning official inspections of products of animal origin. The rules apply only to persons and their activities for which Regulation (EC) No. 853/004 also applies to.

The competent authority shall carry out inspections on compliance with the requirements of Regulation (EC) No. 852/2004, Regulation (EC) No. 853/2004.

Inspections are carried out whether HACCP-based procedures by operators of food are properly applied. There will also be checked if these procedures guarantee that the products comply with the regulations regarding to microbiological criteria, residues and contaminants and are safe to consume.

The identification marks, which are determined in Regulation (EC) No. 853/2004, are assessed to ensure traceability of a product.

Annex IV of Regulation (EC) No. 854/2004 concerns specifically raw milk and dairy products. In this annex states that, animals in milk production companies must be subject to verify that the health requirements for raw milk production, these controls may take place at the occasion of veterinary health checks.

When inspections reveal that the criteria for the total viable count have not been met, food operators get the three months to rectify this. Is there no correction within three months of the total viable count observed, it may no longer supply raw milk until it can be shown that the raw milk criteria have been met.



2.1.5. Regulation (EC) No. 882/2004⁹

In this regulation general rules for the implementation of official inspections are determined. The inspections are aimed at compliance with regulations, which prevent, eliminate or reduce risks for humans and animals to acceptable standards. These inspections are carried out without notice.

2.2. National legislation

National legislation has been prepared to clarify or supplement the international legislation.

2.2.1. Law decision hygiene of foodstuffs (WHL, Abbreviation of Dutch words)¹⁰

The minister approves the hygiene codes under the condition that article 8 of Regulation (EC) No. 852/2004 is met, they are useful for that sector and prepared on the basis of HACCP, as described in Article 5 of Regulation (EC) No. 852/2004.

This law decision contains the following criteria for raw cow milk

- Plate count at 30 ° C ≤ 50,000 per ml
- Staphylococcus aureus < 500 cfu/ml
- Salmonella is absent in 25 g

2.2.2. Law decision preparation and handling of food (BBL, abbreviation of Dutch words)^{1;11}

Products may only be stored in packaging in a clean area. Pathogenic micro-organisms may not be detectable or have to stay under a determined criterion. Storage temperature requirements must be met in order to reduce pathogenic growth. The temperature during transport must also be monitored.

The VWA (Dutch abbreviation of the Dutch food authority) is in the Netherlands the competent authority, as referred to in regulation (EC) No. 178/2002

2.2.3. Law regulation frozen foods¹²

This law regulation is important for horse dairy farms, because horse milk is sold frozen. Regulation (EC) No. 853/2004 states that frozen products must be kept at a cold chain, this law regulation goes further about the cold chain.

This regulation states that the preparation of deep frozen foodstuffs must be done without delay, to avoid chemical, biochemical and microbiological changes.

The temperature of the product may not exceed a maximum -18°C and the temperature must be stable. The food must be packed in a way that dehydration and contamination is prevented. The label of frozen products should be marked temperature at which the product must be kept and how long it can be held at this temperature. The product may not be re-frozen. This needs to be printed on the packing.

3. CONCLUSION

Since 2006 horse dairy farms have to comply with regulation (EC) No. 178/2002 and the hygiene complements. The most important obligations which must be met are stated below.

The produced milk must be safe. The entrepreneur is responsible for the safety of a product. To ensure safe milk, HACCP must be used. Working with HACCP could be implemented by using hygienecodes.



The horses at the horse dairy farms must be healthy. They have no symptoms of zoonoses, intestinal problems, reproduction problems or udder problems. The horses must be subject to verification of health requirements for raw milk production, these inspections must take place during veterinary health checks.

Suitable facilities must be available near the place of milking to enable persons performing milking and handling raw milk to wash their hands and arms. During the milking the nipples and surrounding area must be clean. The milk must be checked on the presence of abnormal properties. The milk from sick or treated animals may not enter the tank for human consumption. After milking the milk must directly be cooled to at least six degrees and be stored at a clean place. Because horse milk is sold frozen, the freezer must also meet the set temperature of -18 degrees.

The horse milk and the product made of horse milk must be traceable. Traceability could be ensured with the required identification mark on the package of the product. This identification mark must contain the code of the country of origin and the recognition number of the horse dairy farm. On the package must also state that frozen milk must not be re-frozen.

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Effectiveness of HACCP at horse dairy farms in the Netherlands and Flanders

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ABSTRACT

Raw horse milk is becoming increasingly popular. This is due to the apparent healing properties. However, because horse milk is consumed raw it can be a threat for the public health.

In 2005 the association for horse milk in the Netherlands and Flanders (SPaN-V) had established a HACCP handbook to apply to regulation (EC) No. 852/2004, which is obliged for all horse dairy farms to work with.

In this research the effectiveness of the SPaN-V HACCP handbook was investigated. Samples of milk were taken from twelve horse dairy farms. These milk samples were investigated for possible microbiologic contamination, which could be a threat for the public health.

From the microbiologic results of the milk samples and the answers to the questionnaire could be concluded that HACCP can only be effective when it is used consciously. At horse dairy farms good hygiene practices are extremely important. Working consciously with HACCP results in a lower total viable count and *Enterobacteriaceae*.

Keywords: Horse dairy farms, HACCP, microbial analyses

1. INTRODUCTION

Because of the alleged health promoting properties, horse milk is consumed raw. During pasteurization the health promoting properties would allegedly disappear. The drinking of raw horse milk becomes more popular in the Netherlands every year. In 2002, there were only ten horse dairy farms in the Netherlands. In 2007, the number of horse dairy farms in the Netherlands has already doubled.¹⁶

Horse milk was considered according to EU-regulation (EC) No. 92/46/EG as a food product. This changed in 2006, with EU-regulation (EC) No. 853/2004 in which the definition of milk was broadened. In this regulation, it was written that dairy products are products which have been produced by the milk gland of animals in production.¹⁶

On basis of article 5 by Regulation (EC) No. 852/2004 entrepreneurs are obliged to prepare food safety procedures which are based on the principles of Hazard Analysis Critical Control Points (abbreviated HACCP). For smaller farms article 8 of the regulation had been established. Article 8 offers the possibility to the entrepreneur to work with hygiene codes based on HACCP. These hygiene codes can be centrally established by a branch organisation.⁸ The critical control points (abbreviated CCP) in a hygiene code were determined according to the HACCP-system. The critical control points should be guarded by the entrepreneur; This guard entails: monitoring of the CCPs, compulsory registration, corrective actions when necessary and periodic verification. After 3 to 5 years the hygiene codes must be evaluated. Checks must be made for the degree of use and usability in practice. On the basis of this information the hygiene codes can be improved.¹⁷

In October 2005, the association cooperating horse dairy farms of the Netherlands and Flanders (SPaN-V, abbreviation of Dutch words) was founded.



SPaN-V had ensured that a HACCP-handbook was established. In this handbook there were determined general control points and critical control points. To the general control points belong: stable hygiene, fodder and drinking water, cleaning and disinfection, environmental hygiene and personal hygiene. To the critical control points belong: the filter and the temperature of refrigeration and freezer.² The association is at this moment working to set up a White Paper concerning the benefits of horse milk and a quality mark, called top quality horse milk (TKPM, abbreviation of Dutch words), will be developed. The aim of the White Paper and the quality mark is quality care and promotion of the image of horse milk products.¹⁶

Most of the horse milk is sold frozen, because frozen milk has a longer shelf life. Most of the bacteria which can enter the milk during or after milking, cannot multiply at freeze temperatures, but the bacteria can survive at this temperature. The temperature of refrigeration and deep freeze is for this reason a CCP. The filter is also a CCP, because the filter makes the milk physical safe.

Bacteria which can be expected in raw milk are: *Enterobacteriaceae*, *Salmonella*, *Campylobacter*, *Listeria monocytogenes*, *Staphylococcus aureus*, *E. coli*O157 and *Bacillus cereus*.^{13;14} Because of these bacteria, consumption of raw horse milk can be a threat to the public health.

The bacteria, which can be in horse milk, can be divided in 2 kinds of microbiological criteria. These criteria are the food safety criteria and the process hygiene criteria. Under the food safety criteria fall *Listeria monocytogenes*, *Salmonella* spp. and toxins of *Staphylococccen*. At overshooting of the criterion of one of these microorganisms a recall of the product must take place. Under the process hygiene criteria fall *Enterobacteriaceae*, *Staphylococccen* and *E. coli*. These are criteria which give an indication if the production process had been done hygienically and acceptably.³

Research was done to determine if the above-mentioned bacteria are really a threat in raw horse milk. It was investigated whether the horse dairy farms work with the guidelines in the HACCP handbook and if these guidelines ensure a lowering of the bacteria in the raw milk. Besides microbiological safety, the HACCP handbook should concern also physical and chemical safety.

2. MATERIAL AND METHODS

2.1. The Horse Dairy Farms

This research concerning horse milk is a continuation of the research of Henricke Krol. The horse dairy farms which had not been investigated by H. Krol(2008)¹¹ were investigated in this research. These horse dairy farms were situated in the Netherlands and Flanders and were associated with SPaN-V.

2.2. Questionnaire

An impression of the horse dairy farms hygiene and HACCP-management was obtained by means of a questionnaire. This questionnaire was send by E-mail to the horse dairy farms. The questionnaire was discussed during the visit when necessary other questions were asked.

2.3. Visual Inspection

During the visit a visual inspection was carried out. The stables, cleanliness of the milking stable and processing room were checked. The method of packaging was also looked into, and if rules and regulations were followed.



2.4. Steri-keri Samples

The hygiene of the milk processing room was assessed by using two kinds of steri-keri plates, namely PCA en VRBG plates. The steri-keri plates were pressed on several objects, which were related to the processing of the milk. These objects ranged from the freezer to the bucket used for pouring of the milk. The used plates were brooded for 1 day at 37°C. After brooding the numbers of colonies on the plates were counted. The total viable count was assessed by means of table 1. When colonies of *Enterobacteriaceae* were found, the object was assessed as bad, whatever the assessment of the total viable count. So, the result of the hygiene of the object was determined by both steri-keri plates.

Number of colonies	Class	Qualification
Less than 3 colonies	0	Excellent
3 till 9 colonies	1	Good
10 till 29 colonies	2	Moderate
30 till 90 colonies	3	Insufficient
More than 90 colonies	4	Bad

Table 1. Scoring of total viable count of the steri-keri plates

2.5. Milk Samples

From each horse dairy farm one sample of approximately 250 ml of frozen horse milk and one sample of approximately 250 ml of fresh tank milk were taken for research. The frozen samples were packed in the same way as sold to consumers.

2.6. Microbial Analysis

In the laboratory of the faculty of veterinary medicine of Utrecht, the milk samples were analysed according to the ISO-protocols: ISO 4833, ISO 21528-1, ISO 6785, ISO 11290-1, ISO 10272, ISO 7932, ISO 16654 and ISO 6888-1.⁷

Frozen samples were thawed in the refrigerator for one day. The milk was well stirred with a sterile spatula before use. The fresh samples were investigated within 24 hours. The milk samples were investigated on total viable count, *Enterobacteriaceae*, *Salmonella* spp, *Staphylococcus aureus*, *Campylobacter* spp, *Listeria monocytogenes*, *E. Coli*0157 and *Bacillus cereus*. For the investigation of total viable count and *Enterobacteriaceae* was made a -1 and -2 dilution.

After one or several days, depending on the ISO protocol, the plates were collected from the stove and the colonies were counted. The bacterium could also be continued on another plate to get specific results. By counting the number of colonies, the amount of bacteria in one millilitre of the milk sample could be determined. The determined amounts of bacteria were compared with the international or national criteria of raw milk or dairy product.



Bacteria	Criteria	Reference
Total viable count	< 500.000 cfu/ml	Regulation (EC) No. 853/2004 ⁹
<i>Enterobacteriaceae</i>	< 1.000 cfu/ml	Hygienecode voor de ijsbereiding ¹
<i>Salmonella</i> spp	Absent in 25 ml	Warenwetbesluit Hygiëne van Levensmiddelen ¹²
<i>E. coli</i>	< 100 cfu/ml	Regulation (EC) No. 2073/2005 ⁶
<i>E. coli</i> O157	Absent in 25 ml	*
<i>Staphylococcus aureus</i>	< 500 cfu/ml	Warenwetbesluit Hygiëne van Levensmiddelen ¹²
<i>Listeria monocytogenes</i>	Absent in 25 ml	Review of the microbiological standards for foods ¹⁰
<i>Campylobacter</i>	Absent in 25 ml	Review of the microbiological standards for foods ¹⁰
<i>Bacillus cereus</i>	< 100.000 cfu/ml	De zuivelketen: juridische aspecten en de voedselveiligheid ¹⁵

Table 2. The determined criteria for raw horse milk

Legend: * based on expert opinions

There is no criterion set for *Enterobacteriaceae* for raw milk. According to the Food Safety Authority in the Netherlands (VWA, abbreviation of Dutch words) is this because *Enterobacteriaceae* are only useful to get an indication if the pasteurization has been sufficient. However, with *Enterobacteriaceae* is it possible to determine how hygienically the work is done. Therefore, criterion for *Enterobacteriaceae* is determined in this research. The criterion for *Enterobacteriaceae* used in this research is determined by using the criterion of cream, which is stated in the hygiene codes for ice-cream. *Enterobacteriaceae* are a large family of gram-negative rod-shaped bacteria, which live in the intestines. This family includes *E. coli*, *Salmonella*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Shigella*, *Yersinia* and many more bacteria. Some of these bacteria may already be pathogens in small quantities and thus cause disease.

2.7. Statistical Analyses^{4;5}

An independent-Sample T-test was done on the results of the researched milk samples. By means of a Levene's test, equality of the variances were checked. If the variables were equal, a T-test for 'Equal variances assumed' was carried out. If variables were not equal, a T-test for 'Equal variances not assumed' was used. An independent-Sample T-test was done to see if there was a significant difference in the *Enterobacteriaceae* and/or total viable count and the working method of the horse dairy farms. It was also used to see if there was a correlation between the *Enterobacteriaceae* and total viable count

3. RESULTS

3.1. Questionnaire

3.1.1. HACCP

Nine of the twelve horse dairy farms (75 percent) said to work with HACCP. Three of these horse dairy farms had an own HACCP handbook. Six of the horse dairy farms worked with the HACCP handbook of SPaN-V.

The abbreviation CCP was often unknown. Four of the horse dairy farms knew what their CCPs were.



However, the CCPs were monitored at three horse dairy farms daily, at four horse dairy farms weekly and at two horse dairy farms monthly. Three horse dairy farms didn't monitor the CCPs. The monitoring of the CCPs was registered at seven horse dairy farms. The other horse dairy farms didn't register the monitoring of the CCPs.

Eight of the twelve horse dairy farms (67 percent) got their feed from a GMP certified farm. Two horse dairy farms got the feed from a non-GMP certified company. The bought feed was visually inspected at arrival by nine horse dairy farms and one horse dairy farm didn't control the bought feed at arrival. Two horse dairy farms used feed from their own ground. Roughage that is molding was not fed to the horses at any horse dairy farms. Tap water was used as drinking water by eight horse dairy farms and spring water by four horse dairy farms.

Seven horse dairy farms had protocols for cleaning and disinfection. The processing room was cleaned and disinfected by eight horse dairy farms daily. One horse dairy farm cleaned the processing room after each milking round. Three of them cleaned and disinfected the processing room each week. The milk equipment was cleaned after each milking round by ten horse dairy farms. Two disinfected at the end of the day. The other horse dairy farms cleaned and disinfected the milk equipment at the end of the day.

All the horse dairy farms had rules about clothing during the processing of the milk. These rules varied from washing hands to wearing special shoes. Special clothes were not always worn during milking, or when the milk was processed. At seven of the twelve horse dairy farms (58 percent) hand gloves were worn during the processing of the milk

Six of the twelve horse dairy farms (50 percent) didn't use a refrigerator for the milk; it went directly to the freezer. Monitoring of the temperature of the freezer and refrigerator (when available) was done by all horse dairy farms. The method of control was by display (50 percent) or with a thermometer (50 percent). The temperature of the refrigerator was monitored daily at six horse dairy farms. At four horse dairy farms the temperature of refrigerator was monitored weekly. The temperature of the freezer was monitored daily at nine horse dairy farms. The other three horse dairy farms monitored the temperature of the freezer weekly. The temperature of refrigerator and freezer was registered by ten horse dairy farms. Two horse dairy farms didn't register the temperature.

Eight of the twelve horse dairy farms (67 percent) had a filter for single use only. Two horse dairy farms used the filter more than once. The other six horse dairy farms replaced the filter after each milk round or filtered all the milk at the end of the day. The other horse dairy farms had other types of filters which they clean after use and checked on possible defect after each milk round or daily. The monitoring of the filter has not been registered at the horse dairy farms.

3.1.2. Milking and processing

Five of the twelve horse dairy farms (42 percent) disinfected the udder of the horses; the other horse dairy farms only cleaned the udders. Two horse dairy farms used one towel for all the horses, one of these horse dairy farms washed this towel between the horses in water.

Two horse dairy farms (17 percent) transferred the milk through pipes to the processing room. The other horse dairy farms took the tank from the milking area to the processing room.

Four of the twelve horse dairy farms (33 percent) processed the milk at the end of the day. The other horse dairy farms processed the milk directly after milking.



Milking is done mechanically at all horse dairy farms and the processing of the milk is always done manually.

3.1.3. Traceability of the products

At ten of the twelve horse dairy farms (83 percent) the products were traceable. Product could be traced by date or supplier. At one horse dairy farm, you could even tell which horses were milked that day. The products were not traceable at two horse dairy farms.

3.1.4. Control of the milk

Nine horse dairy farms control the milk. The control frequencies ranged from once a month to once a year. Three horse dairy farms didn't control the milk. One horse dairy farm kept the milk of each round in the freezer, so if there were any problems with the milk, the milk could still be checked.

3.1.5. Veterinarian

Seven of the twelve horse dairy farms (58 percent) let the veterinarian only come when it is necessary. Two horse dairy farms let the veterinarian come once every three months, two horse dairy farms let the veterinarian come once a month and one horse dairy farm let the veterinarian come more than once a month.

3.1.6. Deworming

Two of the twelve horse dairy farms dewormed their horses during the milking period. They dewormed with Strongid P from Pfizer. There isn't determined a waiting for Strongid P in the milk. However another equine wormer indicated that the horses which produce milk for human consumption should not be dewormed with this equine wormer. The active ingredient of these equine wormers is the same. After personal communication with Pfizer the answer of them was: Pyrantel salts are listed in Annex 2 of EU Directive 2377/90 for equidae, which means that the definition of an MRL (Minimal Residual Level) is not required. A very small quantity of Pyrantel salts is absorbed after oral administration (less than 2% is included in the circulation). The active material, which is absorbed, will be rapidly metabolized and excreted. The possible amount of residue in the milk is thus negligible. The other horse dairy farms dewormed a month for the milking period.

3.2. Visual Inspection

3.2.1. Stable hygiene

At four horse dairy farms the stable was very clean. There was no manure on the ground. At two dairy farms the stable had a lot of manure, and at the other horse dairy farms the stable was fairly clean, but there was a little manure on the ground.

3.2.2. Milk stable

The milk stable was, at five of the twelve dairy farms (42 percent), a space in the court yard. The other horse dairy farms (58 percent) had a stable to milk the horses. The area to milk was, at ten horse dairy farms, next to the stable of the horses. Two of these ten horse dairy farms had a milk stable which could be closed off from the stable of the horses. The milk stable looked visually clean at nine horse dairy farms.



3.2.3. Processing room

The processing room looked visually clean at ten dairy farms. At two horse dairy farms the room didn't look clean, but these horse dairy farms said that they clean before processing the milk.

3.3. Temperature of refrigerator and freezer

The temperature of the refrigerator varied from 2 degrees till 10 degrees. One horse dairy farm had the refrigerator set at 10 degrees. The temperature of the freezer varied from -17.7 degrees till -37.5 degrees. The lowest and highest temperatures were both measured at the same horse dairy farm.

3.4. Steri-keri Samples

All objects at one horse dairy farm were assessed as excellent. At three horse dairy farms the objects were assessed as excellent/good and moderate. The objects of three dairy farms were assessed as excellent/good and bad. At two horse dairy farms the objects were assessed as bad and insufficient. The objects of the other horse dairy farms were assessed as good and insufficient or bad and moderate. The results of the objects are shown in table 3.

Horse dairy farm	Sampled surface	Entero-bacteriaceae	Total viable count	Classification
1	Processing bucket	0	25	Moderate
	Worksheet kitchen	0	8	Good
2	Cooling element	0	6	Good
	Tab knob	5	More than 90	Bad
3	Washbowl	0	0	Excellent
	Measuring jug	0	0	Excellent
	Sieve	0	0	Excellent
4	Bucket, for cleaning the milk equipment	0	2	Excellent
	Button for vacuum milk equipment	0	More than 90	Bad
5	Washbowl	0	61	Insufficient
	Milk tank	1	More than 90	Bad
6	Worksheet kitchen	More than 90	More than 90	Bad
	Handle of freezer	0	38	Insufficient
7	Worksheet kitchen	0	23	Insufficient
	Sieve	0	4	Good
8	Bucket	0	20	Moderate
	Measuring jug	0	0	Excellent
10	Freezer	0	More than 90	Bad
	Table	0	19	Moderate
11	Freezer	0	8	Good
	Kitchen cupboard	0	22	Moderate
12	Worksheet	50	More than 90	Bad
	Measuring jug	0	5	Good

Table 3. Growth on the steri-keri plate.



3.5. Milk samples

The criterion for *Enterobacteriaceae* is set at 1.000 cfu/ml; this is 3 log cfu/ml. Figure 1 shows that horse dairy farms one, five, and seven had *Enterobacteriaceae* in their fresh and frozen milk above the 3 log cfu/ml. Horse dairy farm six had too many *Enterobacteriaceae* in the frozen milk. This horse dairy farm didn't have fresh milk, so the *Enterobacteriaceae* of the fresh milk couldn't be investigated. Horse dairy farm eight had too many *Enterobacteriaceae* in the fresh milk only. At horse dairy farm two, three and ten there were no *Enterobacteriaceae* in their milk.

The criterion for total viable count is 500.000 cfu/ml; this is around 5,6 log cfu/ml. All the horse dairy farms were below this criterion. Horse dairy farm six didn't have fresh milk, so the total viable count couldn't be investigated for this dairy farm. The frozen milk of horse dairy farm six had the highest total viable count. The fresh milk of horse dairy farm two had the lowest total viable count.

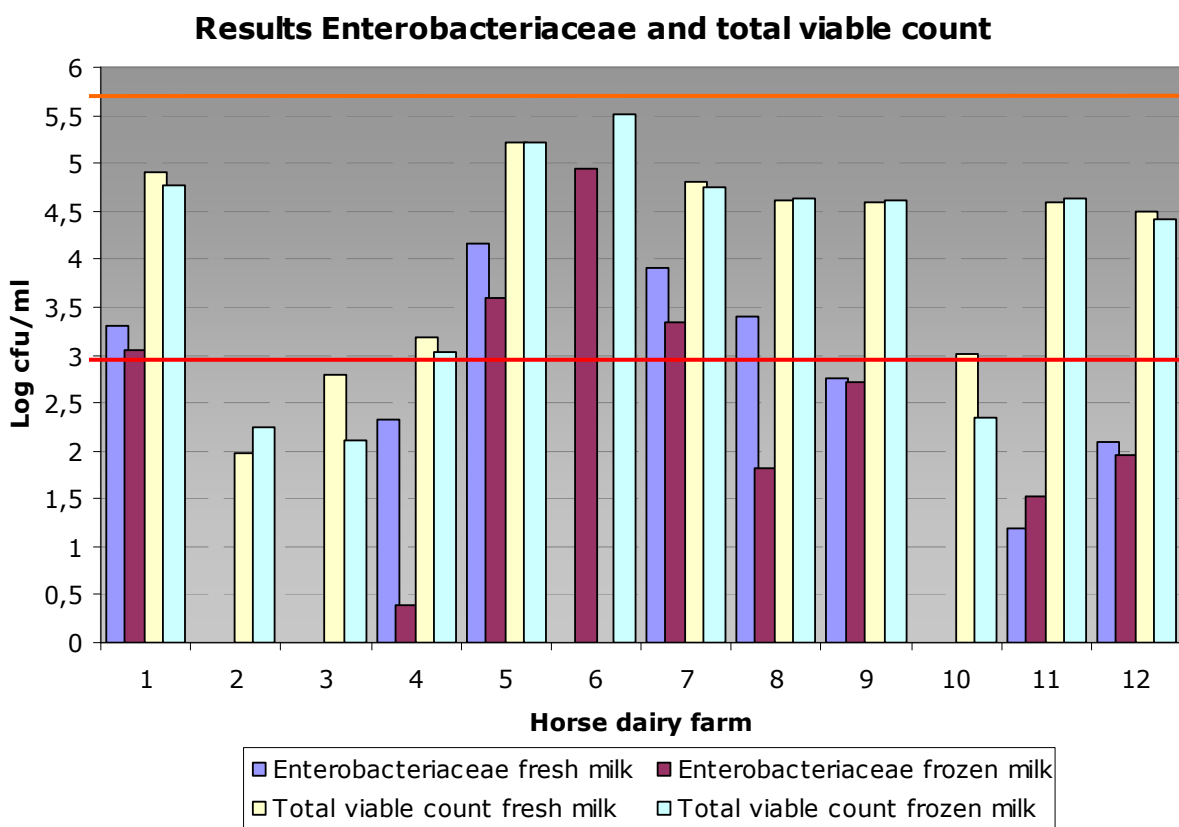


Figure 1. Frequency distributions of total viable count and *Enterobacteriaceae*
 Legend: - - - Criteria of total viable count
 - - - Criteria of *Enterobacteriaceae*

The average of the total viable count for the fresh milk samples is 41.769 cfu/ml, with a minimum of 94,5 cfu/ml and a maximum of 162.350 cfu/ml. For the frozen milk samples there is found an average of 63.395 cfu/ml, with a minimum of 127,5 cfu/ml and a maximum of 326.000 cfu/ml.

Results of the *Enterobacteriaceae* for the fresh milk samples ranged from not detected to 14.700 cfu/ml, with an average of 2.562 cfu/ml. The frozen milk ranged from not detected to 89.750 cfu/ml, with an average of 8.142 cfu/ml.



In addition to the total viable count and *Enterobacteriaceae*, other bacteria were examined. *Staphylococcus aureus* is found in thirteen samples of nine horse dairy farms (N 1-239 cfu/ml). Horse dairy farm twelve had the most *S. aureus* in their frozen milk. The amounts of *S.aureus* were below the criterion. *Salmonella*, *Listeria monocytogenes*, *Campylobacter* and *E. Coli*O157 had not been found in the milk samples. *Bacillus cereus* is found in two samples (N 40-120 cfu/ml). *Bacillus cereus* is only found in the milk of horse dairy farm five. The amount of *Bacillus cereus* stayed below the determined criterion.

3.6. Correlation between *Enterobacteriaceae* and total viable count

To show if there is a correlation between the *Enterobacteriaceae* and total viable count, are they set out together in figure 2.

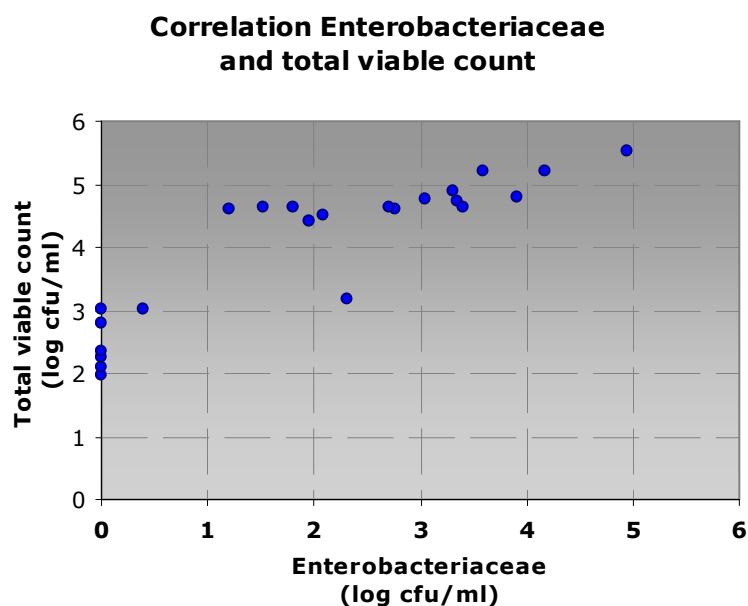


Figure 2. *Enterobacteriaceae* set out against total viable count.

In Figure 2 there is a possible correlation between the *Enterobacteriaceae* and the total viable count. This correlation is calculated. There is a correlation of 0,864 between the *Enterobacteriaceae* and the total viable count. This correlation is significant ($p=0.000$).

3.7. Relation between working method and the microbiological results of the milk

3.7.1. Relation between HACCP and the results

The results of the microbiological outcomes of the milk samples were compared to horse dairy farms which didn't work with HACCP (group 1, $n=6$) and horse dairy farms which did work with HACCP (group 2, $n=16$). There wasn't a significant difference ($p=0,106$ for total viable count and $p=0,672$ for *Enterobacteriaceae*). Horse dairy farms which worked with HACCP had a lower mean of *Enterobacteriaceae*, but the mean of total viable count was higher.

Horse dairy farms which had an own HACCP handbook (group 1, n=6) had a lower total viable count and *Enterobacteriaceae* than horse dairy farms with the HACCP handbook of SPaN-V (group 2, n=10). There was no significant difference for *Enterobacteriaceae* ($p=0,059$), but the *Enterobacteriaceae* at horse dairy farms with an own HACCP handbook was lower than the horse dairy farms with the handbook of SPaN-V. For total viable count the results were significant ($p=0,037$). Horse dairy farms with an own HACCP handbook had a lower total viable count.

3.7.2. Hygienic work method and the results

The groups were divided into those that worked hygienically (group 1, n=7) and those that could work more hygienically (group 2, n=16). This distribution is based on the answers to the questionnaire, additional questions and through a visual inspection. The horse dairy farms which worked hygienically had a lower total viable count ($p=0,000$) and *Enterobacteriaceae* ($p=0,000$) than horse dairy farms which could work more hygienically.

3.7.3. Relation protocols and the results

Comparisons were made to see if there is a difference in microbiological outcomes of the milk samples between the horse dairy farms which didn't work with protocols (group 1, n=10) and horse dairy farms which worked with protocols (group 2, n=12). There was no significant difference between these groups (for total viable count $p=0,169$; for *Enterobacteriaceae* $p=0,171$).

3.7.4. Relation the hygiene of the stable and the results

Group 1 (n=8) had stables which weren't clean or were fairly clean. Group 2 (n=15) are the stables which were very clean. There was no significant difference for *Enterobacteriaceae* ($p=0,211$). For total viable count ($p=0,046$) there is a significant difference. The horse dairy farms with a very clean stable had a lower value of total viable count.

3.7.5. Relation disinfection of the udder and the results

The total viable count and *Enterobacteriaceae* of the milk samples were compared between horse dairy farms which clean the udders for each milking round (group 1, n=14) and those which disinfected the udders for each milking round (group 2, n=8). There is no significant difference (for total viable count $p=0,335$; for *Enterobacteriaceae* $p=0,153$), but the total viable count and *Enterobacteriaceae* were lower at horse dairy farms which disinfected the udder before milking.

3.7.6. Relation hygiene processing room and the results

The results of *Enterobacteriaceae* of the milk samples were compared with the cleanness of the processing room. At group 1 (n=16) there was no *Enterobacteriaceae* in the processing room. At group 2 (n=7) there were *Enterobacteriaceae* in the processing room. There is no significant difference ($p=0,296$), but horse dairy farms where *Enterobacteriaceae* were found in the processing room had a higher *Enterobacteriaceae* value in the milk than horse dairy farms where no *Enterobacteriaceae* was found in the processing room.

The results of total viable count of the milk samples were compared with the total viable count in the processing room. Group 1 (n=12): the steri-keri plates had less than 90 colonies. Group 2 (n=11): the steri-keri plates had more than 90 colonies. There is no significant difference ($p=0,154$), but the processing room with more than 90 colonies had a higher total viable count in the milk.



3.7.7. Relation freezing time and the results

In group 1 (n=7) the milk is directly frozen. In group 2 (n=4) the milk is cooled first and frozen at the end of the day. There is no significant difference between these groups (for total viable count $p=0,599$; for *Enterobacteriaceae* $p=0,521$). The horse dairy farms which cooled the milk first had a higher value of total viable count and *Enterobacteriaceae*.

4. DISCUSSION

4.1. HACCP

Many horse dairy farms said they worked with HACCP, but didn't know what the CCP abbreviation stood for. The CCPs, determined by SPaN-V, were the filter and temperature of refrigerator and freezer. The questionnaire showed that the CCPs were monitored at three horse dairy farms daily, at four horse dairy farms weekly and at two horse dairy farms monthly. Three horse dairy farms said that they didn't monitor the CCPs. In the same questionnaire, a question was asked as to how many times the temperature of the refrigerator and freezer were monitored. The temperature of the refrigerator and freezer was monitored by all horse dairy farms. The temperature of refrigerator and freezer were monitored more often than the CCPs. This showed that there is a lack of understanding what CCP really means. Four horse dairy farms monitored the temperature of the refrigerator (when available) and freezer as much as the CCPs were monitored. The filter is also determined as a CCP, but this CCP is not registered at the horse dairy farms. This revealed that it is not clear what CCPs are.

The temperature of the refrigerator was, at one horse dairy farm, too high. This horse dairy farm had the refrigerator set at 10 degrees. The criterion is 4 degrees, but a temperature of 7 degrees is tolerated.

The temperature of the freezer was different per horse dairy farm. At two horse dairy farms the temperature of the freezer was slightly high. The temperature of the freezer must be at least -18 degrees, but a short period of -15 degrees is allowed. At one horse dairy farm the temperature was risen temporarily. There was some fresh milk in the freezer and the door of the freezer had been open some time. The freezer was trying to cool the temperature back to the set -22 degrees.

4.2. Total viable count and *Enterobacteriaceae*

In Regulation (EC) No. 853/2004 is detained that the criterion of total viable count for raw horse milk must be below the $\leq 500\ 000$ cfu/ml. The average of the horse dairy farms is far below this criterion. None of the dairy farms came around this criterion. The criterion of total viable count for raw cow milk is set at < 100.000 cfu/ml.⁹ When comparing the results of the horse milk with this criteria, there were just two horse dairy farms above it. These horse dairy farms could work more hygienically to get the total viable count down.

When the results of *Enterobacteriaceae* were compared with the results of the research of H. Krol(2008)¹¹ there was a big difference. H. Krol(2008) found *Enterobacteriaceae* in the milk from the tank of horse dairy farm six. In the milk of the tank from the other horse dairy farms, no *Enterobacteriaceae* were found by H. Krol(2008).¹¹ In this research there was a lot of variation in the results of the *Enterobacteriaceae*. At three horse dairy farms there was no *Enterobacteriaceae*. At nine horse dairy farms there were *Enterobacteriaceae* found, of which four horse dairy farms stayed below the determined criteria. With the results of H. Krol (2008)



there were no *Enterobacteriaceae* found at fourteen of the twenty-four investigated horse dairy farms.

4.3. Significant results

By comparing the microbiological results of the raw milk and the working method of the horse dairy farms there weren't many significant results found. The groups were too small to get significant values. When all horse dairy farms are investigated the results should still not be significant, because the group of investigated horse dairy farms is still too small. The results of this research were merged with the results of the research of H Krol¹¹ to investigate whether there were significant results. With the results of both researches there were no significant results.

However there is found a significant lower amount of total viable count ($p=0,037$) by horse dairy farms which worked with their own HACCP handbook. Horse dairy farms with an HACCP handbook had a lower amount of *Enterobacteriaceae*, this difference wasn't significant, but there was a trend ($p=0,059$). These horse dairy farms were more concerned with HACCP and hygiene than the other horse dairy farms. When the horse dairy farms which worked the most hygienically are looked at, it is also clear that these horse dairy farms had a lower total viable count and *Enterobacteriaceae* in the milk.

4.4. Chemical contamination

The horse dairy farms which disinfected the udders before milking had a lower amount of total viable count and *Enterobacteriaceae*. This disinfection of the udder could cause a chemical contamination of the milk. The disinfecting middle which is used can come in the milk during milking. However the chemical contamination isn't investigated in this research.

Like the disinfection of the udders deworming during the milking period can cause chemical contamination. Deworming during the milking period can only be done when the leaflet indicates that this can be applied without problems for human consumption.

4.5. Veterinarian

In regulation (EC) No. 854/2004 stated that: animals in milk production companies must be subject to verify that the health requirements for raw milk production, these controls may take place at the occasion of veterinary health checks carried out pursuant to Community law on public or animal health or animal welfare; and can be done by an authorized veterinarian. The seven horse dairy farms which let the veterinarian only come when it is necessary, do not meet the regulation.

5. CONCLUSION

5.1. HACCP

There is a significant lower total viable count in the milk samples of horse dairy farms which work with their own HACCP handbook. There is also found a significant difference between the stable hygiene of the horse dairy farms and the microbiological results of the milk samples. Horse dairy farms which worked more hygienically than other horse dairy farms, had a lower amount of *Enterobacteriaceae* and total viable count in the milk samples. HACCP is effective when it is executed properly.

From these results could also be concluded that hygiene is very important to produce milk safely.



5.2. Total viable count and Enterobacteriaceae

The total viable count of all the milk samples was far below the criteria of Regulation (EC) No. 853/2004. This criterion could be a lot lower for horse milk.

Five of the twelve horse dairy farms (42 percent) came above the determined criteria for *Enterobacteriaceae*. These horse dairy farms must work more hygienically, to lower this amount of *Enterobacteriaceae*.

5.3. Safety of the milk

Based on the results of the raw milk samples, raw horse milk could be safely consumed by healthy people at seven of twelve investigated horse dairy farms. At these horse dairy farms the total viable count and *Enterobacteriaceae* were below the determined criteria and the other investigated bacteria weren't found in these milk samples. Six of these horse dairy farms worked with HACCP. One of these horse dairy farms didn't work with HACCP, but was working very hygienically.

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Welfare of horses at horse dairy farms

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ABSTRACT

Ever since horses were domesticated, they had to adapt themselves to the circumstances where they were kept. Horses also had to adapt themselves to be milked by humans. When horses can't adapt themselves sufficiently to the circumstances in which they are held, they will show behaviour problems as an expression of stress.

By means of the five freedoms of Brambell an assessment has been made with regards to any possible welfare problems at horse dairy farms. These freedoms were measured by means of a quick scan. When the total score is more than 135 points, no welfare problems are expected for the horses.

At horse dairy farms the mare and foal are separated from an age of approximately six weeks. The separation is built up slowly and not longer than twelve hours. The foal is gradually weaned until at an age of eight months the foal is no longer going back to the mare.

In this research no extreme welfare problems were found. Virtually no horses with behavioural problems have been found. The horses had enough living space to express their normal behaviour.

Keywords: welfare, horse dairy farm, gradually weaning, quickscan

1. INTRODUCTION

Horses are herd animals which used to graze over the meadows without fixed territory. Over the course of time horses were domesticated and had to adapt themselves to the circumstances in which they are held. When the circumstances don't allow the horse to express its natural behaviour, stress might develop. Expressions of stress will result in behavioural problems, such as stable vices. Behavioural problems are an indication of decreased welfare.^{3;7}

What is welfare? The first definition of welfare appeared in 1965 in a report by the Brambell committee, which was set up after the appearance of 'animal machines', to evaluate the bottlenecks in the field of animal welfare measures.⁴ The report of the Brambell Committee is often quoted as the pivotal step forward in recognition of the importance of behaviour in assessing animal welfare.⁵ The report concluded that in intensive farming systems "an animal should at least have sufficient freedom of movement to be able without difficulty, to turn around, groom itself, get up, lie down and stretch its limbs." The Brambell Report also concluded that the welfare of an animal includes its physical and mental state and that any animal kept, must be protected from unnecessary suffering. The committee's criteria of good animal welfare, known as the five freedoms, are still used.

In general it is accepted that the wellbeing of animals is sufficient when the circumstances allow animals to comply with the five freedoms of Brambell. In this research it is assessed, by means of a quickscan based on the five freedoms of Brambell, whether there are welfare problems at the horse dairy farms or if there are situations that could lead to decreased welfare of the horses.



2. MATERIALS AND METHOD

To assess the welfare of the horses at the horse dairy farms the five freedoms have been used.⁴

These are:

- Freedom from hunger, thirst and malnutrition
 - Is fresh water available?
 - What do the horses eat?
 - Condition of the mare and foal?
- Freedom from discomfort
 - Housing?
 - Surface of the stable?
 - Shelters?
- Freedom from pain, injury or disease
 - Vaccination?
 - Veterinarian (once per month, only when necessary)?
- Freedom to express normal behaviour
 - Movement (freedom)?
 - Social contact with each other?
- Freedom from fear and distress
 - Showing of stereotype behaviour?
 - Reduced reproduction (difficult to get pregnant)?
 - Raised sensitivity for sickness?
 - Fear or pain expressions

Information from the horse dairy farms about these freedoms is achieved by a questionnaire and a visual inspection at the horse dairy farms.

On the basis of a quickscan the welfare status in the horse dairy farms is determined. This is done by giving points to above-mentioned criteria, which than were multiplied with the weight of the criteria. At least twenty-seven point per freedom is acceptable. On average a horse dairy farm must score three points for each criteria of freedom. With these achieved points the welfare status at the horse dairy farms can be determined.

The minimum total score of the quickscan is 45 points and the maximum total score is 225 points. A total minimum score of 135 points is needed to be classed as a horse dairy farm with sufficient animal welfare.

The results concerning welfare are reflected with a stacked bar diagram. This diagram shows the amount of points achieved.

3. RESULTS

3.1. Free from hunger, thirst and malnutrition

Nine of the twelve horse dairy farms (75 percent) gave their horses unlimited roughage. The other horse dairy farms gave the horses three times or four times a day roughage.

One horse dairy farm didn't have a pasture. These horses had an outlet on the concrete floor, at another horse dairy farm the pasture was more sand than grass. At ten of the horse dairy farms (83 percent) the horses could go on the pasture for more than ten hours. Overall, the horses were in the pasture at night.

Most of the horses at the horse dairy farms were in good condition, but the horses at four horse dairy farms were overfed. At one horse dairy farm the horses were undernourished, the ribs of the horses were visible.



The points for this freedom varied from 39 till 45 points. Horse dairy farm eight scored at this freedom the fewest points. Horse dairy farms three and seven scored the most points. The other dairy farms were between these values.

3.2. Free from discomfort

Ten of the twelve horse dairy farms (83 percent) had a large course stable, some with outlet on the pasture and some without outlet. The other dairy farms held the horses separated in stables.

The large course stables had more fodder places than there were horses. At two horse dairy farms there were less fodder places, these farms had fodder places for around 90% of the horses.

The hygiene of the stables varied. At one horse dairy farm there was no covering on the floor. At another horse dairy farm there were rubber mats on the stable floor. Most of the dairy farms had a layer of straw at one side of the stable. There were two horse dairy farms with a thick layer of straw in the course stables. Cleanness of the straw differed per horse dairy farm, from a lot of manure to no manure present.

The points for this freedom varied from 27 till 45 points. Horse dairy farm one scored at this freedom the fewest points. The amount of points scored by this horse dairy farm was the minimum points which must be achieved. Horse dairy farm ten scored the maximum achievable points. The other horse dairy farms were between these values.

3.3. Free from pain, injury and disease

Seven of the twelve horse dairy farms (58 percent) only called for the veterinarian when necessary. Horse dairy farms one and eleven called the veterinarian once every three months, horse dairy farms nine and ten called for the vet once a month and horse dairy farm one more than once a month.

Vaccination policies varied at the horse dairy farms. Three of the twelve horse dairy farms did not vaccinate. Four of the twelve horse dairy farms vaccinated the horses for influenza and tetanus. Two horse dairy farms vaccinated the horses for influenza, tetanus and rhino pneumonia. The other three horse dairy farms vaccinated the horses only for influenza.

All the horse dairy farms had less than 5 percent of their horses sick or crippled. Most of the farm owners say that there were no horses sick or crippled.

The points for this freedom varied from 27 till 41 points. Horse dairy farms two, six, seven and eight scored at this freedom the fewest points. The amount of points scored by these horse dairy farms were the minimum points which must be achieved. Horse dairy farms one and nine scored the most point. The other dairy farms were between these values.

3.4. Freedom to express normal behavior

Many causes which can obstruct normal behaviour were already mentioned in the previous freedoms. For example: the surface per horse and/or floor covering of the stable. These examples have influence on the behavior that a horse can express. In general, the horses were calm but alert. They were not scared or in panic. The foals were quiet and curious. The foals were not restless, when the mare came nearby.

The points for this freedom varied from 29 till 45 points. Horse dairy farm four scored the fewest points for this freedom. The horses couldn't forage during the day and could have only social contact with their neighbor. Horse dairy farms two, five, seven and ten scored the most points.



3.5. Freedom from fear and distress

The foals and mares are separated from an age of approximately one month old. The separation is built up gradually, so that the mare can be milked during the day. The mares and foals were separated at nine of the twelve horse dairy farms (75 percent) by a fence. At two of twelve the horse dairy farms the mares and foals could see each other, but couldn't make contact. One horse dairy farm didn't separate the mare and foal.

The separation is at three horse dairy farms for about 12 hours. At five horse dairy farms the separation is between the eight and ten hours and three horse dairy farms separated the mares and foals for less than 8 hours.

During the separation the foal can eat some roughage and when the foal is older also some foal bits. In the evening the foals go back to the mares, which need the latch on the nipples to enhance the production of the milk. The mares are milked during approximately eight months. The foal is finally weaned at the age of eight months.

When it was time to milk, the horses came immediately to the milking stable. While milking the mares were eating, they didn't seem to mind that someone was milking them.

At two horse dairy farms there was one horse with stereotype behavior. The other horse dairy farms had no horses with stereotype behavior.

The points for this freedom varied from 38 till 43 points. Horse dairy farm ten scored at this freedom the fewest points. At this horse dairy farm there was one horse with stereotype behaviour and the foals and mares were separated for twelve hours. Horse dairy farms two, six, nine, eleven and twelve scored the most point.

3.6. Total results of the quickscan

The results from the quickscan are shown below in figure 1. When there are more than 135 points, there should be no welfare problems for the horses. All the horse dairy farms scored above the 135 points. Horse dairy farm ten scored the most points at this quickscan. Dairy farm four had the fewest point at this quickscan. Horse dairy farm ten had 205 points and horse dairy farm four had 169 points.



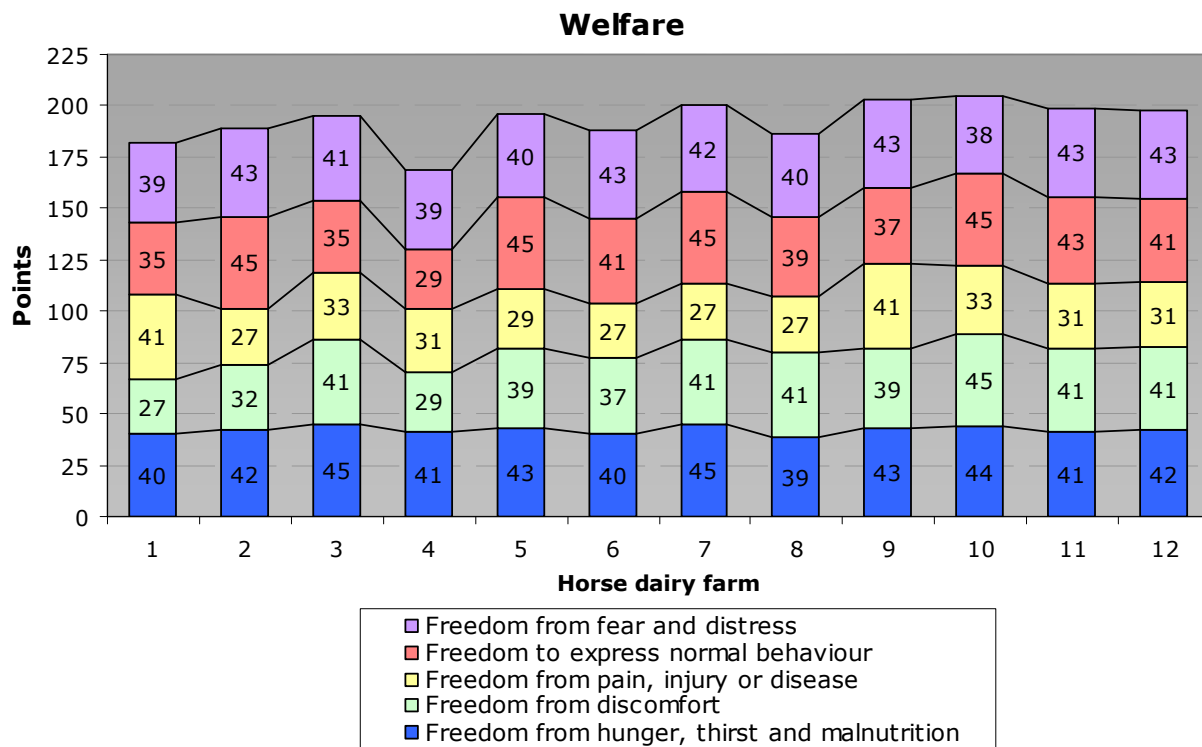


Figure 1. Results of the quickscan about welfare.

4. DISCUSSION

4.1. Free from hunger, thirst and malnutrition

At some horse dairy farms there weren't enough fodder places. When unlimited roughage is available, the horses will eat in groups and not all together. With fodder places for 80 percent of the horses there are enough fodder places to eat (expert opinion). Roughage can also be fed in the stable on the ground. Horses in the wild walk on the meadow while they are searching for food. Horses which are grazing on the pasture take a few bites of grass and walk then to a new area to take another few bites of grass.⁶ If roughage is fed on the ground horses could mimic their natural behavior. The concentrate was fed when the horses were milked.

4.2. Free from discomfort

At horse dairy farm three the horses were kept on the concrete floor, there was no straw for them. However, there was unlimited roughage available. At horse dairy farm four the horses were held on rubber mats. These horses got straw in a corner of the box. At night they were on the pasture. A research of Pederson *et al.* (2004) showed that horses kept on straw bedding lay three times longer than horses kept in shavings. It has been reported that the use of non-straw bedding could increase abnormal behaviour.² If there isn't any straw available, then there must be another soft place where the horses can lay on, for example sand. When there isn't a soft place to lay on, the horses can get decubitus ulcers. Therefore the rubber mats, must be soft enough to prevent decubitus ulcers. If there is only straw in the corner of the stable, as in some course stables, this straw will be used to manure and urinate on instead of lying on the straw. When horses can lay on a soft floor, the second and third freedom of Bambell have not been met.



4.3. Free from pain, injury and disease

In the livestock sector, the veterinarian visits monthly for herd management. What stands out in this research is that there are almost no monthly checks on the horse dairy farms. It might be important that veterinarians visit horse dairy farms monthly in order to resolve any problems, for example mastitis, quickly. To ensure good welfare of the horses, monthly veterinarian control isn't necessary. When an owner is in touch with his horses, he can see the changes of their behavior. Than the periodic veterinarian control of three months should be sufficient. Many diseases can be prevented by a regular, e.g. six monthly, examination of the horse. By doing so, defects, e.g. deficits of nutrients, muscle damage caused by overtraining, liver and kidney disorders, and/or any kind of inflammation are detected at an early stage and further action can be taken.⁹

Vaccination policies varied between the horse dairy farms. Some vaccinated because the horses needed them for competitions. Other horse dairy farms didn't vaccinate because the horses didn't come off the terrain. Horse dairy farms six, seven and eight scored the twenty-seven points for freedom of pain, injury and disease. These horse dairy farms only called for the vet when necessary and didn't vaccinate the horses.

4.4. Freedom from fear and distress

The weaning of the foal begins at a young age. A foal starts eating grass some weeks after birth, but this is not enough to provide in its energy needs. Because of this the foals will get extra food during the separation. From an age of two to three months the degree to which the foal interacts with the mare declines and the time spent with other foals increases. From the age of four months enough energy can be obtained from grass alone. Therefore milk is no longer necessary for energy.^{3;8} Gradually separation from an age of two months, where the foal can still see the mare creates less stress than total weaning at once.^{1;8}

The mares are milked during approximately eight months, this is longer than that the foals at a horse breeding drink milk from the mare. In the wild however a foal will drink for a year from the mare until the mare chases the yearling away to make way for the new-born foal.^{6;7}

5. CONCLUSION

The horses at horse dairy farms have no problems to adapt themselves to the circumstances in which they are kept. The horses didn't seem to mind to be milked; they stood in a row to be milked. The foals were in a good condition. The foals had to get used to the separation, but after some days they weren't calling for their mother anymore. The mares didn't seem to mind to be separated from their foal.

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ATTACHMENT 1

The different types of bacteria in this research

Total viable count: The total viable count from a sample will say how many bacteria that sample contain. It gives an indication concerning the degree of contamination by bad hygiene or storage.

Enterobacteriaceae: *Enterobacteriaceae* are bacteria from the intestine. They give an indication about faecal contamination due to bad hygiene or contamination from the surroundings.

Escherichia ColiO157: *E. coli*O157 exists among horses. *E. coli* may enter in raw horse milk by faecal contamination. The bacterium is not excreted directly in the milk. *E. coli* multiplies between 8°C and 45°C.

Salmonella: *Salmonella* can be excreted by horses. *Salmonella* comes in milk by faecal contamination. It causes one of the most common food poisonings. Between the 5°C and 46°C *Salmonella* multiplies. Until now no cases of *salmonella* contamination have been discovered after drinking raw horse milk.

Campylobacter: *Campylobacter* does not multiply under the 30°C, but can survive under cooling and freezing conditions. This bacterium does not survive freeze drying. It has not been shown that *Campylobacter* is excreted in milk. It may enter the milk from faecal contamination during or after milking. The bacterium causes, just like *Salmonella*, one of the most common food poisonings.

Listeria monocytogenes: *Listeria monocytogenes* is present in the faeces of horses. The bacterium passes directly in milk or through faecal contamination during or after milking. No information about the excretion of *Listeria* in horse milk has been found. *Listeria* multiplies between the -0.4°C and 45°C. This shows that *Listeria* grows at cooling temperature.

Staphylococcus aureus: Horses can be, just like people, bearer of *Staphylococcus aureus*. Bearers can excrete the bacterium and contaminate the environment. *S. aureus* enters the milk through contamination of the environment. If milk is not kept below 7°C or above 48°C the bacterium can develop and will produce toxins. If the bacterium is present in big numbers in the milk (> 100.000 KVE/ml) there is a risk of pathogenic quantities of enterotoxins.

Bacillus cereus: *Bacillus cereus* enters the milk through contamination of the environment. The bacterium can only cause food poisoning when present in large numbers. *B. cereus* multiplies and/or forms tracks between 5°C and 55°C. Concerning the prevention of *B. cereus* in horses no information has been found.



ATTACHMENT 2

Checklist visual inspection

Hygiene

Hygiene of the stables

- How much manure in the stable
- Cleanness of the straw
- Cleanness of the pasture
- How dry is the pasture

Horses

- Cleanness of the horses

Hygiene milk stable

- Is the milk stable dusty or not
- Is the milk stable easy to clean

Hygiene processing room

- Room easy to clean
- Cleaning of the materials at another place than the processing of the milk
- Opportunities to wash your hands
- Work clothes available
- Hygiene of the refrigerator/freezer

Personal hygiene

- Washing hands
- Hair away from the face
- Wearing clean clothes

Welfare

Housing

- What kind of stables
- Living area per horse
- Sufficient fresh air in the stable

Horses

- Condition/feeding situation mares
- Condition/feeding situation foals
- Cripple horses
- Behaviour mares
- Behaviour foals

Feed

- Feed present
- How many fodder places
- Quality feed
- Drinking water available



Milking

- Behaviour mare during milking
- Comes mare voluntarily to the milk stable
- Way of milking (machine/by hand)
- Cleaning/disinfection of the udder
- Pre-milking or not
- Route of the milk (from milking to freeze)
- Temperature refrigerator/freezer



ATTACHMENT 3

Questionnaire about HACCP, hygiene and welfare

The horses

How many horses are present on the horse dairy farm?

- Mares?
- Foals with mare?
- Own stallions?
- Yearlings?
- How many mares give milk?

Have the mares been vaccinated?

If so, against what?

- Influenza
- Tetanus
- Rhino pneumonia
- Different namely.....

When after deworming do the horses give milk?

- Right after deworming
- A day after deworming
- A week after deworming
- A month after deworming
- They are not dewormed while they give milk

How often does a veterinary visit the horse dairy farm?

- Once a week
- Once a month
- Once every two months
- Once every three months
- Only when necessary

Have in the last year any horses been sick?

- No
- Yes, how many?

What is done with the milk of a sick or treated horse?

- Sick horses are milked
- Sick horses are not milked, the milk is for the foals
- Sick horses are milked at the end and the milk is thrown away
- Sick horses are milked at the end and the milk will be used for product, not for raw milk for human consumption.

Are horses with sick foals milked?

- Yes
- No
- Depends on type of illness



Are there horses with stable vices?

If so, how many animals?

- Weaving? How many?
- Sucking air? How many?
- Crib biting? How many?
- Stable turning? How many?
- Different, namely How many

Are there mares on the farm with difficulty in getting pregnant?

- No
- Yes, how many mares?

Stables

How frequently are the stables cleaned?

- Each day
- Weekly
- Monthly
- When necessary
- Only removing of the manure

Are there shelters present on the pasture?

- Yes
- No
- No pasture available

Feed and water

Is feed purchased?

- Yes
- No

Is feed visually inspected on arrival?

- Yes
- No

Is the fodder company GMP certified?

- Yes
- No

How is the fodder (concentrate and roughage) been stored?



What happens with mouldy fodder?

- It is fed to the milk mares
- It is fed to the other horses
- It will not be given to any horse

What gets the foals to eat, when they are separated?

- Foal bits
- Milk
- Grass
- Hay/silage
- Different, namely

How frequently is the drinking water of the horses refreshed?

- Automatic drinking trough
- Daily
- Weekly
- Monthly
- Not, refill only

What kind of water is used for the drinking barges?

- Source water
- Tap water
- Rainwater

From milking to transport

How frequently is the filter inspected?

- After each milking round
- Each day
- Every other day
- Weekly
- Monthly
- Different, namely.....

How frequently is the filter being replaced?

- After each milking round
- Each day
- Every other day
- Weekly
- Monthly
- When necessary
- Different, namely.....

How does the packaging of the milk take place?



How much time is there between the milking and the cooling of the milk?

- Nearly immediately
- 10 minutes
- 15 minutes
- 30 minutes
- An hour
- Different, namely....

At which temperature is the refrigeration set?

..... °C

Is the temperature of the refrigeration checked?

- No
- Yes, how?
How frequently?
 - Daily
 - Weekly
 - Monthly
 - Every 3 months
 - Every half year
 - Every year

How long is milk cooled stored?

- One day
- Two days
- Three days
- Four days
- Five days

How much time is there between the milking and the freezing of the milk?

- Nearly immediately
- 15 minutes
- 30 minutes
- One hour
- More than one hour, namely

At which temperature is the freezer set?

..... °C



Is the temperature of the deep freeze checked?

- No
- Yes, how?
How frequently?
 - Daily
 - Weekly
 - Monthly
 - Every 3 months
 - Every half year
 - Every year

Are the measured temperatures of refrigeration and freezer recorded?

- Yes
- No

What is the shelf life of frozen milk?

- A week
- A month
- 3 months
- 6 months
- One year

Are the products labelled, with date and place?

- Yes
- No

Is tracking of the packing possible?

- Yes, how?
- No

Does sampling of the milk take place, by means of a milk sample?

- Yes, how frequently?
- No

Cleaning and disinfection

How frequently is the space, where milking take place, cleaned and disinfected?

- Each milking round
- Every day
- Every other day
- Every week
- Monthly



How frequently is the milk equipment cleaned and disinfected?

- Each milking round
- Every day
- Every other day
- Every week
- Monthly

Persons

Which measures are taken concerning the personal hygiene in the milk space?

- At entering wash hands
- Wearing gloves
- Hair away from the face (bound in a tail)
- Special clothing
- Special shoes
- No jewels
- Different, namely

Visitors can only enter the premises when accompanied?

- Yes
- No

General control points (GCP) and Critical Control Points (CCP)

Is there an analysis of the possible risks of food safety on your farm?

- Yes, what are these risks?
- No

Does the horse dairy farm work with protocols?

- Yes, which protocols?
- No

Are all employees aware of these protocols?

- Yes
- No
- Not sure

Which CCPs are there at the horse dairy farm?



How frequently are the CCPs checked?

- Weekly
- Monthly
- Every 3 months
- Every half year
- Every year

Are these inspections registered?

- Yes
- No

How is the staff informed about the HACCP handbook?

What do you think of the HACCP handbook of SPaN-V?



ATTACHMENT 4

Quickscan concerning animal welfare

Free from hunger, thirst and malnutrition

How frequently roughage a day

weight 2

- 1) None
- 2) Once a day
- 3) Two times a day
- 4) Three times a day
- 5) Unlimited

Fodder places

weight 1

- 1) <80%
- 2) 80-90%
- 3) 90-100%
- 4) 100-110%
- 5) >110%

Quality of fodder

weight 2

- 1) Is mouldy and rots
- 3) Looks clean, but much weed
- 5) looks clean

Drinking water available

weight 1

- 1) no
- 3) yes, but very dirty
- 5) yes, clean drinking water

Condition/feeding situation

weight 3

- 1) Underweight
- 2) Overweight
- 3) Undernourished
- 4) Exuberant feeding situation
- 5) Good condition

Free from discomfort

Housing

weight 3

- 1) Separate in the stable no meadow
- 2) 24 hours meadow pace (no stable)
- 3) Separate in the stable with meadow pace
- 4) Large course stable no meadow
- 5) Large course stable with meadow pace



Living area

weight 2

- 1) < 4 m²
- 2) 4-6 m²
- 3) 7-9 m²
- 4) 10-12m²
- 5) > 12 m²

Hygiene in the stable

weight 2

- 1) A lively disorder
- 2) no on straw or flax
- 3) A rather clean bed
- 4) A clean bed
- 5) A generous, clean bed

How many hours a day on the pasture

weight 1

- 1) None
- 2) 1-3 hrs
- 3) 3-6 hrs
- 4) 6-10 hrs
- 5) > 10 hrs

Are there shelters on the pasture

weight 1

- 1) no
- 3) no pasture
- 5) yes

Free from pain, wounds and illnesses

Visit frequency veterinary surgeon

weight 2

- 1) When necessary
- 2) 1x per 3 months
- 3) 1x per 2 months
- 4) 1x per month
- 5) More often

Vaccination

weight 2

- 1) Not vaccinated
- 2) Only against influenza
- 3) Against influenza and tetanus
- 4) Against influenza, tetanus and rhinopneumonia
- 5) All vaccinations available



Number of sick animals per year (without crippled)

weight 2

- 1) > 20%
- 2) 15-20%
- 3) 10-15%
- 4) 5-10%
- 5) < 5%

Number of crippled animals per year

weight 1

- 1) > 20%
- 2) 15-20%
- 3) 10-15%
- 4) 5-10%
- 5) < 5%

How rapidly will be called for a veterinarian

weight 2

- 1) Not (sees the mare during regular checks)
- 2) After a couple of days when no improvement
- 3) The next day
- 4) First see if the situation improves, if no improvement the same day
- 5) Immediately

Free to be able to show normal behaviour

Behaviour of the mares

weight 2

- 1) Isolates itself
- 2) Only busy with foal
- 3) No attention for anything
- 4) Quiet, but alert
- 5) Curious and alert

Behaviour of the foals

weight 2

- 1) Shows stereotype behaviour
- 2) Is only screaming
- 3) Tries to get contact with a mother
- 4) Quiet
- 5) Is playing with the other foals

Is forage possible

weight 2

- 1) No
- 3) some
- 5) yes



- Can the mare have social contact with other horses weight 3
- 1) no
 - 3) With one or two horses
 - 5) yes

Free from fear and chronic stress

- Stereotype behaviour* weight 3
- 1) > 10%
 - 2) 5-10%
 - 3) < 5%
 - 4) < 2 %
 - 5) None

- Hours of separation between mare and foal* weight 2
- 1) > 14 hrs
 - 2) 13-14 hrs
 - 3) 11-12 hrs
 - 4) 8-10 hrs
 - 5) < 8 hrs

- Is contact between mare and foal possibly, when they are separated* weight 1
- 1) No contact, they also cannot see each other
 - 3) They are able to see each other
 - 5) Yes, concerning the fence/wire

- Willingness to be milked* weight 3
- 1) Under coercion to the milk stable
 - 2) After touché, mare comes to the milk stable
 - 3) After calling, comes to the milk stable
 - 4) Comes after hesitation
 - 5) Mare comes immediately walks to the milk stable



ATTACHMENT 5

What is HACCP?

HACCP stands for Hazard Analysis Critical Control Points. HACCP is a method for systematic and structured identifying, evaluating and mastering possible hazards, which can influence food safety.

A hazard is a biological, chemical or physical contamination, or situation which can lead to contamination. This contamination can make a product unsafe for consumption.

A hazard analysis is a process of collecting and assessing hazards and conditions, which can lead to the presence of hazards. Food safety hazards must be identified and if necessary must these hazards be monitored.

Critical control points are points, steps or procedures whereby important specific control measures are necessary for the food safety. These control points are necessary to avoid, eliminate or reduce hazards to an acceptable level. For this reason these critical points must be assessed.

In short HACCP means that hazards can be assessed and are monitored by means of inspections. The emphasis lies on the prevention of contamination of the end product.

HACCP-principals have been built from:

- 1) Determine all potential hazards and risks.
- 2) Identify CCP to check the identified hazards.
- 3) Establish critical limits of each CCP.
- 4) Establish how CCPs are monitored.
- 5) Establish the corrective action which must be taken when a CCP exceeds a certain limit.
- 6) Registration and documentation of the HACCP plan
- 7) Verification to examine if the HACCP plan is effective. (verification is a periodic examination to determine whether the CCP is effectively controlled)

Remark point 1

All possible biological, chemical or physical hazards which can be expected (including purchase and storage of raw materials and ingredients), must be determined.

For a HACCP-plan hazard analysis must be carried out in order to recognize any hazards.

The hazard analysis must be pay attention to:

- The potential hazards and the seriousness of it for the public health.
- The presence of hazards must be evaluated.

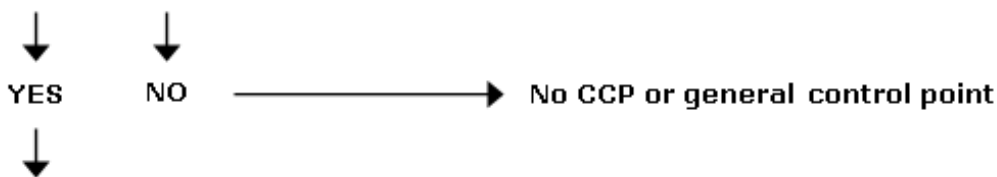


- Surviving or multiplying of pathogenic micro-organisms and the emergence of unacceptable chemical substance in the products or in the environment must be established.

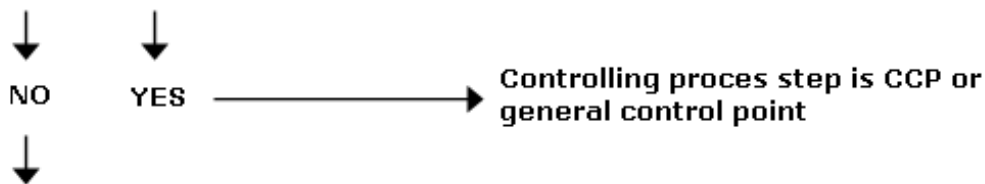
Remarks point 2

After identifying the hazards control points must be taken. Control points include steps and activities to prevent, to eliminate or to reduce hazards to an acceptable level. Control points can be classified in specific or general control points. Determining a critical control point (=specific control point) or general control point to control a hazard is possible by means of a decision tree.

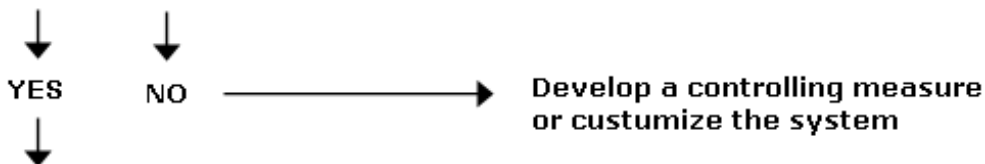
Is controlling of this section necessary for food safety?
(chance x impact = risk high)



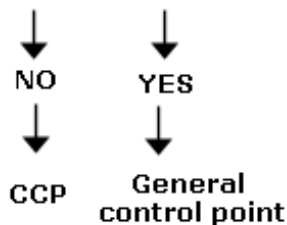
Is there a next process step that the hazard eliminated, controlled or reduced to an acceptable level?



Are there effective controlling measures?



Is the hazard dominated by one or more general control points? (no specific control points)



Decision tree to determine if a control point is specific or general
Source: HACCP handbook SPaN-V²



Remarks point 3

The critical limits correspond to the acceptable extremes of product safety. They are expressed in observable or measurable parameters.

Limits of critical control points can be established on the basis of:

- International regulations, which contain acceptable standards. (E.g. Codex Alimentarius)
- National regulation, which contain acceptable standards.
- Experience.

Remarks point 4

For the monitoring of CCPs continuous or periodic observations or measurements can be used. The method, the frequency of the observations or measurements and the registration procedure should be established. In short, each CCP must indicate by whom, when and how the control and supervision are carried out.

Remarks point 5

When a critical control point limit is reached a description of the correcting action or measures carried out must be made. The products which have exceeded the limit should be discarded. A written record of the actions taken, such as: date, time, type of measure, involved person and following inspection must be established. The correcting measures after exceeding the determined marginal limit may include destruction of the product/party or adjusting or repairing of the process.

Remarks point 6

HACCP documentation contains the determined CCPs and general control points, the HACCP-based procedures and records of measurements and analysis. When a critical marginal limit is exceeded, the correcting measure, which has been taken, must also be mentioned. The registration of data is important for the competent authorities to determine whether a food business is well functioned concerning food safety procedures. Registration is required to determine that it meets the requirements of the HACCP-based food safety system.

Remarks point 7

Verification is the application of procedures and tests afterwards to determine that production took place with the set conditions and the HACCP system is functioning as intended. Verification must be repeated at least once a year so that any changes and/or renewals of the process can be established.

In addition to verification, there is also validation. Validation is determining in advance that the critical control points and the general control points of the HACCP plan are effective and show that these control points are effective.



ATTACHMENT 6

Determined HACCP measures from the HACCP handbook for horse dairies (commissioned by SPaN-V)

General control points (GCP)

GCP 1: Stable hygiene

Hazards: microbiological contamination of the milk

Method monitoring: daily check visually and clean if necessary

Critical limits: good stable hygiene: visually clean with dry places to lie, enough fresh air and light.

Action at deviation: at cleaning: infections which are transmissible from mare to mare: disinfecting

GCP 2: Fodder and drinking water

Hazards:

- A) chemical contamination
- B) by microbiological outgrowth fungi in silage
- C) microbiological storage of food

Method monitoring:

- A) arrangements with the supplier
- B) follow instructions, use appropriate materials
- C) store dry and clean

Critical limits:

- A) GMP requirements
- B) ensiling and use as such that silage of air remains concluded
- C) clean and dry, according to manufacturer's advice

Action at deviation:

- A) returning fodder
- B) fodder get another destination, not for the milk mares
- C) fodder get another destination, not for the milk mares

GCP 3: Cleaning and disinfection

Hazards: physical and microbiological contamination

Method monitoring: visual inspection

Critical limits: visually sufficiently cleaned and disinfected

Action at deviation: re-cleaning and/or disinfecting of the component/device, removal of contaminated milk

GCP 4: Environmental hygiene

Hazards: microbiological and physical contamination

Method monitoring: visually clean, work according cleaning and disinfection procedures

Critical limits: visually good environment hygiene

Action at deviation: re-cleaning and/or disinfection of the processing room, milk tank and refrigerator



GCP 5: Personal Hygiene

Hazards: physical and microbiological contamination

Method monitoring: continually aware of it

Critical limits: good personal hygiene

Action at deviation: cleaning and/or disinfecting the hands and footwear

Critical control point (CCP)

CCP 1: Temperature refrigeration

Hazards: microbial growth in the milk

Method monitoring: temperature registration

Critical limits: max 7 degrees

Action at deviation: removal of the milk if above the 7 degrees

CCP 2: Temperature freezer

Hazards: microbial growth in the milk

Method monitoring: freezer checked by an expert, temperature registration

Critical limits: maximum -15 degrees during for a short time

Action at deviations: removal of the milk

CCP 3: The filter

Hazards: physical contamination

Method monitoring: after every milk round check the filter for defects

Critical limits: no foreign particles, intact filter

Action at deviation: filter once again

