◇ CAUSES OF DEATH RELATING TO WELFARE ISSUES IN 2 MONTHS TO 3 YEARS OLD PET GUINEA PIGS *(CAVIA APEREA F. PORCELLUS)* IN THE NETHERLANDS ◇

Research project of the Master Companion Animal Medicine
M.A. van Vugt, 3260208
Supervisor: Dr. J. IJzer
Faculty of Veterinary Medicine, Department Pathobiology
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SUMMARY

Because guinea pigs, rabbits, ferrets and rats in the Netherlands are often kept as a pet and because these animals often decease at relatively young age without knowing the cause of death, The Veterinary Pathology Diagnostic Centre of the Faculty of Veterinary Medicine at Utrecht University started a research about causes of early death of guinea pigs, rabbits, ferrets and rats. This report is a summary of the results of 30 guinea pigs submitted to this welfare project from October 1st, 2009 until February 17th, 2011. The aim of this report is to investigate the most common causes of death of guinea pigs. Further on, these causes of death are compared with those of 133 guinea pigs of a retrospective study and with data found in literature. Besides that, there is checked whether or not there is a relationship between death causes and welfare.

The most common cause of death of guinea pigs in literature, retrospective study and this welfare project, is lung problems. Heart en kidney problems are also frequently found in the welfare project-guinea pigs, but are not often found in literature and retrospective study. Besides that, it is remarkable that possibly in accordance with nutrient imbalance in 23.3% of the guinea pigs of this project metastatic calcifications were found. Except suspicions about nutrient imbalance, living space, and stress indicators (such as changes in temperature, humidity or diet, overcrowding, type of housing and intercurrent diseases) that increase the susceptibility to disease, there are no direct welfare related problems found.

Following these results, recommendations will be given, designated for owners, to prevent stress and the most common diseases of young guinea pigs through a checklist.

1. INTRODUCTION

In the Netherlands small mammals (guinea pigs, rabbits, ferrets and rats) are often kept as a pet. Unfortunately, many animals often decease at relatively young age. For example; many books and articles give a mean lifespan of 3 to 8 years for guinea pigs (Quesenberry, Carpenter 2004, Bishop 2002, Harkness, Harkness 2010, Anderson 1987, Laber-Laird, Swindle & Flecknell 1996). However, in a home environment they rarely reach an age of more then 5 years (Harkness, Harkness 2010). Also, Caneel et al. showed in their research that 50% of the guinea pigs of owners in the Netherlands die before they even get 4 years old (Caneel et al. 2000)). The causes are usually unknown, because a complete post mortem examination is hardly ever done.
In the Netherlands, there is an increased interest in welfare of companion animals. It is possible that small mammals decease because of impaired welfare and there is insufficient knowledge of health- and welfare problems by pet owners (Raad voor Dieraangelegenheden 2006). Therefore, at October 1st 2009, the Veterinary Pathology Diagnostic Centre (VPDC) of The Faculty of Veterinary Medicine at Utrecht University started a research about the causes of early death of guinea pigs, rabbits, ferrets and rats (Welfare research of small mammals) commissioned by the Ministry of Economic business, Agriculture and Innovation Agriculture (Former: Ministry of Agriculture, Nature and Food quality) (Universiteit Utrecht, Wageningen Universiteit en Research Centrum December 2007). This report is a summary of the results of 30 guinea pigs submitted to this welfare project from October 1st, 2009 until February 17th, 2011.

The aim of this report about the results of 30 guinea pigs submitted to the welfare project, is to investigate the most common lethal ailments of guinea pigs between 2 months and 3 years old (through evaluation of the necropsy reports). Besides that, there is investigated whether differences exist between the causes of death in the retrospective study and the current report, as well as between literature and the current report. Further on, there is checked whether or not there is a relation between death causes and welfare. Following these results, recommendations will be given, designated for owners, to prevent the most common diseases of young guinea pigs.

To compare and discuss the results of this report with causes of death found in literature and with welfare issues, common diseases and welfare issues found in literature are described below.

Common diseases
The most common diseases that cause death in domestic Guinea pigs (*Cavia aperea f. porcellus)* found in literature are: Dental malocclusion, Urinary Calculi, Ovarian cysts, Pneumonia and Hypovitaminosis C (Mitchell, Tully 2009).

The incisors and cheek teeth of guinea pigs are open-rooted and grow continuously (called full elodont), which is why Guinea pigs are more susceptible for malocclusion. Genetics, trauma and poor nutrition can lead to insufficient wear, pain, weight loss and anorexia (Legendre 2002, Jenkins 2010).

Urilithiasis is a common health problem in guinea pigs (Hawkins et al. 2009). The underlying causes are not completely understood, but they are possibly related to a high calcium diet and/or genetic predisposition (Pizzi 2009).

Ovarian cysts are very common in sows of 2-4 years old. They develop spontaneously and increase in size as the animal ages (Quesenberry, Carpenter 2004).

Probably the most common disease in guinea pigs is pneumonia. The animals are mostly affected by the agents *Bordetella bronchiseptica* and *Streptococcus pneumoniae* (Quesenberry, Carpenter 2004) .

Guinea pigs are completely dependent on ascorbic acid (Vitamin C) in their food or drinks, because they lack the enzyme L-gulonolactone-oxidase, which is essential for the conversion of glucose to ascorbic acid. Ascorbic acid is necessary for crosslinking lysine and proline in collagen, which is important for strong bones, joints, blood vessels and muscles. Therefore, a lack of ascorbic acid can cause many problems (Jenkins 2010).

Welfare

Wild guinea pigs are group-living animals. In the wild, guinea pigs live in groups of 5 to 10 individuals (mostly 1 man and many sows with their unweaned offspring). Pairs, harems, female groups of 2 or more females, male groups of 2 males, small mixed sex groups or large mixed sex groups are recommended housing conditions, because social housing systems reduce stress reactions (Kaliste 2004). Especially in crowed conditions or in the presence of a female in estrus, unfamiliar males placed together can start a fight (Harkness, Harkness 2010). To prevent fighting, males kept in groups should have been raised together since weaning (Laber-Laird, Swindle & Flecknell 1996). In many households the animals are kept alone, which can be an indicator of welfare infestation. The effect of environmental changes on guinea pigs kept together with congeners is minimal or nonexistent (Donnelly, Brown 2004).

Guinea pigs are shy animals. In the wild, they inhabit burrows of other animals. Therefore, in captive a place to hide is recommended (hay or objectives in their cage) (Donnelly, Brown 2004).

Guinea pigs are susceptible for environmental changes. Stress responses have an immunosuppressive effect and contribute to susceptibility of diseases. Therefore, it is important to habituate the animal slowly to new conditions (Kaliste 2004) and protect the animal from overcrowding, improper temperature and humidity (Laber-Laird, Swindle & Flecknell 1996).

According to the European convention Appendix A, the minimum floor area per adult guinea pig bigger than 700 grams is 900 cm^2. The minimum enclosure size is 2500 cm^2 (Council of Europe 2006). These minimum measurements are helpful in preventing overcrowding and allowing the animals to practise their natural behaviour.

The recommended temperature to keep guinea pigs is 21˚C (range 18-26 ˚C). Low temperature extremes and high humidity (because of wet bedding) predispose respiratory disease. High temperatures (27 ˚C and higher) and housing in direct sunlight can cause heat stress and sterility (Harkness, Harkness 2010, Laber-Laird, Swindle & Flecknell 1996). Guinea pigs can tolerate cool temperatures better than high temperatures (Quesenberry, Carpenter 2004). Therefore guinea pigs can be kept outside, but then it is recommended to give the animal covering (hay or a shelter for example). It is also important to acclimatize them gradually (Laber-Laird, Swindle & Flecknell 1996).

As mentioned above, guinea pigs are dependent on Ascorbic acid in food or drinks. Vitamin C is water soluble and is not stored in the body. So the food should contain ascorbic acid. The activity of Ascorbic acid in water decreases for 50% during 24 hours and faster than the room temperature increases. Pellets with added Ascorbic acid must be stored cool and dry and must be eaten 90-180 days after production (Harkness, Harkness 2010). For sufficient Vitamin C intake, guinea pigs should eat freshly milled, properly stored, complete commercial pellets. This food can be

replenished with hay, vegetables or supplements (Laber-Laird, Swindle & Flecknell 1996).

It is also important to give the animal food pellets, carrots or wooden branches regularly to ensure wearing of their teeth and to prevent overgrowth of their teeth (Kaliste 2004).

Besides that, guinea pigs are fastidious animals. They only eat things which they get in an early stage of their life (Harkness, Harkness 2010). This is something to take into account.

Guinea pigs should have permanent access to fresh drinking water. It is not recommended to provide the water in a bowl, pan or crock, because guinea pigs can contaminate the water with faeces or urine; a bottle with nozzle is better. To mix food and water, the animals put their whole mouths around the end of the sipper. Sometimes the food slurry goes back into the nozzle and blocks it. This can lead to dehydration. Tubes with ball bearings in the tip help to reduce this problem, but water bottles may need to be rinsed and refilled one or more times daily and should be cleaned at least 2-3 times a week (Laber-Laird, Swindle & Flecknell 1996).

Newspapers, shredded paper, wood shavings and straw are used as bedding materials. Testerink and Klaver pretend that abietic acid found in wood shavings possibly causes liver injuries and respiratory problems. Abietic acid can damage the epithelium of the trachea and bronchi, and because of that viruses and bacteria can cause infection more easily. The toxic acid also enters the bloodstream and can cause liver damage (Klaver 2005, Testerink-Baas 2010). The research design is not really strong, therefore it is not clear to what extend wood shavings really cause organ problems.
Dust of wood shavings can also cause respiratory problems, because of bacteria from urine, faeces, hairs and feed release via dust particles (Wirth 1983), (Kaliste et al. 2004). To what extent guinea pigs absorb the dust, and in which amount this can cause health problems, is not clear.

The development of ammonia, carbon oxide and growth of harmful bacterial toxins decreases if the bedding material can absorb moisture from urine and faeces. Ammonia is immunosuppressive and can cause respiratory and eye problems (Laber-Laird, Swindle & Flecknell 1996, Burn, Mason 2005). Ammonia can develop when there is a high density of animals, when cages are not cleaned frequently enough, or when ambient temperatures and/or humidity are favourable for bacterial growth (Burn, Mason 2005).

2. MATERIAL AND METHODS

There are two research groups. The first group contains 30 guinea pigs from 2 to 36 months old submitted to the present welfare project from October 1st, 2009 until February 17th, 2011.
The second group contains 133 guinea pigs of a retrospective study that have been previously submitted for necropsy to the Faculty of Veterinary Medicine, Department of Pathobiology at Utrecht University from 1993 until October 2009.

2.1 Welfare project

From October 2009 until October 2012, owners of young, deceased guinea pigs are allowed to bring the animal to the faculty where the animal is examined to participate in this particular research. There are a few demands: the contributed animals must be between 2 and 36 months old, and the animals must be brought to the faculty 24 hours (at maximum) after they died. At that time, the animals must be stored at 4 degree Celsius.
Each deceased animal becomes a complete post mortem examination, including macroscopic and microscopic research for free. At first, the animal is inspected on the outside, and after that it is inspected on the inside (from organ to organ). All the organs are examined macroscopically and many organs are collected in formalin for microscopic histology (see for the necropsy protocol appendix I). A few organs are frozen for additional examinations (for example: bacterial analysis, electron microscopy or PCR). The liver, lung, spleen and colon become a cytological evaluation. If needed, a bacterial culture can be cultivated. All the findings are recorded at the general necropsy protocol (appendix II). Furthermore, the owner is obligated to provide a completely filled anamnesis form with information about the group size, husbandry, feeding conditions and clinical record (Appendix III). This form is important to detect possible relations between diseases and welfare.
The necropsy reports, that contain clinical data, an anamnesis, macroscopic and microscopic findings, cytology of the lungs, liver, spleen and hindgut and a definitive conclusion, are filled into Glims database. After this, all the files are imported into the Filemaker Pro database, which was especially made for the welfare research project (Baijens 2008). The data from this database are then exported to Excel, to be analyzed.
The welfare project group contains 30 welfare guinea pigs collected from October 1st, 2009 until February 17th, 2011.

2.2 Retrospective study

The retrospective study contains data from the report of Freek Jurg 'Vroegtijdige sterfte onder cavia's' (Jurg 2010), who analyzed data from guinea pig post-mortem examinations from the archive of the Faculty of Veterinary Medicine, Department of Pathology from 1993 until October 2009. In that period, 814 guinea pig necropsies were done according to standard protocol (appendix II) and reported in Micros database (in October 2008 this database was replaced by Glims database). As in the welfare research, each necropsy report contains clinical data, macroscopic and microscopic findings, cytology of lungs, liver, spleen and hindgut and a definitive conclusion. Freek Jurg selected for his retrospective study guinea pigs which were 6-36 months old. Laboratory animals and necropsies of one organ and biopsies were excluded. With that, 114 relevant reports were left.
In November 2010, the age limit of the welfare project was reduced from 6 to 2 months old guinea pigs. Therefore, in this report the archives of 2-6 months old guinea pigs are added to the retrospective study group (19 animals). In total, 133 animals were selected for the retrospective study group from 1993 until October 1st, 2009.
All the files were imported into the Filemaker Pro database. The data from this database will then be exported to Excel to be analyzed.

3. RESULTS

This chapter gives an overview of the results from two groups of guinea pigs. First, the results of 30 guinea pigs of the welfare project are discussed. Secondly, the results of 133 guinea pigs of the retrospective study are shown.

3.1 Results welfare research

This paragraph provides an overview of the results of the guinea pigs of the welfare research of October 1st, 2009 until February 17th, 2011. The group contained 30 guinea pigs with an age of 2-36 months. First, the causes of death were classified according to organ system. There is a distribution of the etiology (infectious, non-infectious and unknown), as well. Furthermore, remarkable findings are reproduced. Besides that, there is a separation between euthanized or non-euthanized animals.

3.1.1 Causes of death classified to organ system

Figure 1 provides an overview of the causes of death classified according to organ (system). The organs which are most involved in causing death are lungs, heart, kidneys, liver and teeth. Occasionally there are two main organ systems involved in which case no single organ system is the cause of death. Therefore, in total there are more causes of death than guinea pigs in the group.

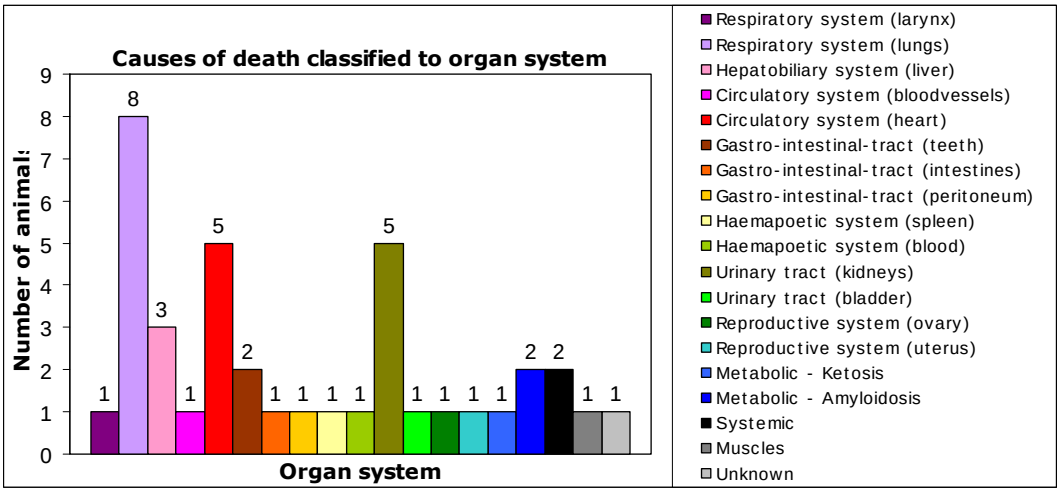

Figure 1 – Causes of death classified to organ system

The seven most involved organs that caused death are discussed below.

For 27.7% (N=8) of the guinea pigs the lungs were affected (pneumonia) and caused death. 4 of 8 animals had an infectious bronchopneumonia. Three of them were of bacterial basis (*Bordetella bronchiceptica*, grampositive coccidia and unknown) and one of them was an unknown virus. No indications for infectious agents were found in the remaining four affected animals; the etiology was unknown. In 4 of these 8 guinea pigs, a second cause of death was identified. This concerned chronic interstitial nephritis, subacute transmural myocarditis, myocarditis and metabolic amyloïdosis.

For 16.7% (N=5) of the guinea pigs, the heart was the affected organ of death. One animal had a ventricle septum defect, the other animals had another cause of death together with the heart diseases. One animal had an acute myocarditis and a decompensatio cordis without signs of infectious agents together with an acute peritonitis around the gallbladder. The next animal was died to an endocarditis and myocarditis combined with fibrosis of the kidneys. Another animal had died on an extended subacute transmural myocarditis and a subacute bronchopneumonia.

Besides that, one animal died from a myocarditis and an interstitial pneumonia caused by a virus.

In 16.7% (N=5) a problem of the kidneys caused death (two times a chronic interstitial nefritis, and one time of kidney fibrosis, glomerulosclerosis or nefritis).
In none of the ailments, an etiology was detected. 4 of these 5 animals died of a combined illness. Except for a kidney disease, these animals had a metabolic amyloïdosis, an endo- and myocarditis, a pneumonia or liver fibrosis.

10% (N=3) of the guinea pigs died on a liver disease. There was no etiology detected in all of these animals. One animal died of portoportal bridging fibrosis, one of liver fibrosis combined with glomerulosclerosis and one of a chronic hepatitis together with an extended atherosclerosis of the lungs, liver, uterus and heart.

6.7% (N=2) of the animals died on dental problems. One guinea pig had an osseous widening of the mandible with deviate position of the pre-molaries. The other animal had no specific dental problem.

In 6.7% (n=2) of the guinea pigs a metabolic amyloïdosis was found as cause of death. In both animals, a second problem caused death (pneumonia and nephritis). The amyloïd depositions are found in the kidneys, spleen, pancreas, thymus, liver and parts of the intestinal tract.

In 6.7% (N=2) of the guinea pigs a systemic disease had led to death. In one animal they found transsudate/blood in the thorax, pericard and abdomen, possibly caused by trauma. This animal also had a minor myocarditis and a chronic pneumonia. There was no etiologic agent detected and there were no indications of infectious agents found. The other animal had particular ulcerative laesions on the back, which caused ischemic liver necrosis and a disastrous sepsis. According to the anamnesis, the skin laesions were caused by the together living rabbit.

3.1.2 Etiology of death

Figure 2 shows the etiology of death classified to infectious, non infectious and unknown. In total there were 45 causes of death in 30 animals.

- Infectious: 15.6% (N=7)
- Non-infectious: 20% (N=9)
- Unknown: 64.4% (N=29)

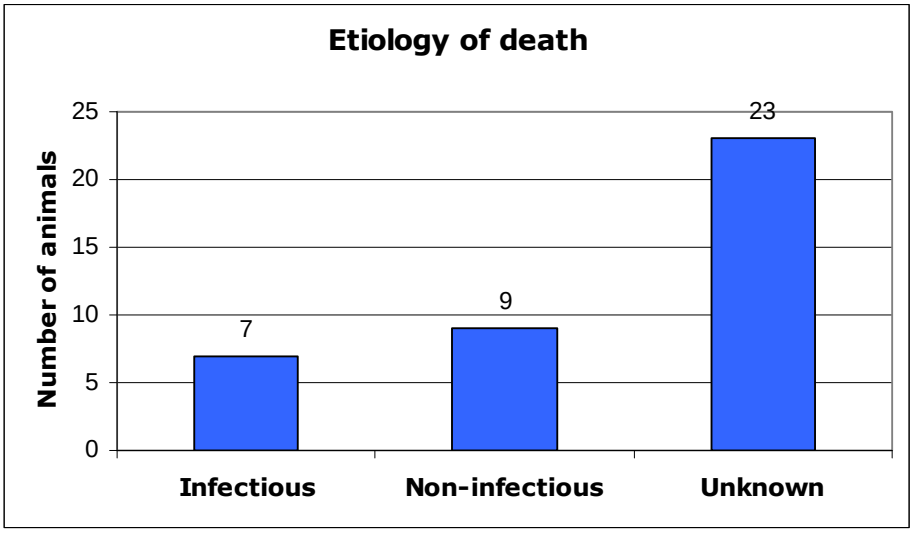

Figure 2 – Etiology of death

In certain post-mortem examinations indications of infectious agents as pathogen of the affected organ are found. Incidental the pathogens are cultivated. *Bordetella bronchiseptica* (lungs), *Klebsiella spp.* (trachea), anaerobic gram positive cocci (lungs), unknown virus (heart, lungs) are found one time. One animal had sepsis due to trauma.

The found non-infectious causes are: trauma, tumour, ketosis (gestation toxaemia), torsio uteri, congenital (ventricle septum defect), rupture, cachexia and atherosclerosis.

The etiology of many affected organs was not known. These are defined as unknown.

3.1.3 Remarkable findings

After reading the post mortem examinations some findings drew attention. In 40% (N=12) of the guinea pigs, calcium depositions in different organs were present. Besides that, in 10% (N=3) of the animals amyloidosis was found.

3.1.3.1 Calcium depositions

In 13 of 30 guinea pigs calcium depositions were found in different organs (43.3%). In 13.3% this was dystrophic (found in heart, lungs, colon and liver). In 6.7% of the cases, it was metastatic and as a consequence of a kidney problem. In 23.3% it was metastatic as well, but possible in accordance with nutrient imbalance. Calcium depositions were found in muscles, gall bladder, kidneys, colon, lungs and heart.

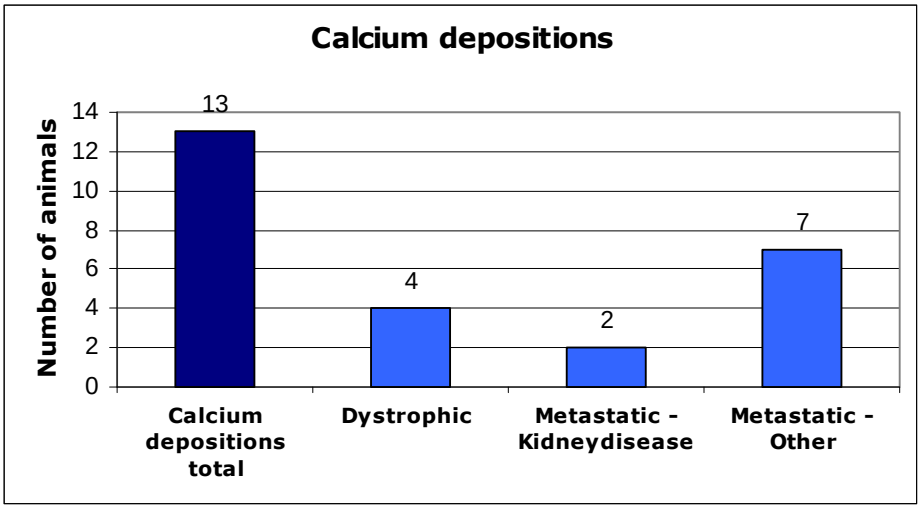

Figure 3 – Calcium depositions

3.1.3.2 Amyloidosis

In 3 of 30 animals amyloïd depositions were found (10%). Two of these animals are discussed before (3.1.1), because the amyloïd depositions were one of the causes of death. The third guinea pig has a spleen amyloïdosis as secondary ailment.

3.1.3.3 Other findings

Because in literature it is found that ovarian cysts are common in guinea pigs and Freek Jurg paid attention to liver fatness, these two ailments are mentioned here. In this welfare project an ovarian cyst is found in one guinea pig (3.3%, N=1). In three animals, there was a fatty liver as secondary finding (10%, N=3).

3.1.4 Types of death

Figure 4 shows that 70% (N=21) of the animals died a natural death and that 30% (N=9) of the animals was euthanized.

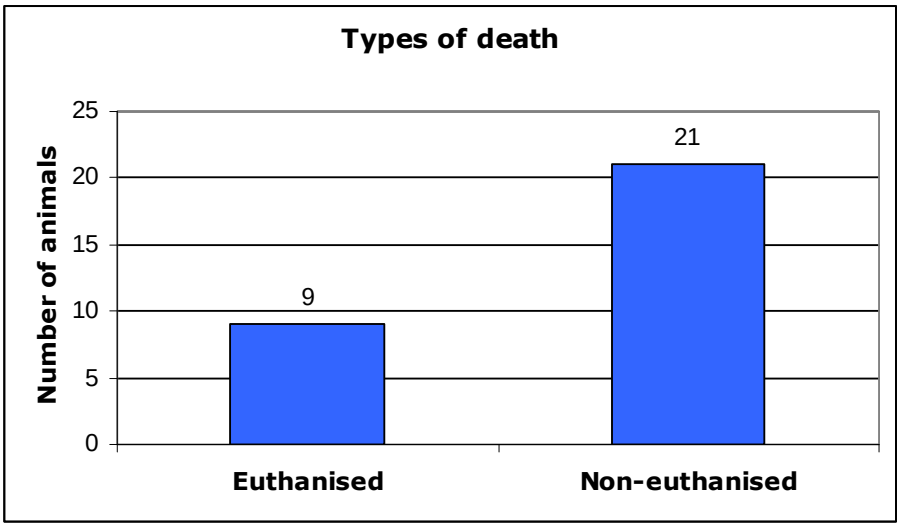

Figure 4 – Types of death

3.2 Results retrospective study

In this paragraph, the necropsy results of the retrospective study of Freek Jurg (guinea pigs of 6-36 months old from 1993-2009) replenished with the results of the 2-6 months old guinea pigs in the same period are analyzed, which include 133 guinea pigs in total. The same classification as Freek Jurg is used.

3.2.1 Found diseases

Figure 6 shows frequently found diseases and literary interesting problems. The found diseases could have been causes of death, but could also have been secondary ailments.

In the welfare project, 27.7% of the animals are deceased on a lung problem. In the diagram of Freek Jurg's study, lung problems are not listed separately but fall among the remaining group. Therefore, it was investigated how many of the animals had lung problems. 30.1% of the animals (N=40) happened to have a lung problem.

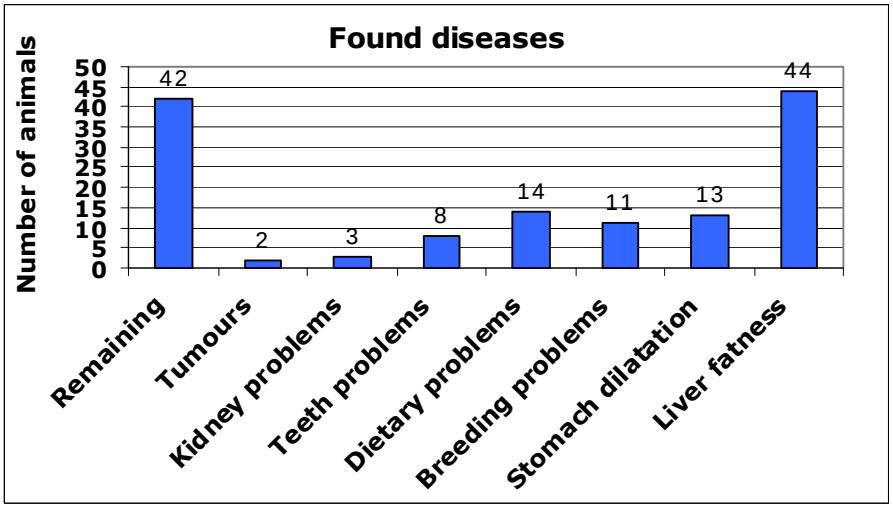

Figure 6 – Found diseases (retrospective)

3.2.2 Etiology of death

Figure 7 shows the etiology of death classified to infectious, non-infectious and unknown. This results in:

- Infectious: 31.6% (N=43)
- Non-infectious: 29.3% (N=38)
- Unknown: 39.1% (N=52)

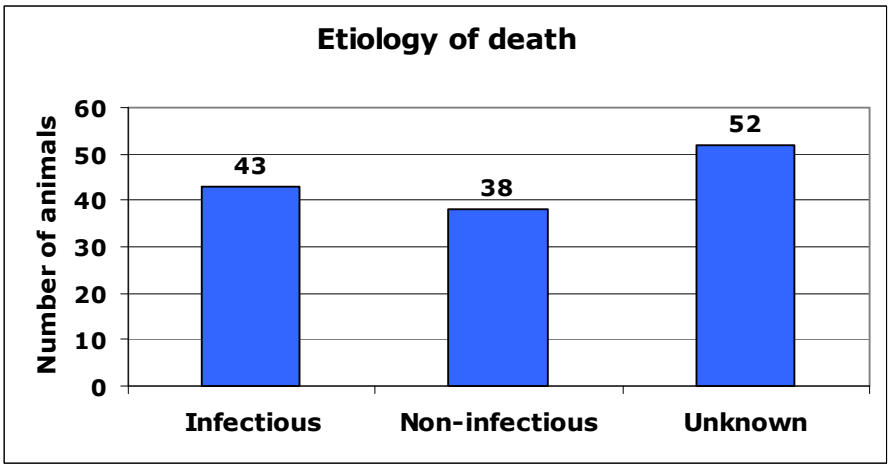

Figure 7 – Etiology of death (retrospective)

All found infectious agents that caused death are shown in figure 8 (N=43).
The four most found agents are:

- Not specified bacteria: 37.2% (N=16)
- Yersinia: 14% (N=6)
- Pasteurella: 9.3% (N=4)
- Streptococcen: 7% (N=3)

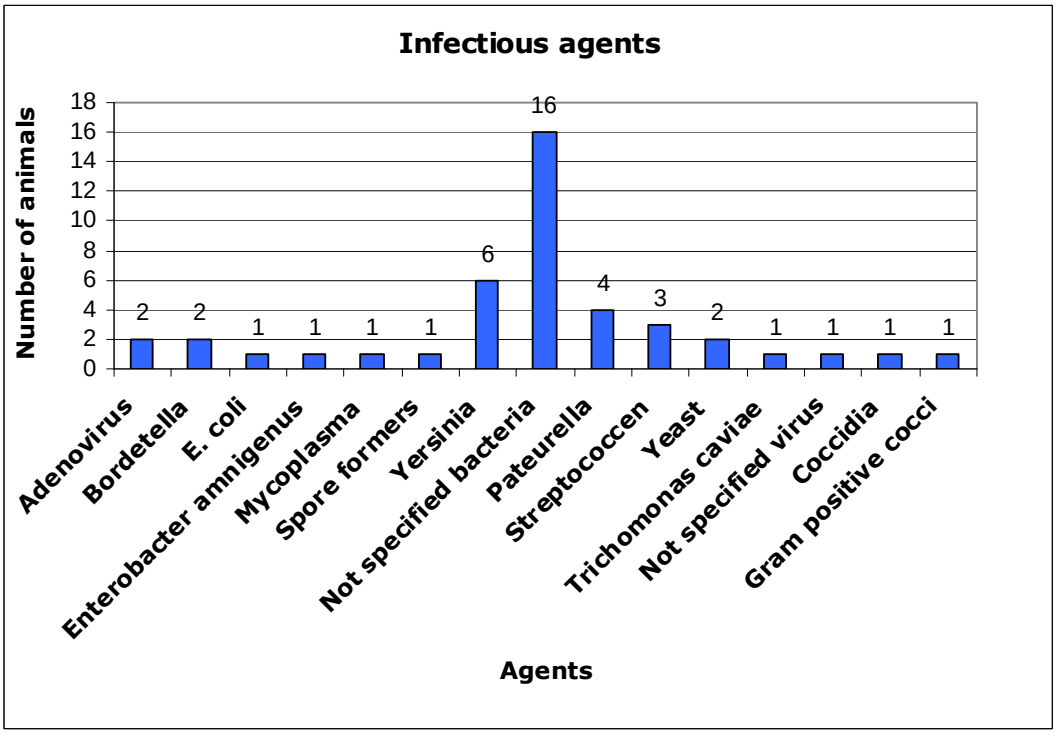

Figure 8 – Infectious agents (retrospective)

Figure 9 shows in which organ(system) the infectious agents were found. The most infected organs or organ systems are the lungs, gastro-intestinal tract and liver. 46.5% (N=20) of the guinea pigs had infectious disease in the lungs. 25.6% (N=11) of the animals had an infection of different parts of the gastro-intestinal tract. 14% (N=6) of the animals had an infection of the liver.

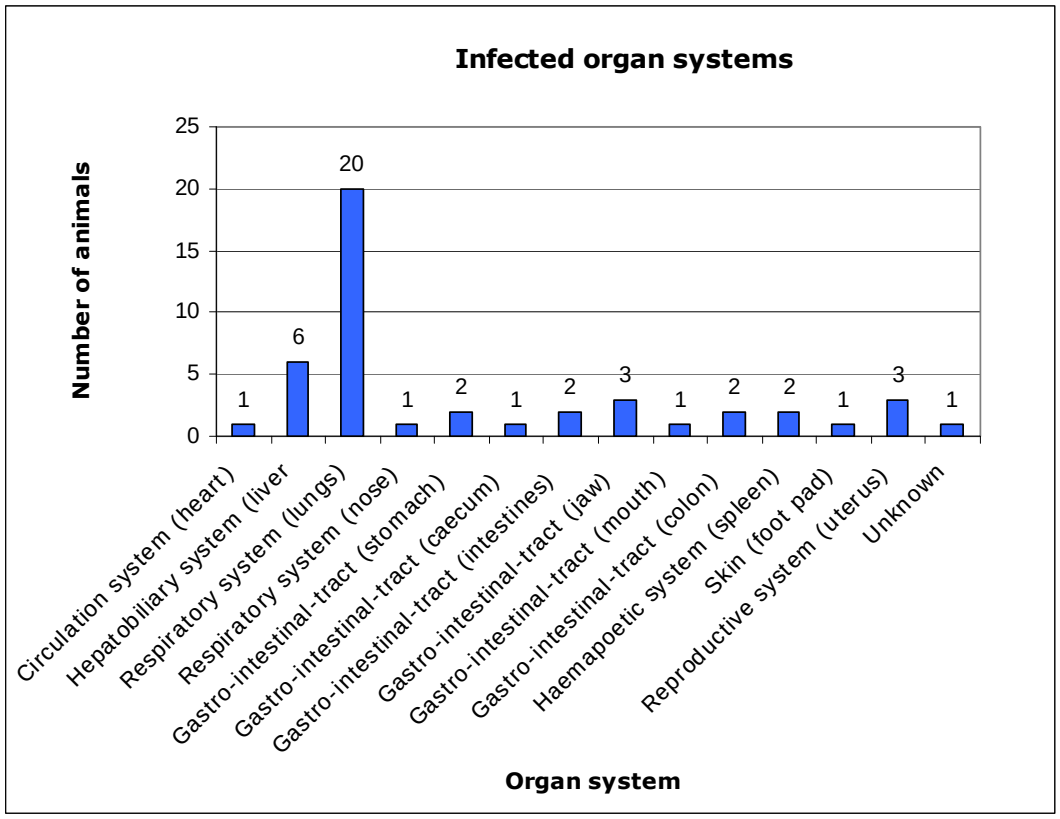

Figure 9 – Infected organ systems (retrospective)

3.2.3 Types of death

Figure 4 shows that 45% (N=60) of the animals died a natural death and that 19% (N=25) of the animals was euthanized. 36% (N=48) of the animals had an unknown type of death.

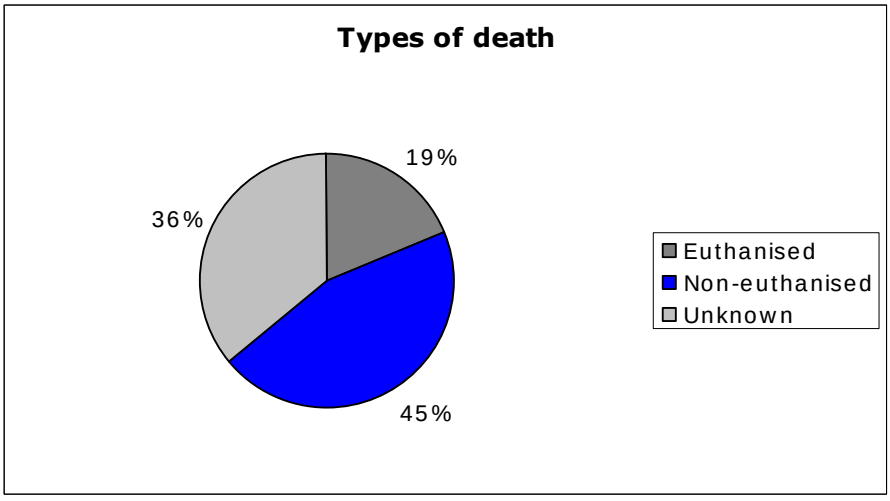

Figure 10 – Types of death (retrospective)

4. DISCUSSION

In this chapter there will be a discussion about the found causes of death in comparison with literature and the retrospective study and about relations between causes of death and welfare.
All the findings of this project are based on a group of 30 guinea pigs, which is too small to draw statistically valid conclusions.

4.1 Most susceptible organs and comparison with literature findings

The most susceptible organs in this study of 30 animals are the lungs (27.7%), heart (16.7%) and kidneys (16.7%). Furthermore, calcium depositions possibly caused by feeding imbalances are frequently found (23.3%).
The most common diseases that cause death in domestic guinea pigs found in literature are: dental malocclusion, urinary calculi, ovarian cysts, pneumonia and hypovitaminosis C (Mitchell, Tully 2009).

As found in this project and in literature, pneumonia is common in guinea pigs. Wood shavings possibly have a deleterious effect on the lungs (see introduction and 4.4). Besides that, stressors (as nutritional imbalances, overcrowding, abrupt changes in temperature, huminity, or diet, intercurrent diseases or type of housing) increase the susceptibility to disease, and young guinea pigs are most often affected by it (Quesenberry, Carpenter 2004, Harkness, Harkness 2010, Rigby 1976). It is difficult to say that these findings correspond with the results of the welfare project, because there are no obvious stressors found; the anamnesis form gives not enough information about it. But there are well nutritional imbalances and also intercurrent diseases found. By now, there can be said that pneumonia was and is a big point of interest.

Heart ailments in guinea pigs are not frequently found in literature. According to John E. Harkness in his book: 'heart disease is rarely seen in guinea pigs' (Harkness, Harkness 2010). Nevertheless, in this welfare project 16.7% of the guinea pigs were deceased on heart problems; one of them on a congenital heart problem and four on a myocarditis. These four had another cause of death combined with the heart diseases. It is difficult to see heart laesions macroscopically, therefore in this project the heart standardly becomes a histological evaluation (normally, this does not happen standardly). Consequently, heart ailments are not often found in literature, but concluding the results of this project, heart ailments in guinea pigs are a point of interest.

Less is described about kidney failures in literature, and the material that is found is about older guinea pigs. Chronic renal disease is common in older guinea pigs of both sexes and the disease can be caused by inappropriate diets, dystrophic mineralization following inappropriate vitamin D supplementation, fatty infiltration of the kidney following pregnancy toxemia, or other toxicities according to Harkness and Wagners (Harkness, Harkness 2010). Chronic nephrosclerosis (predisposed by a dietary calcium:phosphorus imbalance) and chronic renal failure are also common incidental ageing lesions, described M.J. Huerkamp et al (Laber-Laird, Swindle & Flecknell 1996). Nevertheless, in 16.7% of the animals of this project a kidney disease is found (see 3.1.1). The affected animals were 6, 15, 18, 24 and 29 months old. Those results are difficult to explain with the above literature findings. Despite it, there are possibly more kidney failures, also in young guinea pigs, than supposed so far.

Metastatic calcification is one of the diseases of ageing guinea pigs in Handbook of rodent and rabbit medicine (Laber-Laird, Swindle & Flecknell 1996). According to 'Guinea pig and chinchilla care and husbandry' metastatic calcification occurs most often in guinea pigs older than 1 year (Donnelly, Brown 2004). In this research,

metastatic calcifications were found in 30% of the guinea pigs (in 6.7% as consequence of a kidney problem and in 23.3% possibly in accordance with nutrient imbalance). A low magnesium and high phosphorus diet and high calcium or high Vitamin D intake have been implicated (Donnelly, Brown 2004). Possibly the mineral ratio of the diet of the project animals was not so good, therefore this is a point of interest.

Dental malocclusion is not often found in the project-guinea pigs in contrast with findings in literature. Possibly because there was another cause of death found, through which the dental problems were overshadowed or the animals that had teeth problems were not sent in for this project, because the cause of death was clear.

According to literature, urolithiasis is common in guinea pigs (Quesenberry, Carpenter 2004, Harkness, Harkness 2010, Hawkins et al. 2009), but only once found in this project-guinea pigs. Hawkins said in her study that urinary calculi are more seen in females older than 2.5 years old. Besides that, the animals that deceased or suffered from urolithiasis were not sent in for this project, because the ailment was clear. Therefore it is obvious that this ailment is one time found in the project-animals (Hawkins et al. 2009).

Ovarian cysts are commonly found in guinea pigs of 2 to 4 years old (Keller, Griffith & Lang 1987). Therefore it is not so surprising that there is one guinea pig found in this research with cystic ovaria.

Hypovitaminosis C is much found in literature, but no indications (hemorrhage, swollen and paintful joints, no tight teeth) of this are found in necropsy reports of the welfare project-animals. Because it is a fact that guinea pigs are dependent on Arcorbic acid in food or drinks, it remains a point of interest.

4.2 Comparison welfare project, retrospective study and literature

In this paragraph, the results of the welfare project will be compared to the results of the retrospective study.

The results of the retrospective study and the ones of the welfare project are not classified in the same way, therefore they are difficult to compare. Freek Jurg has classified his results on frequently found ailments and literary interesting ailments. The found problems could have been causes of death, but could also have been secondary ailments. The arrangement of the welfare project is based on causes of death classified to organ system.

Furthermore, the previous study contains a large ‘remaining’ group. Interestingly, a large proportion of those animals (30.1%, N=40) had a lung problem. Concluding in both studies almost one third of the animals deceased on a lung problem.

In both studies, tumours do not occur often; 1.5% (N=2) in the retrospective study and 3.3% (N=1) in the welfare project. Also, in literature, it is usually known that tumours in guinea pigs under 4 years old are virtually nonexistent (Laber-Laird, Swindle & Flecknell 1996).

On the other hand, kidney problems are more found in the welfare project than in the retrospective study (16.7% (N=5) respectively 2.3% (N=3)). Possibly because the extensiveness of the autopsy reports of the retrospective study was limited.

Heart problems are not found in the retrospective study, possibly because they were classified in the remaining group.

In both research groups dental problems occur as often (6,7% in the welfare project respectively 6.0% in the retrospective study). According to literature it is

an important problem, but in these studies it was not seen. As previously said (4.1) these animals were possibly not sent in for those projects, because the ailment was clear. For owners it remains important to take a look at the teeth frequently, in that way they can detect problems in time.

Nutrition problems (detected through calcium depositions in different organs, see 3.1.3.1) are more common in the welfare project than in the retrospective study (23.3% respectively 10.5%). The calcium depositions caused by nutrition problems are not the cause of death, but are a welfare problem. Also in comparison with literature findings it is a relatively high percentage (see 4.1). Concluding, it is important to give the animals the effective food with the right Ca/P ratio.

Reproduction problems and stomach dilatations are more common in the retrospective study. None of the animals of the welfare project have died on these two problems, while in the retrospective study 8.3% died on reproduction problems and 9.8% on a stomach dilatation.

A fatty liver is determined on 33.1% (N=44) of the animals in the retrospective study and in 10% (N=3) of the animals of the welfare project as a secondary ailment.

The etiology of death is classified in the same way for both research groups. In both groups, the 'unknown' group is the biggest, but in the welfare project group the unknown group is bigger; 64.4% against 39.1% in the retrospective study.
In the retrospective research group, there is almost no difference in infectious or non-infectious causes. In the welfare project group, the infectious group is the smallest. In the welfare project there were less infectious agents diagnosed than in the retrospective study, presumably because in the past there always was a bacterial analysis. Nowadays, a bacterial analysis only takes place when there are indications for it.

In both research groups there are more naturally deceased animals than euthanatized animals. The retrospective research group contains a number of animals from which nothing is known about the type of death. In all animals of the welfare project group, this is well known.

4.3 Welfare

Welfare is a broad concept. In order to find a possible relation between causes of death and welfare, a total of 12 welfare parameters from the Welfare Quality Project® is used to make assumptions about the welfare of guinea pigs in this research. The Welfare Quality project has developed a standardized, science-based index which makes it possible to objectively determine animal welfare (Blokhuis 2008).

Principles	Welfare criteria
Good feeding	1. Absence of prolonged hunger 2. Absence of prolonged thirst
Good housing	3. Comfort around resting 4. Thermal comfort 5. Ease of movement
Good health	6. Absence of injuries 7. Absence of disease 8. Absence of pain induced by management procedures
Appropriate behaviour	9. Expression of social behaviours 10. Expression of other behaviours 11. Good human-animal relationship 12. Absence of general fear

Table 1 – Welfare principles and criteria Welfare Quality Project (Blokhuis 2008)

Living space
Environmental parameters, such as housing systems and living space, can not define how animals feel, but act as boundary conditions for good welfare. As animals have enough space, they can act their natural behaviour, have lesser stress and there is no overcrowding. As mentioned previously (introduction), the enclosure size of one adult animal should not be less than 2500 cm^2 and the floor area per animal must be 900 cm^2 (Council of Europe 2006). These are guidelines for laboratory animals and therefore minimal sizes. All the cages in this welfare project have an enclosure size above 2500 cm^2, but not every animal has 900 cm^2 floor area, therefore living space is a point of interest.

Number of animals in group
Wild guinea pigs are group-living animals. To reduce stress, it is good to shelter the animals in pairs or groups (Kaliste 2004). Most of the animals of this project have lived together with one or more congeners. 5 of 30 guinea pigs have lived alone (16,7%). It is unknown from which gender the together living congeners were and for how long they have lived together. Therefore, it is unclear whether living together reduces stress or increases stress (as result of fights).

Hiding places
Guinea pigs are shy animals. In the wild, they inhabit burrows of other animals. Therefore, in captive a place to hide is recommended (hay or objectives in their cage) (Donnelly, Brown 2004). Nothing about this is mentioned in the anamnesis form, but it can be advised in the owner recommendations.

Bedding material
Testerink and Klaver pretend that abietic acid found in wood shavings possibly causes liver injuries and respiratory problems (Klaver 2005, Testerink-Baas 2010) (see introduction). Most of the animals had wood shavings as bedding material. 6 of 30 animals (20%) lived at other bedding material (wood cat litter pellets, Carefresh, only straw, Little friends pellets, woodpulp). All the guinea pigs that had lung problems lived at wood shavings, but not all animals that lived on wood shavings had a lung problem. Therefore, it is not possible to draw a conclusion about this. All the animals that lived on other bedding materials did not have lung problems and 2 of 3 animals with liver problems lived at wood shavings and 1 of 3 lived on wood pellets, but because of the small research group size it is not possible to draw a conclusion about this either.

Cleaning frequency
Ammonia is immunosuppressive and can cause respiratory and eye problems (Laber-Laird, Swindle & Flecknell 1996, Burn, Mason 2005). Ammonia can for example develop when cages are not cleaned frequently enough, or if ambient temperatures and/or humidity are favorable for bacterial growth (Burn, Mason 2005). All cages have been cleaned at least once a week, in 9 of the 30 cases (30%) even 2 to 4 times a week. Two animals whose cages have been cleaned 2 to 4 times a week have died of lung problems. Nothing is known about the temperature and humidity, which makes it impossible to draw conclusions about this.

Housing location
7 of 30 (23.3%) animals live outside. The remaining animals live inside. One could think that animals living outside more often die on lung problems than animals that live inside, but this is not the case: the outside living animals deceased on different problems. One on a spleen rupture, one of cachexia, one of urolithiasis, one of nefritis and amyloïdosis, one of a pneumonia and myocarditis, one on pneumonia and one on myocarditis and peritonitis. Looking at these results, there can be concluded that there is no difference between diseases in animals living in- or outside.

Extra ventilation

One of the questions of the anamnesis form is about extra ventilation. 6 animals had no extra ventilation, 10 animals had extra ventilation and 14 forms had no information on it. It is not known what the owners mean with extra ventilation, but it is possible to think that extra ventilation means draft and gives a higher risk for lung problems than living without extra ventilation. 2 of the 8 animals with pneumonia had extra ventilation. The other animals with extra ventilation deceased on different other problems (myocarditis and peritonitis, dental problems, spleen rupture, urolithiasis, cachexia, liver problems and kidney problems). Looking at these results, it is not possible to draw a conclusion about this.

Food types and food supplements

There are only two guinea pigs with dental problems and those animals get pellets (Kasper fauna food and Science direct), hay and vegetables to eat. One of them also got rodentmix and the other one fruit (melon and apple). But it is not clear in which amount, frequency and brand they received it. Therefore, it is not possible to draw conclusions about this.

It seems that all animals got the right food; almost all animals got pellets, vegetables, fruit and hay. But, as mentioned before, it is mostly not known in which amount, frequency and brand the animals receive it. Besides that, 13 of 30 animals (43.3%) got food supplements, 9 got no food supplements (30%) and of 8 animals (26.7%) it is unknown. There were no guinea pigs with typical Vitamin C deficiency problems (leaking blood vessels, and hemorrhage in joints, gums and intestines). Therefore, it is not possible to draw conclusions about this either.

Despite the correct food types, in 30% of the guinea pigs of this research metastatic calcifications were found (in 6.7% as consequence of a kidney problem and in 23.3% possibly in accordance with nutrient imbalance) and a low magnesium and high phosphorus diet and high calcium or high Vitamin D intake have been implicated (Donnelly, Brown 2004). Therefore it is important to give food with minerals in the right ratios. In conclusion, feeding is a big point of interest.

Watersupply

Guinea pigs should have permanent access to fresh drinking water (Laber-Laird, Swindle & Flecknell 1996). Almost all animals of this project have access to tap water through a drinking nipple. More than half of the owners change the water daily or more. The remaining group had no answer about this.

5. CONCLUSION

The conclusions of the three main questions are described in this chapter.

5.1 Most common causes of death

Just like the literature, the most common cause of death of guinea pigs in this project is a lung ailment (27.7%, N=8). Heart problems (16.7%, N=5) and kidney problems (16.7%, N=5) together take the second place. In literature, they are both less described in guinea pigs. Kidney problems are sometimes described in older animals. Besides those problems, in 23.3% (N=7) of the guinea pigs calcium depositions are found, which are possibly caused by nutrient imbalance. In literature, this is only reported in animals older than one year.

5.2 Results welfare project compared with retrospective study results

After comparison of the results of the welfare project and the results of the retrospective study, it can be concluded that in both studies almost one third of the animals deceased on a lung problem. On the other hand, kidney problems are more found in the welfare project than in the retrospective study (16.7% (N=5) respectively 2.3% (N=3)) and heart problems are not mentioned in the retrospective study. In both research groups dental problems occur about as often (6,7% in the welfare project respectively 6.0% in the retrospective study). Nutrition problems are more common in the welfare project than in the retrospective study (20% respectively 10.5%). Reproduction problems, stomach dilatations and liver fatness as secondary ailment are more common in the retrospective study.
Looking to the etiology of death in both research groups, the 'unknown' group is the biggest. In the welfare project there were less infectious agents diagnosed than in the retrospective study.
In both research groups there are more naturally deceased animals than euthanatized animals.

5.3 Causes of death relating with welfare

Except suspicions about nutrient imbalance, living space and stress indicators (such as changes in temperature, humidity or diet, overcrowding, type of housing and intercurrent diseases) that increase the susceptibility to disease, there are no direct welfare related problems found. Of course, recommendations can be given to owners to guarantee proper care of them and to prevent problems in the future.

6. RECOMMENDATIONS TO OWNERS

To prevent stress and diseases of young guinea pigs, recommendations about housing, feeding, watering and health are given for owners. First, all the recommendations are described, after that a short checklist (Table 2), specifically for guinea pigs owners will be given.

6.1 Housing

Temperature and housing location
Guinea pigs can be kept in- or outside, but the recommended temperature to keep guinea pigs in, is 21˚C (range 18-26 ˚C). Therefore, for outside living animals it is important to make sure they have a warm place to hide (by providing enough hay or a shelter for example). It is not recommended to house the animals in direct sunlight and they must be gradually acclimatised.

Living space
To reduce stress and overcrowding and to make sure the animal can act their natural behaviour it is important to give the animal enough living space. There are no data known about minimal cage sizes for pet guinea pigs, but cages of laboratory animals have a minimal enclosure size of 2500 cm^2, and a living space of 900 cm^2 per animal. Therefore it is advised to give a bigger enclosure size than 2500 cm^2 and more living space of 900 cm^2 per animal.

Hiding places
Guinea pigs are shy animals and they are susceptible for environmental changes, therefore it is considered to give the animal a hiding place as a shelter or enough hay and it is important to habituate the animal slowly to new conditions.

Number of animals
Wild guinea pigs are social group-living animals, therefore and to reduce stress, it is good to shelter the animals in pairs or groups. Pairs, one man and more females, (harems), female groups of 2 or more females, male groups of 2 males, small mixed sex groups or large mixed sex groups are recommended housing conditions. Sometimes males start to fight and to prevent this, males kept in groups should have been raised together since weaning.

Ventilation
The room or place in which the animal lives should be adequately ventilated, but the animal must be protected from drafts.

Bedding material
To reduce the development of ammonia, carbon oxide and growth of harmful bacterial toxins, the bedding material must be able to absorb moisture from urine and faeces. Softness is another characteristic of the bedding material. Wood shavings have all these characteristics, therefore this material is recommended.

Cleaning frequency
It is important to prevent ammonia production, therefore it is recommended to clean before it smells, and the bedding material must be dry and clean as much as possible. The cleaning frequency is at least once a week depending on the number of animals and the bedding material which is used.

6.2 Feeding and watering

Feeding
For sufficient Vitamin C intake, guinea pigs should eat freshly milled, properly stored, complete commercial pellets. This food must be replenished with hay, vegetables or supplements.
Something to take into account: guinea pigs are fastidious animals. They only eat things which they get in an early stage of their life.
Another thing to take into account: the activity of Ascorbic acid in water decreases for 50% during 24 hours and faster than the room temperature increases. Pellets with added Ascorbic acid must be stored cool and dry and must be eaten 90-180 days after production.

Water
Guinea pigs should have permanent access to fresh drinking water in a bottle (to prevent contamination of the water with faeces or urine) with nozzle and ball bearings (to prevent blocking of the nozzle).

6.3 Health

Weight
It is recommended to weigh the animal every week. This is a non-invasive way to detect ailments early.

Observation
It is advised to observe the animal during the day/every day to detect behaviour- or health problems (for example look at the teeth, fur, foot, eyes (tears), nose (nasal discharge), faeces, eat and drink). It is important to contact the veterinarian if the animal does not drink or eat and when the animal exhibits other unusual behaviour.

Vaccination
There are no vaccinations for guinea pigs known.

6.4 Checklist

HOUSING	RECOMMENDATION	CHECK
▪ Housing location	Inside: not in direct sunlight or Outside: with shelter possibilities and not in direct sunlight	
▪ Temperature	As kept inside: 21˚C (range 18-26 ˚C)	
▪ Living space	> 2500 cm^2 enclosure size and > 900 cm^2 per animal	
▪ Hiding places	Sufficient hay or a small shelter.	
▪ Number of animals in group	> 1	
▪ Ventilation	Adequately ventilated, without draft.	
▪ Bedding material	Soft and good absorptive (wood shavings).	
▪ Cleaning frequency	At least once a week, before it starts to smell and before the bedding material is too wet and dirty.	
FEEDING AND WATERING	**RECOMMENDATION**	**CHECK**
▪ Feeding	Freshly milled, properly stored, complete commercial pellets. Replenished with hay, vegetables or supplements.	
▪ Watering	Permanent access to drinking water	
HEALTH	**RECOMMENDATION**	**CHECK**
▪ Weight	Weigh every week	
▪ Observation	Every day	

Table 2 – Checklist guinea pig to owner

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APPENDIX I – NECROPSY PROTOCOL WELFARE RESEARCH SMALL MAMMALS

Macroscopy	Microscopy: put in cassettes	Freeze
Identification chip/ tattoo body weight		
Skin, nails	Skin left flank	
Bone (right femur)	Right femoral head, decalcifying	
Skeletal muscle	Dorsal back muscles, left	
Eyes	OS and OD	
External ears		
Nose, larynx	Conchae	
Mouth, teeth		
Pharynx and oesophagus	Oesophagus	
(Para) thyroid	(Para) thyroid	
Trachea	Trachea	
Lung	Lung	Lung
Heart	Heart, full circle at 1/3 height	Heart
Large vessels		
		Fat
Intestinal tract	Stomach, duodenum, jejunum, caecum, colon	Colon
Pancreas	Pancreas	Pancreas
Liver and gall bladder	Liver and gall bladder	Liver
Kidney end urinary tract	Kidney, bladder	Kidneys
Spleen, lymph nodes	Spleen, Lnn. Mesenteriales	Spleen
Bone marrow (right femur)	Bone marrow R. femur, distal and middiafyse	
Adrenal gland	Adrenal gland	Adrenal gland
Reproduction organs	Testicles/uterus/ovaries	
Brain	Brain	Brain
Pituitary gland	Pituitary gland	
Other pathologically changed areas	Other pathologically changed areas	

APPENDIX II – GENERAL NECROPSY PROTOCOL

Number: Species:

Notes:
Called to submitter (by whom, which announcement):

Cassette 1: CYTOLOGY
Cassette 2: HC liver:
Cassette 3: HC spleen:
Cassette 4: HC lung:
In formalin: HC colon:
Frozen: Native colon:
IFT: yes/no

MACROSCOPY
Section date: Path. / sio: Student:
Chip:
Exterior skin:
General aspect:
Weight:

Head and neck
Nose:
Ears / eyes:
Mouth / teeth:
Tongue:
Brains:
Thyroid:

Thorax/respiration and circulation
Position organs / free fluid
Trachea:
Pleura / diaphragm:
Lungs:
Heart:

Abdomen/ other intern organs
Position organs / free fluid
Stomach:
Duodenum / pancreas:
Jejunum / ileum:
Colon:
Caecum:
Liver:
Spleen:
Lnn:
Kidneys:
Adrenals:
Urine bladder:
Gender: M / F

Skeleton/ extremities
Mineralisation:
Joints:
Feet:

Preliminary conclusion after macroscopy:

APPENDIX III – ANAMNESIS FORM

VPDC, Postbus 80158, 3508TD UTRECHT **Tel. 030- 253 3195** Fax: 030-2534774

Praktijk: ..... Relatienummer:.....
Dierenarts: ..... E-mail:.....
Tel. Nr.: .....

Eigenaar ..... Konijn/ cavia/ fret/ rat (omcircelen wat van toepassing is)
Straat: ..... Naam/ nr.....
Postcode: ..... Leeftijd/ Geb. datum:.....
Woonplaats: ..... Geslacht: M/ ex-M/ V/ ex-V
Tel. Nr.: ..... In bezit sinds:.....
Code eigenaar: .....

Crematie □ nee/ □ ja, naar.....
□ **Euthanasie/** □ **Gestorven** □ dd:.....

Individu ziek sinds: :.....
Aantal dieren in groep:.....
Aantal dieren in eenheid ziek:.....
Reeds behandeld met: .....
Tijdsduur behandeling: .....

Verschijnselen □ Diarrhee □ Ademhalingsproblemen □ Huidproblemen
□ Kreupelheid □ Slechte groei □ Plotseling dood
□ Slecht eten □ Slecht drinken

Huisvesting:
Lokatie: □ Binnen □ Buiten
Afmetingen: L: ..... B: ..... H: .....
Materialen: □ Hout □ Metaal □ Plastic/Fiberglas
□ Glas □ Anders.....
Bodembedekking:.....
Extra ventilatie: □ Nee □ Ja
Frequentie schoonmaken huisvesting:.....
Gebruikte schoonmaakmiddelen/desinfectia:.....

Voederfrequentie:.....
Voersamenstelling:
0 Pellets: Merk..... Hoeveelheid:.....
0 Hooi Merk..... Hoeveelheid:.....
0 Groente Soort..... Hoeveelheid:.....
0 Fruit Soort..... Hoeveelheid:.....
0 Snoepjes Merk..... Hoeveelheid:.....
0 Anders Beschrijving.....
Wat wordt hiervan gegeten?.....
Voedingssupplementen (incl. vitamine C): □ Nee □ Ja
Watervoorziening: □ kraanwater □ water uit een fles □ regenwater □ anders
Type: □ Schaaltje/kom □ Drinknippel
Frequentie verversen van water:.....

Anamnese:
.....
.....
.....

Klinische diagnose:
.....
.....
.....