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Contents

Front Cover: **Dorper Sheep Breed**

The Dorper was developed in South Africa in the 1930's by crossing the Dorset Horn with the Blackhead Persian. The sheep is easy to care for and is well adapted to survive arid conditions. They have a high fertility and maternal instinct along with a high growth rate and resilience. They are outstanding browsers utilizing to the upmost the available pasture. Dorper sheep may have either black or white heads. The name is derived from the first syllables of the parent breeds. This breed has been introduced into the Israeli herd and contributed its characteristics to the variety of culinary treats in our country. The two pictures were photographed in the Namaqualand region of South Africa in September 2014 during the annual flowering season.

Editorial

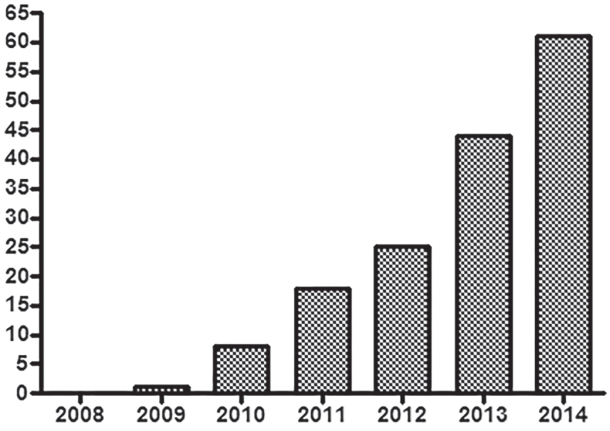
Since my commencement as editor of the Israel Journal for Veterinary Medicine (IJVM) in 2010 I have chosen a species of animal each year to be portrayed on the cover of the journal. For example in the year 2010 different breeds of horses appeared on the cover, in 2011 dogs and in 2013 cats. The year 2015 has been declared the "Year of the Sheep" by the Asian communities and it is just by chance that I also decided to choose this species. The sheep ranks in the eight position of animals in the Chinese zodiac and represents solidarity, harmony and calmness. I welcome all our readers to submit pictures of sheep for inclusion on the cover of the journal during this year, 2015.

The present issue presents four articles on bovine medicine and three on equine medicine. This is very pleasing and I welcome more articles relating to these species. Two articles deal with exotic species medicine, both articles are from overseas. One article from Italy through the gracious support of Prof. Mario Giorgi a good friend of Israel and the other through Dr. David Eshar a former student of the Koret School of Veterinary Medicine. Dr. Monica Mazuz from the Kimron Veterinary Institute presents an interesting first case report of pups infected concurrently with Neospora and Hepatozoon. Prof. Ibrahim Balkaya has presented a survey on the presence of Toxoplasma and Neospora in wild boars in Eastern Turkey. So there is a lot for everyone to read.

The endeavor of a number of countries to boycott Israeli academics is discussed frequently in the media. I want to remind our Israeli veterinary scientists that this issue may one day affect all of us and also future veterinarians. I raise this issue to emphasize the importance achieving a good professional Israeli veterinary journal of a high standard. In order to achieve this, the journal must receive support from veterinarians in Israel.

The status of the journal is improving annually and this year we have reached another record in the number of citations which have been on the ascent since 2010 since I assumed the editorship of the IJVM. I have included a graph of the number of citations per year from 2008 to demonstrate our achievements. This increase would have been even more significant if only the Journal had the support of senior academic veterinarians, which I urge and for look forward to in the very near future. This is not my first request for this cooperation and a group-effort from academic veterinarians as documented in many of my editorials will hopefully signal a change in their minds and attitude.

NUMBER OF CITATIONS OF ARTICLES BY YEAR



The excellent standard of veterinary research in Israel is reflected in the abstracts of the 37th Symposium of the Koret School of Veterinary Medicine. I am proud to publish these abstracts and I congratulate the organizers in gathering such admirable research presentations.

The Editorial board of the IJVM wishes all its readers a pleasant and safe spring. To those going on holiday and attending conferences, wishing you safe travels and successful conferences.

Chag Pesach Samach.

Trevor (Tuvia) Waner
Editor-in-Chief
Israel Journal of Veterinary Medicine

Review Article

Perioperative Antibiotics Prophylaxis in Equine Surgical Colic Patients – Are We Doing the Right Thing?

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ABSTRACT

Perioperative prophylaxis is one of the most common reasons for antimicrobial administration. Its goal is to reduce postoperative infection at the surgical site, thereby reducing morbidity, mortality and treatment costs. When perioperative prophylactic treatment is used, several issues should be decided upon, including the drugs that are used, timing of first administration, re-administration after 2 half-lives of the drug if surgery is still ongoing and duration of treatment. Equine colic surgery is typically an emergency procedure, classified in the best circumstances as a clean-contaminated surgical procedure and carries a high rate of surgical site infection as an important short term complication. Information regarding the compliance with prophylaxis guidelines in veterinary medicine is limited and is often not detailed enough. The duration of prophylactic treatment is a more complicated topic due to the high rate of post-operative complications in horses undergoing colic surgery. Guidelines for the judicious use of antimicrobial therapy recommend that antimicrobials should be administered for the shortest effective period possible to prevent the development of resistant pathogens. A recent study in surgical colic patients has shown no difference in the rate of incision infection with the use of perioperative antimicrobial therapy for 72 hours and for 120 hours, resulting in the conclusion that there is no benefit for the longer duration of prophylactic antimicrobial administration. Clinician's awareness for guidelines or standard protocols for antimicrobial drug use for equine patients undergoing surgery for colic is important. Implementing such guidelines, which should be reviewed and updated regularly, and reducing the amounts of perioperative antimicrobials that are being used are important goals which we all should strive for in order to reduce the emergence of resistant strains of bacteria that could affect our patients.

REVIEW

Perioperative prophylaxis is one of the most common reasons for antimicrobial drug administration. Its goal is to decrease the likelihood of infection after exposure to bacteria during the surgical period (1). The use of perioperative prophylaxis has evolved greatly in the last 30 years. Improvements in the timing of initial administration, the appropriate choice of antibiotic agents, and shorter durations of administration, define its value in reducing surgical site infections while minimizing the risks for side effects (2). On the other hand, inappropriate use results in unnecessary costs, increased antimicrobial resistance, and development of superinfections (3). Whereas much information is available in human medicine, objective scientific studies which evaluate the use of perioperative prophylaxis in veterinary medicine are limited and more studies are warranted. An American College of Veterinary Internal Medicine (ACVIM) consensus statement on antimicrobial drug use in veterinary medicine was issued several years ago (4). In recommendation I, it is stated that the committee believes that prophylactic and metaphylactic use of antimicrobial drugs is appropriate for control and prevention of infectious diseases

in animals. However, it is also stated that its use should be conservative and should emphasize drugs assigned to the primary use category. It is also stated that it is not necessary to use antimicrobial drugs in all surgical cases to prevent infections, which is especially true for clean surgeries, as opposed to clean-contaminated or contaminated procedures (4). Clean surgical wounds have an infection rate less than 5%, so antimicrobial prophylaxis is generally not necessary (3). Clean-contaminated wounds are those in which contaminated areas of the body (eg. gastrointestinal system, genitourinary system) are entered under controlled conditions without unusual contamination (3). Colic surgery in horses is typically an emergency procedure, classified in the best circumstances as a clean-contaminated surgical procedure (4). Surgical site infection (SSI) is one of the most common short-term complications in colic patients and can occur in as many as 22-40% of all cases (5, 6, 7).

When using prophylactic antibiotics, several aspects should be taken into accounts including the selection of appropriate agent, the timing of the first dose, re-administration of antibiotics during surgery after 2 half-lives of the drug have passed, and duration of treatment. Regarding the timing of the first dose, prophylactic antimicrobials must be present in appropriate levels at the surgical site during the time of contamination (3). In human patients, administration of the first dose of antibiotics more than two hours before surgery or three hours after surgery resulted in six times higher complication rate as opposed to patients that were treated 0-2 hours before surgery (8). Current guidelines for use in horses suggest that prophylactic antibiotics should be administered, preferably intravenously, within one hour of first incision (9). As for re-administration during surgery, in human patients undergoing colorectal surgery, the incidence of infection was decreased in patients that were administered repeated intraoperative dosing of antibiotics (10). Regarding re-administration of antimicrobials in veterinary patients if surgery is ongoing after 2 half-lives of the drug have passed, prophylactic beta-lactam antimicrobial administration should be repeated during surgery (3).

Information regarding the compliance with prophylaxis guidelines in veterinary medicine is limited. In a study reporting on prophylactic antimicrobial use in horses undergoing elective arthroscopy, only 6.3% received preoperative antibiotics within 60 minutes of the first incision (11). Although it is recommended that a second dose should be administered in colic surgeries if the surgery is ongoing after 2 half-lives of the drug have passed in order to assure adequate levels for the duration of surgery, intraoperative antibiotic administration did not occur in any horse, in that study (11). These discrepancies can be partially explained by logistical reasons but it was suggested by the authors that adhering to these recommendations is preferable (11). In a recent study that investigated antimicrobial use in horses undergoing emergency colic surgery, only 88 (11.6%) horses received the appropriate preoperative dose within 60 minutes of the start of surgery and only 8 horses (1.8%) were re-dosed correctly (1).

The duration of prophylactic treatment is a more complicated topic due to the high rate of post-operative complications in horses undergoing colic surgery. Guidelines for the judicious use of antimicrobial therapy recommend that antimicrobials should be administered for the shortest effective period possible to prevent the development of resistant pathogens (4). Continued use of prophylactic antimicrobials beyond the conclusion of surgery contributes to the development of resistant bacteria, superinfections and nosocomial infections (3). While in horses undergoing elective arthroscopic surgery, most (66%) were treated for 24 hours or less (11), this is clearly not the common practice in colic patients. In 2006, Santschi suggested that equine surgeons should seriously consider reducing the duration of prophylactic antimicrobial use in their patients (9). Due to various reasons, among which is the high post-operative complication rate, it is still common practice in many equine hospitals to administer prophylactic antimicrobial therapy for 5 days, postoperatively, mostly aiming to reduce incisional complications (6, 12). This is also the current protocol at the Koret School of Veterinary Medicine – Veterinary Teaching Hospital (KSVM-VTH). In a recent study, the use of perioperative antimicrobial therapy for 72 hours and for 120 hours was compared, in order to evaluate its effect on the development of postoperative incisional infections. High post-operative incisional complication rate (42.2%) was found, but no differences were seen between the two tested groups, resulting in the conclusion that there is no benefit for the longer duration of prophylactic antimicrobial administration (6). The authors of that study suggested that further studies are warranted to identify the minimum effective duration of antimicrobial therapy as it is possible that even a shorter course of 1-2 days would be equally beneficial (6).

The last but not least decision when administering prophylactic antimicrobial is the selection of appropriate agent. As stated earlier, in the ACVIM consensus it is recommended that prophylactic antimicrobial use should be conservative and should emphasize drugs assigned to the primary use category. The most common antibiotic combination used in adult colic patients is penicillin G and gentamicin (1) which is in accordance with the ACVIM consensus. This is the standard antibiotic combination in the Royal Veterinary College and at the Bell Equine Veterinary Clinic, both in the UK, in colic patients (6). In a survey of 761 horses undergoing emergency colic in the New Bolton Center of the University of Pennsylvania, 89.3% were given potassium or procaine penicillin G and gentamicin and very few were given other drug combinations (1).

As stated earlier, inappropriate use of perioperative prophylaxis results in unnecessary costs, increased antimicrobial resistance, and superinfections (3). We cannot conclude inclusively, but it is possible that among other reasons, the recent emergence of multi-drug resistant (MDR) bacteria in the Large Animal Department of the KSVM-VTH is the result of such use of antimicrobials. During 2013 49% (27/55) isolates from different body sites in hospitalized horses at the KSVM-VTH were MDR (Berlin, personal communication). Multi-drug resistant bacteria are classified as those that are resistant to at least 3 groups of antimicrobials (13) and included 6/12 *Escherichia coli* isolates, 4/7 *Enterobacter* isolates, 7/8 *Klebsiella* isolates and 1/1 a *Salmonella* isolate which further emphasizes the problem of nosocomial infection and question the current prophylaxis antimicrobial use routine. Methicillin resistant *Staphylococcus aureus* was also isolated from both carrier horses and infected wounds during this year. Strict guidelines for the use of prophylaxis antimicrobial use in horses undergoing colic surgery is needed.

In a survey that was conducted little more than a decade ago among diplomats of the American College of Veterinary Surgeons, 28 of 32 respondents (88%) reported that they were unaware of any written guidelines or standard protocols for antimicrobial drug use for equine patients undergoing surgery for colic at their veterinary teaching hospital (14). A decade later, in a survey among UK equine surgeons, less than 1% of the practices had antimicrobial use guidelines (15). The authors emphasized the importance of implementation of such guidelines in any institution where antimicrobials are prescribed in order to maintain their effectiveness (15). Implementing such guidelines, which should be reviewed and updated regularly, and reducing the amounts of perioperative antimicrobials that are being used is an important goal which we all should strive for in order to reduce the emergence of resistant strains of bacteria that could affect our patients.

In conclusion, in horses undergoing colic surgery, it is important to administer the first dose of antibiotics, within 60 minutes of the first incision. A second dose should be administered if the surgery is ongoing after 2 half-lives of the drug have passed. Prophylactic antimicrobial administration should be limited to no more than 72 hours and further studies are required to determine if even shorter duration is accepted.

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Translocation of Rabies Virus in Israel by Cattle: A Threat for the Public Health

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ABSTRACT

Rabies is endemic in Israel, the only country in the Middle East that implements a nation-wide anti-rabies campaign. However, between 2002 and 2013 about 32 rabies virus isolates belonging to genetic variant V1 were recovered within Israel. The present study describes for the first time the translocation of the rabies virus strain, fox V1, by infected cattle born on "Tzfon HaGolan dairy farm" located in Kibbutz Ortal on the Golan Heights to two farms situated in the western Yezre'el Valley, emphasizing that cattle may serve also a source of human rabies infection.

Keywords: Rabies; Cattle; Translocation; Diagnosis; Post exposure vaccination.

INTRODUCTION

Rabies virus is a member of the Rhabdoviridae, genus Lyssavirus. All mammals are susceptible to rabies infection. Rabies is enzootic throughout the Middle East, including Israel. Rabies is also a serious enzootic disease in Jordan, Syria, Lebanon and Iran and Irak where stray dogs maintain rabies virus in circulation, with frequent spillover to wildlife, including jackals, squirrels, stone-martens, foxes, wolves and infection of domestic animals and human (1, 2, 3, 4, 5, 6).

Since 1979, red foxes (*Vulpes vulpes*) have become the most important reservoir of rabies virus in Israel (7). An oral vaccination (ORV) program directed at wild animals has been implemented since 1998 in northern Israel (8). In 2004 the program was extended and it currently covers all the territories controlled by Israel and the Palestinian Authority. Israel is the only country in the Middle East that implements ORV program. To insure its success, extensive rabies surveillance along Israel's borders is carried out. Despite the efficacy of the ORV in controlling fox rabies in north Israel, a new outbreak occurred in 2004 in this region, in which stray dogs (*Canis familiaris*) where shown to be main reservoir and transmitter of genetic variant V7 (9, 10).

Molecular epidemiological studies of rabies in Israel between 1993 and 1998 revealed four fox strain genetic lineages (V1-V4), were distributed within four geographical regions (11). Molecular analysis of rabies isolates on the borders of Israel and neighboring countries revealed the presence of three genetic variants V5, V6 and V7 (12). Due to the ORV program, fox rabies genetic variants V1 and V2 specific to northern Israel region were eliminated in 2003, while fox strain genetic variants V3 and V4 enzootic to central and southern of Israel were eliminated by 2005 (9).

However, incursions of fox rabies variant infected animals persisted across Israel's borders. Between 2002 to 2013 about 32 isolates of fox rabies virus belonging to the genetic variant V1, were detected within Israel and its neighboring countries.

All mammals are susceptible to rabies virus. Bovine rabies has a direct economic impact (13) on the livestock industry and represents a public health threat in rabies endemic area. In Israel between the years 1996-2014, 104 rabies cases were reported in cattle, most of them on the borders with neighboring countries.

Recently two human rabies mortalities, one in Iran and

the second in Brazil were reported from human handling rabies infected livestock (14, 15). The two veterinarians involved were not vaccinated against rabies and did not received post exposure prophylaxis.

There are several examples of long distance transmission translocation of rabies virus by human mediated animal movements and trade. Recently there was a number of reported translocations of infected bovine from Romania to Croatia (16), and in Ohio, USA (17). Translocation of dog rabies from northern Israel to Jerusalem (18) and to the center of the country was also reported (19).

In the present report, we describe for the first time rabies translocation by infected cattle from a dairy herd on the Golan Heights to two farms located in the western part of the Yezre'el Valley, as well as a rabies infected case in cattle in Israel. In addition to the economic damage caused to the dairy industry by rabies infection, the zoonotic aspects are discussed.

CASE REPORT

On September 29, 2013, two 11 weeks old calves one from a village Bet Zaid and the other form a village, Kfar Yehushua in the western Yezre'el valley showed clinical signs of change in behavior, salivation, difficulty in swallowing, vocalization and recumbence. Rabies was suspected and the two calves were diagnosed rabies positive by direct immunofluorescence assay (DFA) on the brain tissue, at the Israeli National Rabies Laboratory, Kimron Veterinary Institute (KVI), Bet Dagan, Israel.

In addition on November 1st 2013, two more calves, one 8 months old, from Kibbutz Ortal, and the second from Kfar Yehushua were rabies positive by DFA (Fig. 1).

A case investigation revealed that on August 26, a Jackal had entered an orchard near the Kibbutz Ortal it was shot and the carcass sent to National Rabies Laboratory (20). The Ortal dairy farm is one of the largest and most modern in Israel. Male calves are sold at a few weeks of age to other

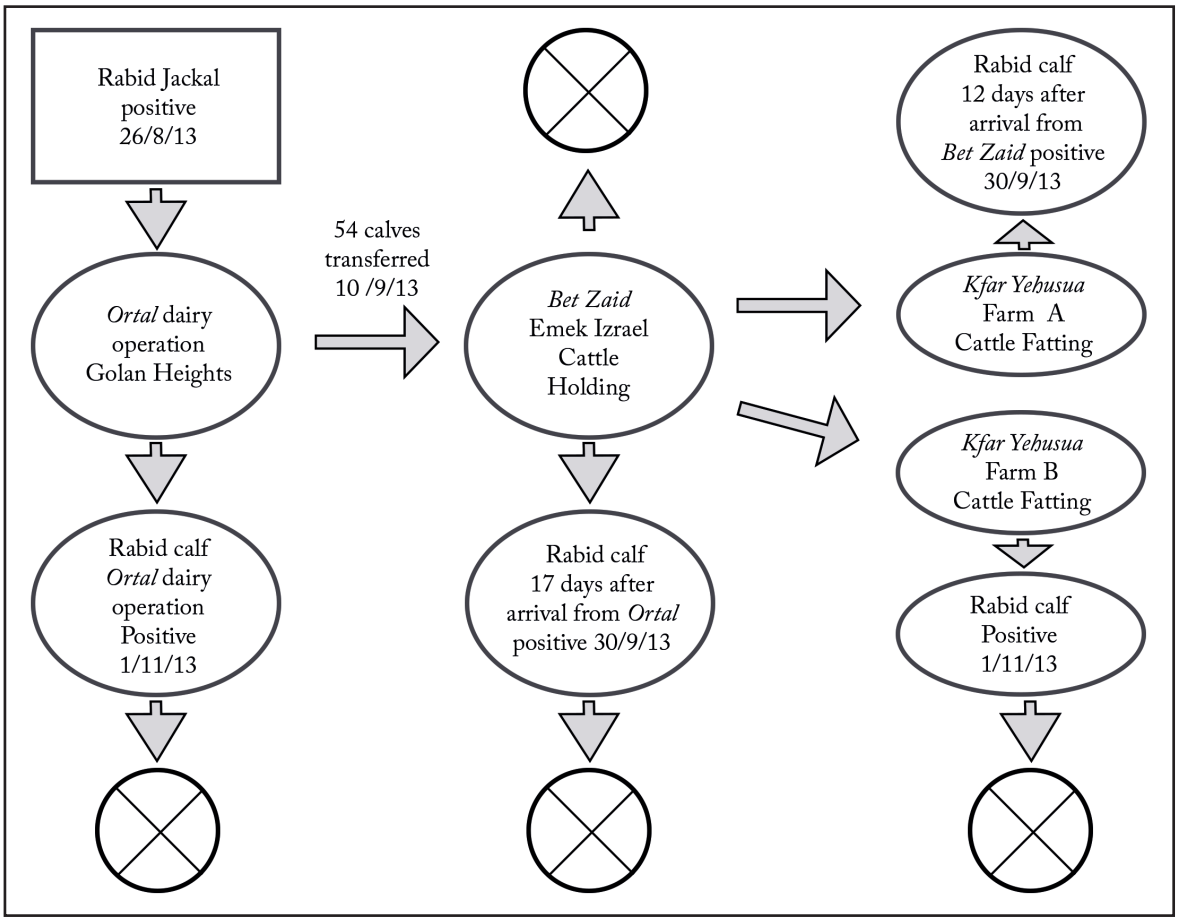

Figure 1: Succession of events following entry of a rabid jackal into Kibbutz Ortal dairy farm.

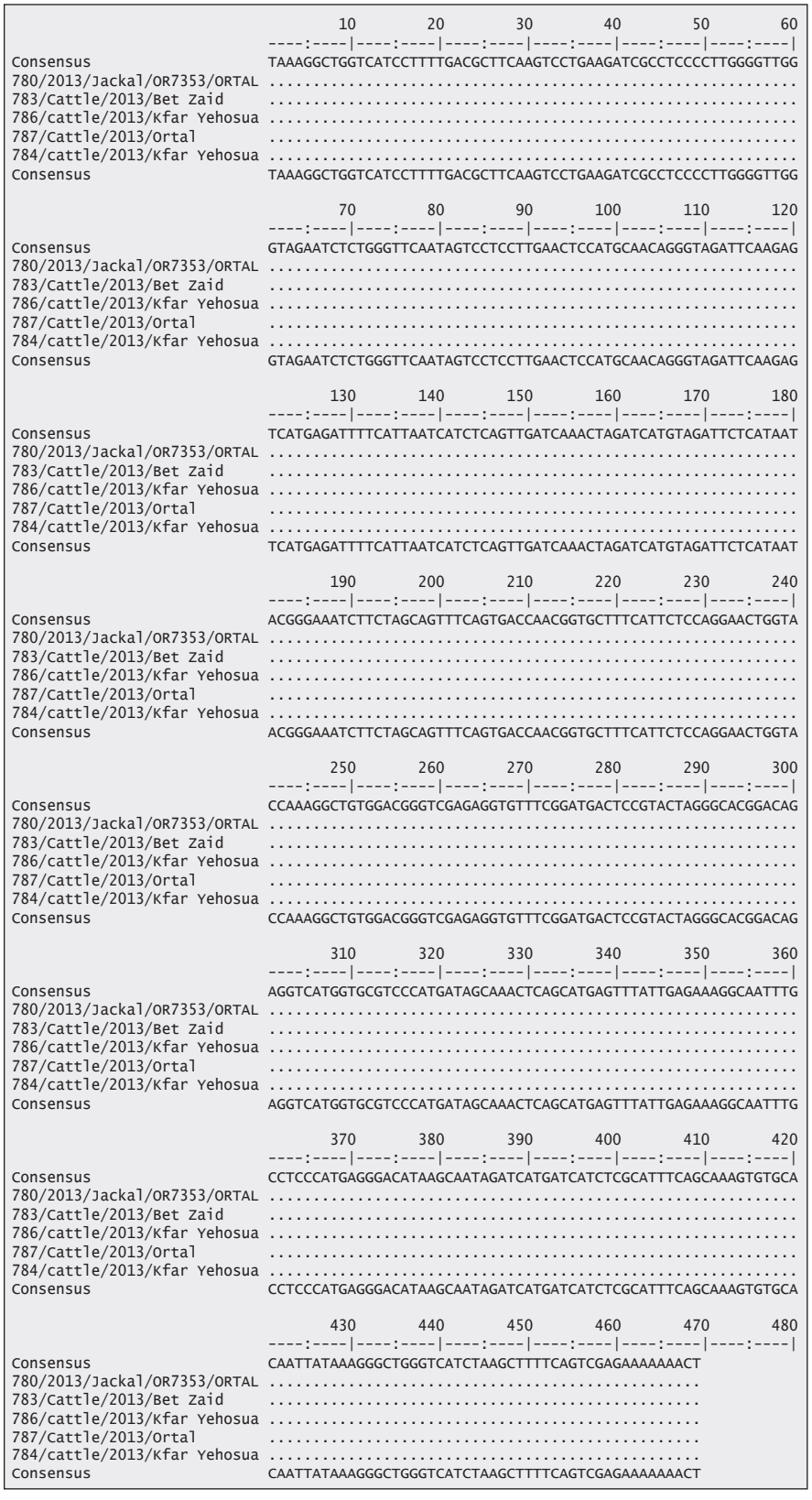

Figure 2: Comparison of 469 base pairs of the G-L intergenic fragments from the sequences of the four calves and the Jackal isolates.

locations throughout Israel for fattening. A group of 54 rabies unvaccinated calves was transported form Kibbutz Ortal on September 10, 2013 to a cattle holding farm in Bet Zaid and then distributed to several farms in Kfar Yehushua.

Four calves and the jackal were diagnosed rabies positive by DFA. Diagnosis was confirmed by virus isolation in tissue culture, and its inoculation into suckling mice (21). Reverse transcriptase – PCR and direct sequencing were applied to a 469 base-pair (bp) G-L intergenic region fragment (19). A phylogenetic tree was constructed by the neighbor–joining method, with the distance calculated using the Kimura-2 parameter with the computer program MEGA, version 4.1 (22). The reliability of the phylogenetic groupings was evaluated using bootstrapping with 1000 replicates.

The molecular analysis showed that the viral sequence obtained from the 4 calves and the Jackal belonged to the V1 genetic variant (Fig.2). Following the diagnosis of rabies, post-exposure vaccination was administered to a group of 12 people, who had come into contact with the three calves and the jackal (Fig. 3).

DISCUSSION

Rabies is endemic in Israel and since 2004 stray dogs were found to be the main reservoir and transmitter (7, 10). However the ORV program of wildlife eliminated the fox rabies variants V1 to V4 from almost all rural areas in Israel. Nevertheless, there is still incursion of fox genetic variants V1 across the borders with neighboring countries. The genetic variant V1 caused mortality of 11 cattle, 2 sheep and 2 horses during the last 12 year on the border but was never detected in the Yezre'el valley region (Fig. 3).

Based on molecular and epidemio-

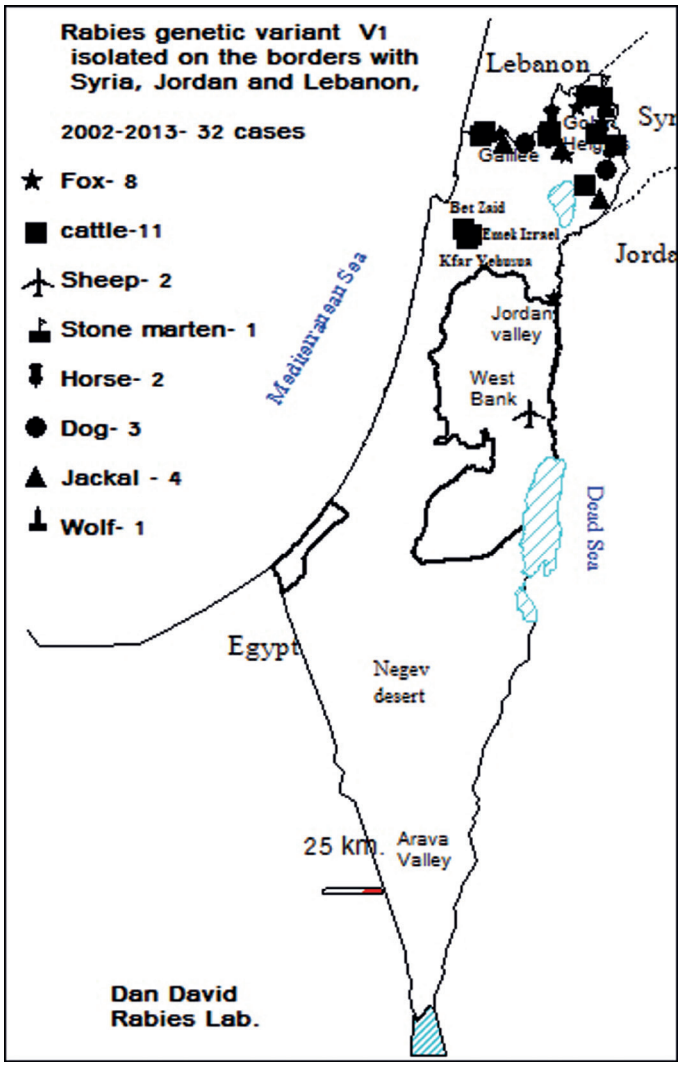

Figure 3: Map of Israel showing the area of northern Israel where 32 rabies virus belonging to the VI genetic variants were isolated during 2002 through 2012 on the borders with neighbors countries; the three positive calves isolated in Emek Yezre'el the Jackal and the fourth calf in Kibbutz Ortal.

logical analysis the 4 calves reported here were most likely bitten by the rabid Jackal. We therefore assumed that the calves were infected by the rabid jackal that had entered Kibbutz Ortal. This notion is supported by the chain of events described above and the identical molecular findings in the infected calves and Jackal.

The incubation period of rabies virus in cattle varies from 20 to 165 days (23). In our case the period of incubation was between 1 to 3.5 months. Various sanitary measures were implemented in Ortal and two farms in Emek Yezre'el as a result of this case: quarantine of cattle at the Ortal dairy farm and quarantine and vaccination of all the calves at the two farms in Emek Yezre'el.

Vaccination of cattle is not mandatory in Israel however, this case illustrates the importance of the current recommendation of the Israeli Veterinary Services and Animal Health to vaccinate cattle in rabies areas, where a high risk exists of rabies-infected wildlife penetrating its borders.

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Determination of Macroelement Parameters in Different Productive Stages of Simmental Cows

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ABSTRACT

The results of inorganic blood parameters are presented in the periparturient period and during lactation of Simmental dairy cows. Serum calcium and inorganic phosphorus in blood of dairy cows in puerperium were significantly lower ($p<0.05$) compared to the values in the blood of dairy cows in advanced pregnancy and during peak of lactation, probably indicating the increased use of these macro-elements by the Simmental cows' mammary gland at the early stages of lactation. When the cows were in the periparturient period serum magnesium levels were significantly lower ($p<0.05$) compared to the values of dairy cows during their maximal lactation probably indicating the increased use of magnesium by the Simmental dairy cow during the periparturient period.

Keywords: Simmental cows; Blood Serum; Calcium; Phosphorus; Magnesium.

INTRODUCTION

Deviation from the normal values of the inorganic blood parameter status of cows in early lactation, as well as their deficiency in the diet can lead to subclinical or clinical manifestations (puerperal paresis, tetany), which have a negative impact on health and fertility (1, 2, 3, 4, 5). Late pregnancy and the beginning of lactation present a burden for the cow's physiology, as it has increased demands in calcium, which is necessary for the construction of the fetal skeleton. Phosphorous represents the second most important macro-element for bone tissue production. In addition, it participation in the cellular process of phosphorylation and energy production, affects acid-base balance and plays a role in the detoxification process (6, 7).

Determination of calcium, magnesium and inorganic phosphorus in the blood serum is of important diagnostic value in preventing puerperal paresis and other diseases (8, 9). However, their values can differ in cattle for many reasons, among which is the breed (10). The values of the above mentioned macro-minerals in Simmental cows particularly at different production stages are lacking in the literature. This study was aimed at determining and comparing levels of serum calcium, inorganic phosphorus and magnesium preparturienly, peri-parturiently and during the peak of lactation in Simmental dairy cattle.

MATERIALS AND METHODS

Animal selection and study design

The study was conducted in a dairy cattle farm consisted of 150 Simmental cows. In total, 45 clinically healthy cows of 3 different productive stages were selected. The animals of the 1st group (15 cows - group A) were close-up cows, at the

last 15 days before the expected parturition. The animals of the 2nd group (15 cows - group B) were fresh cows 1 to 15 days after calving, while the 3rd group (15 cows - group C) consisted of high-production cows, from the 90th to 100th day of lactation. All the included cows were in their 3rd lactation and their average milk production was 6,825± 305 liters per lactation period.

Feeding and housing were in accordance with the intended use of the animals. Diet composition was adjusted to the requirements of cows during pregnancy and lactation. The feeding regime of the cows at different production stages is presented in Table 1.

Cows were judged to be clinically healthy for the duration of the study.

Table 1: Feeding cows before and after calving

Cows before the calving were fed daily	Cows after calving and during lactation consumed daily
3 kg alfalfa hay	1,800 Kg alfalfa hay
Wheat straw 3 kg, 10 kg	15 kg of corn silage (30% dry weight)
Corn silage (30% dry weight)	8 kg of alfalfa silage
4 kg alfalfa haylage	4 kg of maze ear silage (68% dry weight)
2 kg maize ear silage (68% dry weight)	2 kg of dry sugar beet pulp
0.5 kg dry sugar beet pulp	2 kg exstruded soybeans
1.5 kg of additional mixtures (30% of total protein)	4.5 kg additional mixtures (30% of total protein)

Blood sampling

Blood sampling of all the tested cows was conducted once, by jugular veni-puncture into 20 ml sterile vacuum glass tubes. All samples were drawn between 10:00-12:00 a.m. The samples were placed in ice and were forwarded to the laboratory within an hour; sera were separated by centrifugation at 2,500 × g for 15 min. Thereafter the sera were placed in plastic tubes (1.5 ml) (Eppendorf, Germany) and stored at -20°C until analysed. The concentration of calcium and magnesium was determined by atomic absorption spectrophotometry (AAS), and inorganic phosphorus by a spectrophotometrical assay kit (Inorganic Phosphorus Assay Kit, Bioo Scientific Corporation, USA) using the spectrophotometer, UV-1800 (Shimadzu, Japan). Serum levels of calcium, magnesium and phosphorus in sera were determined by the standard method of by atomic absorption spectrophotometry (Serbian SRPS ISO 6869 2002 using UNICAM 969, USA).

Statistical analysis

The ANOVA procedure followed by LSD *post hoc* was used to analyze the difference between analyzed groups of Simmental cows. The differences with p-values of $p<0.05$ were considered significant (95% significance). Statistical analysis was carried out by Ver.5.0 Microsoft Statistical Stat.Soft.Inc.1995.

Ethical compliance

Statement of ethical compliance: The experiment was done in compliance with Serbian Law on Animal Welfare (Official Gazette of the Republic of Serbia No 41/09) and Ordinance on the conditions for registration for experimental animals and the keeping of such register, training programs on welfare on experimental animals, request forms for approval of conducting experiments on animals, standing, treatment and killing experimental animals and reproduction, circulation, or implementation experiments on animals (Official Gazette of the Republic of Serbia No 39/10).

RESULTS

Results of the concentration of inorganic phosphorus in serum of tested Simmental cows are shown in Table 2. Significantly higher ($p<0.05$) levels of phosphorus were in

Table 2: Serum inorganic phosphorus concentrations (mmol/l) from all the sampled cows (groups A, B and C)

Productive stage	Group	N	X	SD	CV	P<0.05
Close-up dry cows	A	15	2.09	0.43	32.55	A:C
Fresh cows	B	15	1.97	0.39	30.98	B:C
High-production cows	C	15	2.40	0.38	18.93	

Table 3: Serum magnesium concentration (mmol/l) from all the sampled cows (groups A, B and C)

Productive stage	Group	N	X	SD	CV	P<0.05
Close-up dry cows	A	15	1.02	0.26	43.25	
Fresh cows	B	15	1.09	0.32	44.76	A:C
High-production cows	C	15	1.28	0.20	29.23	

Table 4: Serum calcium concentrations (mmol/l) from all the sampled cows (groups A, B and C)

Productive stage	Group	N	X	SD	CV	P<0.05
Close-up dry cows	A	15	2.35	0.24	21.98	
Fresh cows	B	15	2.17	0.22	23.56	B:C
High-production cows	C	15	2.53	0.26	19.23	

N = Number of cows
X = Mean
SD = Standard deviation
CV = Coefficient of variance

group C compared to group B. The mean serum phosphorus concentration of close-up dry cows of group A was significantly higher ($p<0.05$) compared to fresh cows of group B. Variations in serum inorganic phosphorus concentration was not high within tested groups; the most pronounced variability was recorded in the fresh cows group (CV = 32.55%).

Results of serum magnesium concentration are shown in Table 3. The highest mean magnesium concentration was found in high-production lactating Simmental cows (1.28±0.20 mmol/l). Mean magnesium values were significantly higher ($p<0.05$) in high-production lactating cows (group C) compared to close-up dry cows (group A). Magnesium levels in the blood of tested cows had relatively high variations within tested groups: in range from 0.20 to 0.32 mmol/l and the coefficient of variation varied from 29.30% to 44.76%.

Results of serum calcium concentrations are shown in Table 4. Mean calcium concentration of fresh cows (group B) was significantly lower in comparison with those of high-production cows of group C ($p<0.05$). The variability of serum calcium concentrations among the three groups of cows was not high, being most pronounced in the fresh cows (CV=23.56%).

DISCUSSION

Lactating dairy cows use large quantities of calcium for milk synthesis (11). Hypocalcemia in intensively managed dairy cows causes periparturient paresis and contributes to "downer cow syndrome", while during lactation it can also cause fertility disorders (2,6). The results of the present study indicate that in a group of Simmental dairy cows in puerperium, lowest calcium levels were found, and these values were significantly lower than the level of calcium in the blood in Simmental cows in advanced pregnancy and during maximum lactation. These findings are similar to the findings of other researchers, who suggest that a sudden loss of calcium from the body occurs in the puerperal cows, as for every liter of colostrum 1.0-2.0 g of calcium is consumed (7), while a physiological decline of calcium in the blood is held steady for several days and on account of intensive production, serum calcium decrease is correlated with the appearance of puerperal paresis (7). Calcium metabolism is closely related with phosphorus metabolism and is responsible for the proper metabolism of vitamin D (12, 13).

Inorganic phosphorus levels in the blood of cows are at low concentrations in the range of 2.1-3.0 mmol/l, while significantly more phosphorus is bound to organic compounds such as phospholipids (14, 15, 16). Similarly to serum calcium, inorganic phosphorus values were significantly lower in Simmental cows in puerperium compared to the values of inorganic phosphorus in the blood of cows in advanced pregnancy and during maximal lactation. Grünbergand coworkers (17) found that hypophosphatemia is present in the blood of highly productive Holstein-Friesian cows throughout the postpartum period which is associated with the entry of glucose in the glycolytic path of peripheral tissues, as well as to supply the necessary quantities of mammary gland phosphorus. The authors believe that hypophosphatemia allows proper glucose metabolism which prevents ketosis immediately after calving (6, 18). The sudden loss of phosphorus from the body occurs in the puerperal cows when 0.8-1.9 g phosphorus per liter of colostrum is spent. This physiological decline of phosphorus in the blood is held steady for several days and the intensity of phosphate fall is correlated with previous findings that phosphate blood level falls in older animals (19). The results of these tests show a decrease in serum calcium and inorganic phosphorus in the blood of Simmental cows in early lactation, suggesting increased use of these macroelements by the mammary glands. Our results show slightly higher calcium, phosphorus and magnesium blood serum levels than in Jersey cows but similar to levels of Holstein breed cows (12, 20), on the other hand calcium and magnesium blood sera levels were found to be lower than in beef cattle such as Angus, Charolais and Hereford, or Limousin (10).

Magnesium, like calcium reduces neuro-muscular irritability and a drop in its concentration in the blood results in spontaneous muscle contractions or tetany (1). The results of this study indicate that the lowest magnesium levels were detected in Simmental cows in advanced pregnancy and that greater values of magnesium in the blood were determined in cows in puerperium. However the differences of these values were not significant. The highest values of magnesium in the blood were detected in high production Simmental cows during the peak of lactation which were significantly higher compared to the values of the cows in advanced pregnancy and the puerperium. The results obtained in this study indicate that the magnesemia was within the normal range in all tested cows, and lower values were found in a group of cows in the periparturient period. This probably points to the

increased use of magnesium during negative energy balance in early lactation.

CONCLUSION

Serum calcium and inorganic phosphorus in the blood of Simmental dairy cows in puerperium were significantly lower ($p<0.05$), compared to the values of cows in advanced pregnancy and during maximum lactation, indicating the increased use of these macro-elements by the mammary gland of cows in early lactation. For the cows in the periparturient period, values of magnesium in the blood were significantly lower ($p<0.05$), compared to the values during the periparturient period.

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The Influence of Subclinical Hypocalcemia on Production and Reproduction Parameters in Israeli Dairy Herds

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ABSTRACT

A large percentage of mature dairy cows experience some degree of hypocalcemia during the first days post-calving. In some cases calcium concentrations decline to levels that disrupt neuromuscular function, resulting in the clinical syndrome known as parturient paresis or milk fever. Post-parturient hypocalcemia is divided into clinical and subclinical forms. It has been established that cows suffering from clinical milk fever are susceptible to a variety of secondary conditions, however to the best of the authors' knowledge there has been no evaluation of the impact of the subclinical form on production and reproductive parameters. The objective of this study was to investigate the association between subclinical hypocalcemia and post-parturient disorders, production and reproductive parameters in Israeli dairy herds. Blood results for corrected calcium concentrations were analyzed from 634 mature cows from 5 farms. The subclinical hypocalcemic cows produced 3.2, 2.7 and 1.9 kg more milk in the first three milk recordings than the normocalcemic cows. Subclinical hypocalcmic cows did not show an increased risk for post-parturient diseases nor compromised reproduction parameters in comparison to normocalcemic cows. It was concluded that there was no negative impact of subclinical hypocalcemia on production and reproductive parameters in Israeli dairy cows after parturition.

Keywords: Bovine; Milk Fever; Calcium; Hypocalcemia; Milk Production.

INTRODUCTION

Parturient paresis is a metabolic disorder occurring close to parturition especially in high producing dairy cows. The disease is characterized by a rapid decline in blood calcium (Ca) concentrations. Nearly all mature cows experience some degree of hypocalcemia during the first day after calving as the intestine and bone adapt to the Ca demands of lactation (1). In some cows, the mammary drain of Ca causes extracelluar and blood Ca concentrations to decline to levels that disrupt neuromuscular function, resulting in the clinical syndrome of "Milk Fever". This Ca decline lasts in some cases for several days postpartum (2).

Post-parturient hypocalcemia is divided into clinical and subclincal forms (3, 4). The literature indicates that the clinical form is associated with an increase in post-parturient diseases (5, 6, 7, 8). In large parts of the United States and some European countries it is assumed that the subclinical form is also related to post parturient diseases (5, 9, 10, 11) and has a negative impact on profitability. Therefore, many dairies use expensive feed additives to reduce the incidence of this form (12, 13). Fatty acid metabolism might differ between cows with subclinical hypoclacemia and their normocalcemic counterparts (14) however, there is conflicting evidence regarding the impact of subclinical blood calcium levels on milk production, reproduction parameters and post-parturient disorders (10, 14, 15). Most of the research

published targets methods to improve calcium homeostasis through manipulations of dietary cationic anionic difference or through calcium binders (16, 17, 18, 19) however to the best knowledge of the authors' the direct impact of the subclinical form on production and reproductive parameters has not been adequately investigated. The objective of this study to investigate whether the subclinical hypocalcemia state has an influence on post-parturient diseases, reproductive parameters and milk production parameters in Israeli dairy herds.

MATERIALS AND METHODS

Animals and study design

The study was comprised of two separate entities: The first study, the preliminary study was conducted on a 60 cow dairy farm in the northern part of Israel. Cows were housed in large covered loose housing systems and fed dry cow total mixed ration (TMR) pre-calving and a standard milking TMR post-calving both manufactured by Givaat Yoav Feeding Center located in Moshav Givaat Yoav, Israel. Blood was drawn from the coccygeal vein of 11 mature Holstein cows at a 4 hour interval from the beginning of first stage of labor and up to 12 hours postpartum. Blood was drawn once more at 24 hours postpartum. Samples were immediately centrifuged and serum was harvested and frozen at -20°C for laboratory analysis at the Kimron Veterinary Institute, Beit Dagan. According to these results a post-calving calcium curve was prepared to determine the time of nadir levels of serum calcium.

The second study, the main study was conducted on 4 commercial Israeli dairy herds of 200-450 milking cows each between June 2006 and July 2007. Blood was drawn from 633 mature Holstein cows between 8-20 hours postpartum when serum Ca levels were expected to be the lowest based on the results of the first preliminary study. Samples were immediately centrifuged and serum was harvested and frozen at -20°C for further laboratory analysis.

All cows were either housed in free stalls or large covered loose housing systems. All herds were fed dry cow TMR pre-calving and a standard milking TMR post-calving. All farms received their feed from different feed manufactures (Table 1).

In all herds cows were milked 3 times daily identified by ear tags and freeze marking in computer controlled milking parlors. The annual milk production was 10,000-12,500 kg/cow. The herds were within the practice area of the "HaChaklait", a mutual society for veterinary medicine which provides a complete herd-health service. Visits are conducted by a veterinarian to the farms during the study period at least twice weekly.

Table 1: Farm name and feed manufacture

Farm	Feed manufacturer
Kibbutz Geshur	Givaat Yoav Feeding Center, Moshav Givaat Yoav, Israel
Kibbutz Beit Zera	Amabar Feeding Center, Moshav Kefar Yehezkel, Israel
Kibbutz Givaat Haim Meuhad	Amatz Feeding Center, Moshav Amatz, Israel
Kibbutz Afikim	Kibbutz Afikim, Israel

All cows were examined after calving by trained veterinarians who diagnosed, treated and recorded all the periparturient disease conditions. Cases of retained fetal membranes (RFM) were defined as the presence of placental tissues 24 hours or more after calving as observed by trained farm employees or the attending veterinarian. Animals with observed or suspected RFM were submitted for veterinary examination on the next routine veterinarian visit (1-4 days postpartum). Animals without a history or diagnosis of RFM were submitted for examination between 6 and 9 days postpartum. At this examination, body condition scoring (BCS) of all animals was recorded and cows were comprehensively examined by intravaginal palpation after thoroughly cleaning the perineal area. The diagnosis of clinical endometritis (CEM) was based on the combined characteristics of vaginal discharge obtained by manual examination of the vagina. Affected cows with CEM had a watery or purulent, fetid vaginal discharge as previously described (20).

All cows were examined for ketosis by placing a drop of urine obtained with a sterile disposable plastic catheter on a reagent strip (Ketostix, Bayer, Germany). The color reaction was compared to the standardized color chart after 15 seconds. Cows with urine aceto-acetate concentrations above 15 mg/dl were recorded as ketotic (3). Cows with lower than expected milk production and poor appetite were examined for displacement of the abomasum (LDA) by auscultation and percussion. BCS was further recorded approximately 40-60 days after calving and before the dry-off period. All animals not observed in estrus by the end of the voluntary waiting period at approximately 60 days postpartum were recorded and submitted for examination.

Clinical, reproductive, production and management data were computer recorded by the herd manager and the attending veterinarians. Cows not observed in estrus were recorded for further reproduction calculation as cows not showing heat. Once a month, each cow's milk was sampled and analyzed for fat, protein, lactose and somatic cell count by the Central Laboratory for Milk Recording at the national service for udder health and milk quality located in Caesarea industrial park, Israel.

Reproductive management was solely based on artificial insemination performed by trained technicians employed by "Sion" Israeli Company for artificial insemination and breeding, Migdal Ha'emek, Israel. In all herds, cows were mainly inseminated on observed estrus or computerized pedometry system. Conception rates and cumulative pregnancy were based on pregnancy diagnosis performed by rectal palpation of the uterus and its contents 40-50 days post-insemination.

Blood analysis

Total serum calcium levels were determined using Arsenazo III method and calcium levels were corrected for serum albumin which was determined using the Bromocresol Green method. Correction was done using the following equation (3).
Corrected Calcium (mg/dl) = Measured Calcium (mg/dl) – Albumin (g/dl) + 3.5

Statistical analysis

All data editing and analysis were performed using SAS version 9.0 (21). Results were considered to be of statistical significance if the relevant *P*-value was < 0.05.

In general, data analysis followed a 3 step approach: (1). Descriptive statistics which included calculation of the mean, standard deviation and histogram for continuous variables, and frequency tables for other variables (2). Bivariate analysis in which associations between a dependent variable and an independent variable were assessed using the chi-square test for categorical variables and t-tests when one of the variables was on a continuous scale. For time to event data, i.e. days from calving to conception, survivor functions were compared using the Kaplan-Meier method and log-rank test (3). Multivariable analysis in which associations between the dependent variable and two or more independent variables were assessed. Multivariable analysis was only performed if, for the same dependent variable, two or more significant associations were found in the bivariate analysis. In our case, this only occurred in the analysis of milk production data. For the latter, average test-day milk (kg) was estimated from monthly test-day data using a linear model with a marginal effect to account for repeated measurements from the same cow. A maximum of 10 test-days was allowed per cow. Lactation number was grouped into four categories, ie. second, third, fourth and fifth, or greater lactation. Summer months were considered June to September, inclusive.

Subclinical hypocalcemia was defined in a cow with a corrected serum Ca level of < 7.5 mg/dl and without clinical hypocalcemia 12-24 h postpartum. Somatic cell counts (cells/mL) were grouped in 4 categories: ≤ 100,000; 101,000-200,000; 201,000-400,000 and > 400,000. Farms were modeled as a fixed effect and the correlation matrix used for R was autoregressive. The model we used was:

Y = farm (4 index variables) + summer (2 index variables) + lactation (4 index variables) + MIM (10 index variables) + HCOR (2 index variables) + MIM + HCOR*MIM + SCCL (4 index variables) + CEM (2 index variables) + e.

Y was test-day fat percentage, summer represents test days occurring in the summer months, lactation was lactation group, MIM was month in milk, HCOR was subclinical hypocalcemia, SCCL was somatic cell count level, and "e" a complex error term representing the within-cow correlation of test-day fat percentage and the residual error. Significance of the fixed effects was determined using the *F*-test (21).

RESULTS

Preliminary study

Corrected serum calcium levels obtained from all 11 cows were combined into an average level for each 4 hour interval. These average levels were plotted by time from calving. Calcium levels decreased between calving and reached a nadir at 8 hours-calving and stayed low until 20 hours post-calving. Although not statistically significant, based on these results, 8-20 hours post-calving was chosen as the period of the nadir of serum calcium levels for the main study. (Figure 1).

Main study

Data sets included measurements from 634 cows. 247, 186, 103 and 97 cows from second, third, fourth, fifth or greater lactations, respectively. There was missing data on calving disease incidence for one cow and 16 cows suffered from clinical

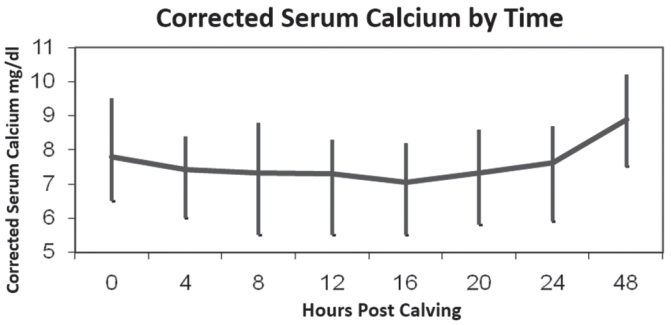

Figure 1: Corrected serum calcium (mg/dl) by time from calving (hours)

milk fever. Therefore a total of 617 cows were included in the final data set.

Of all cows 18.9% suffered from subclinical hypocalcaemia. Milk fever incidence was 7.63% and 1.36% for the subclinical hypocalcemic and normocalcemic cows respectively. The probability for development of clinical milk fever was found to be statistically higher in the subclincial hypocalcemic group as opposed to the normocalcemic group of cows. ($P < 0.0007$) (Table 2).

The probability for a cow developing subclinical hypocalcemia increased with lactation number ($P < 0.0001$) (Table 3).

Table 2: Milk Fever by Subclinical hypocalcaemia

Subclinical Hypocalcemia	Milk Fever		Total
	No	Yes	
No	508	7	515
%	98.64	1.36	
Yes	109	9	118
%	92.37	7.63	
Total	617	16	633
%	97.47	2.53	100

Table 3: Subclinical hypocalcaemia by lactation

Lactation Number	Subclinical Hypocalcemia		Total
	No	Yes	
2	235	12	247
%	95.14	4.86	
3	164	22	186
%	88.17	11.83	
4	63	40	103
%	61.17	38.83	
>=5	53	44	97
%	54.64	45.36	
Total	515	118	633
%	81.36	18.64	100

Calving diseases

Of the normocalcemic cows, 12% versus 11% of subclinical hypocalcemic cows suffered from retained placenta. There was no statistical difference between normocalcemic and subclinical hypocalcemic cows ($P < 0.897$). Of the normocalcemic 27.6% cows versus 19.3% of subclinical normocalcemic cows suffered from metritis however there was no statistical difference in the probability of a subclinical hypocalcemic cow suffering from metritis ($P < 0.087$). 21.3% of normocalcemic cows versus 25.7% of subclinical normocalcemic cows suffered from ketosis however there was no statistical difference in the probability of a subclinical hypocalcemic cow suffering from ketosis ($P < 0.376$). Only 2 cows from the normocalcemic group suffered from an LDA. 5.7% of normocalcemic cows versus 3.7% of subclinical hypocalcemic cows had stillbirths. There was no statistical difference in the probability of a subclinical normocalcemic cow to have stillbirths ($P < 0.533$).

Although subclinical milk fever (defined as corrected serum calcium below 7.5 mg/dl) was associated with milk fever incidence no association was found between serum calcium and calving diseases.

Reproduction

Thirty-two cows received a "do not breed" code or were culled before first insemination and therefore 585 cows were included in the reproduction analysis study.

There was no statistical difference ($P < 0.755$) between groups for the first artificial insemination (AI) conception rate. 29.7% vs. 27.6% for the normocalcemic and subclinical hypocalcemic cows respectively. There was no statistical difference ($P < 0.453$) between groups for cows not showing heat. 33.7% vs. 38.1% for the normocalcemic and subclinical hypocalcemic cows respectively. There was no statistical difference ($P < 0.958$) in the cumulative conception until 180 days in milk between both groups (Figure 2).

No associations were found between subclinical milk fever (defined as corrected calcium below 7.5 mg/dl) and reproduction parameters.

Milk production

Subclinical hypocalcemic cows produced significantly more milk when compared with normoclacemic cows. 3.17, 2.71 and 1.90 kg more milk was produced on the first, second and third test days, respectively (Table 4).

Table 4: Milk production by calcium group.

Test day	Subclinical Hypocalcemia	Estimate Kg	Standard error	P-value
1st test day	No	-3.1673	1.1607	0.0064
1st test day	Yes	0		
2nd test day	No	-2.7079	1.1508	0.0187
2nd test day	Yes	0		
3rd test day	No	-1.8951	1.1368	0.0956
3rd test day	Yes	0		

Subclinical hypocalcemic cows produced more milk in the first 6 milk test days (Figure 3).

DISCUSSION AND CONCLUSIONS

Many physiological pathways are dependent on blood ionized calcium levels. Decreased ionized calcium levels have been found to be associated with increased fat mobilization around calving (14) and these decreased levels could presumably influence the gastrointestinal track motility (22) leading to decreased feed intake and as a result an increased prevalence of metabolic disorders and other post-parturient diseases. It has also been shown that decreased levels of calcium stores in peripheral blood mononuclear cells precedes measurable hypocalcemia and that hypocalcemia at parturition further exacerbates the ability of these cells to release intracellular calcium in response to intracellular signals therefore impairing these cells' ability to be activated (8). These changes collectively could probably contribute to the impaired immune system of the periparturient cow and its increased susceptibility to infectious diseases (5, 6, 7, 8).

It would be reasonable to assume that these cows would show impaired reproductive performance and decreased milk production later in their lactation. However, our results demonstrate that in the Israeli high producing cow this subclinical hypocalcemia is not a risk factor for the development of postpartum diseases, furthermore we have demonstrated that subclinical hypocalcemic cows produce more milk. These results agree with previous studies which showed that hypocalcemia at calving is not a significant risk factor for decreased milk yield (14, 23). Our results of higher milk production for the hypoclacemic cows could be related to a higher genetic merit causing these cows to produce more milk and subsequently exhibit lower circulating calcium levels. On the other hand it must be pointed out that these milk parameters do not rule out the possible damage caused by these low circulating blood calcium levels. It has been demonstrated that lame cows produce more milk in comparison to their non-lame herd mates (25, 26). Despite these findings it is clear that lameness is a risk factor for decreased milk production. When comparing the impact of these two diseases it could be that the hypocalcemic cows in our study, although higher in milk than their normoclacemic herd mates could have produced even more milk had we corrected their circulating blood calcium levels. Furthermore, we did not analyze the quality of milk and whether calcium level had any effect on the immunoglobulins or other parameters of milk quality.

Our study had several limitations. First, due to the number of cows in the first study we made a subjective decision on the time of sampling which could have influenced the number of cows being defined as subclinical hypocalcemic. Second, we could not measure ionized calcium and therefore had to

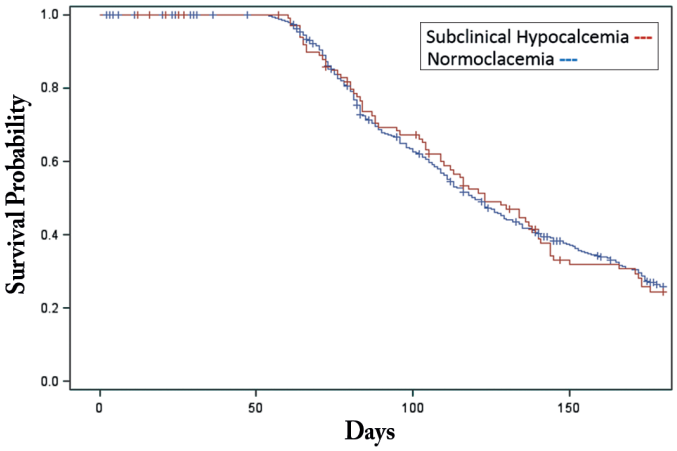

Figure 2: Cumulative conception until 180 days in milk

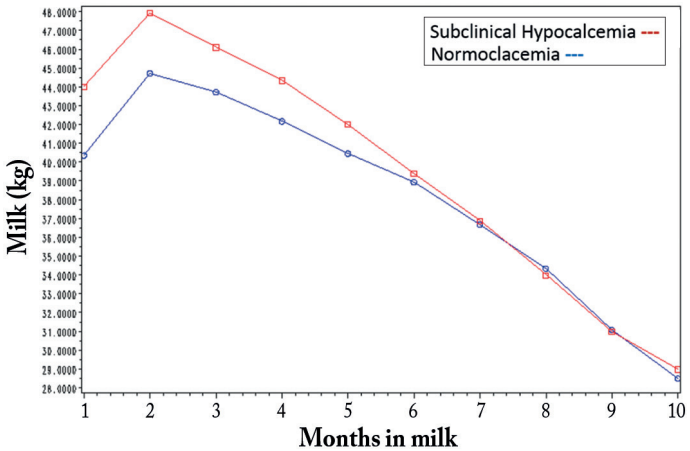

Figure 3: Milk production (Kg) by month

use the correction equation according to albumin levels. It is possible that this calculation of corrected calcium resulted in an underestimation of the true level of ionized calcium in the blood and that some cows in the subclinical calcium group should have actually been included as normocalcemic cows. On the other hand our study was done on several farms feeding from different rations, feeds and feed suppliers which gave substantial power to our results.

Taking into consideration other research done in this field, our results should be interpreted with caution and ideally should be validated by future large scale studies and further research.

In conclusion, subclinical hypoclacemia in the Israeli dairy herd does not seem to impair production and reproduction parameters. Therefore the authors do not find it reasonable or necessary to incorporate feed additives that reduce the incidence of this disorder as long as milk fever incidence remains in the normal range for Israeli dairy herds.

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Reproductive Performance in Dairy Cows Synchronized with the Ovsynch Protocol at Different Stages of the Estrus Cycle

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ABSTRACT

The objectives of the study were to evaluate reproductive performance in cows synchronized with the Ovsynch protocol at different stages of the estrus cycle as detected by pedometry, and to compare the reproductive performance of cows inseminated by Ovsynch in different stages of the estrus cycle (no observed estrus, 0-4, 5-12, 13-17 and 18-24 days after estrus) to cows inseminated by pedometry in Israel. Timing the beginning of Ovsynch protocol did not affect first artificial insemination (AI) conception rate, proportion of cows empty at 150 days in milk (DIM) or the time from calving to conception. Cows without observed estrus 25 days or more before initiation of the Ovsynch protocol had rate of cows not pregnant by 150 DIM in comparison to the control group (Odds Ration (OR) = 1.885, $P = 0.013$) and longer calving to conception interval than the control group (155.4 days and 135.7 respectively, $P = 0.018$). It was concluded that reproductive performance cannot be improved by combination of pedometry and timed artificial insemination (TAI); Ovsynch has low efficacy as a treatment for anestrus. However, other anestrus treatment options should be compared to Ovsynch.

Keywords: Bovine; Reproduction; Ovsynch; Anestrus; Estrus Detection; Reproduction; Timed Artificial Insemination; Pedometry; Ovsynch; Dairy; Anestrus.

INTRODUCTION

Reproductive efficacy is a key element in dairy farm management. A major component in reproductive management is estrus detection and artificial insemination (AI) of the cow at the correct time in the estrus cycle. In Israel, the vast majority of large dairy farms rely mainly on electronic estrus detection by pedometry or activity meters (1, 2). Recently, automated estrus detection aids have become more available and adopted worldwide (3-5). In farms without automated estrus detection, timed AI (TAI) has become widely accepted as a reproductive management tool. The Ovsynch protocol, first described by Pursley *et al.* in 1995 (6), is most widely used either on its own or in combination with pre-synchronization (7-10) or post insemination protocols (11, 12). The Ovsynch program consists of 2 injections of Gondotropin-releasing hormone (GnRH), 7 days before, and 48 hours after an injection of Prostoglandin F2 alpha ($PGF_{2\alpha}$). Cows are inseminated 16 to 25 hours after the second injection of GnRH (6). The advantages of Ovsynch include the lack of need for estrus detection by visual or by expensive electronic means, high insemination rates, reduced losses due to trauma or mastitis that are estrus related, and flexibility in the number of cows to be synchronized. Additionally, AI at a fixed times allows for on farm concentrated work effort for hormone injections, for AI in a predicted time, and Ovsynch was also described as a treatment for acyclic cows (13-15). It has been reported that cows inseminated using the Ovsynch protocol conceived in comparable rates to the conception rates (CR) of cows

detected in estrus, but with higher pregnancy rates (PR) due to the higher insemination rates of the Ovsynch treated cows (1, 6, 10, 16-18).. The best conception rates of synchronized insemination are achieved when the Ovsynch protocol is initiated at days 5-12 of the estrus cycle. Pre-synchronization protocols evolved to improve conception rates with the aim of the most commonly used pre-synchronization protocol, Presynch, to achieve higher proportions of cows that are 5-12 days post estrus at the beginning of the Ovsynch protocol. This is done by injecting $PGF_{2\alpha}$ twice 14 days apart and initiation of the Ovsynch protocol 11-14 days after the second $PGF_{2\alpha}$ treatment, thus contributing to higher conception rates at TAI (7, 9).

The extensive use of estrus detection systems in Israeli dairy farms makes the sweeping use of TAI protocols redundant. However, cows not detected in heat or repeat breeders are synchronized in some farms. The intelligent use of TAI in cows that are detected in estrus and are not inseminated due to technical reasons (religious considerations – Saturdays and holidays, or other reasons) according to the known estrus date may yield better reproductive performance. Knowledge of the estrus date (recorded by pedometry) could be used as a substitute to the Presynch protocol in order to time the beginning of the Ovsynch protocol to days 5-12 in the estrus cycle.

The objectives of this study were to compare conception rates between cows treated with Ovsynch protocols initiated at different periods of the estrus cycle detected by pedometry and to compare these to conception rates in cows inseminated by pedometry alone. Finally, we attempted to evaluate the efficacy of Ovsynch as a treatment for anestrus.

MATERIALS AND METHODS

Study design and animals

The study was designed as a retrospective cohort study in a sample of cows that were inseminated for the first time in lactation between January 2006 and December 2007. The study was conducted in two commercial Israeli dairy herds consisting of 450 and 550 Israeli Holstein cows. Cows were housed in loose housing systems in large, completely covered open sheds and fed total mixed ration (TMR) *ad libitum*. In both herds, cows were milked three times daily in computer controlled milking parlors. Mean 305 day milk production was approximately 11,500 kg per cow. All cows were identified by ear tags, electronic identification tags (SAE, Afikim, Israel) and freeze marking. The herds were within the practice area of the Ambulatory Clinic of The Koret School of Veterinary Medicine, which provided a complete herd-health service. Both herds were visited at least three times weekly during the trial period. Clinical, reproduction, production and management data were recorded to the farm management software ("NOA", Israeli Cattle Breeders Association). Both farms performed monthly milk recordings through the Central Laboratory for Milk Recording. Reproductive management was solely based on AI performed by highly trained technicians employed by the Artificial Insemination Service of the Israel Cattle Breeders Association (ICBA). In both herds, cows were equipped with computerized pedometry systems (SAE, Afikim, Israel). Pregnancy diagnosis was performed by transrectal palpation of the uterus and its content 45-51 days post insemination.

Clinical Examination

All cows were examined routinely between 5 and 12 days after calving by trained veterinarians, who diagnosed, treated and recorded periparturient diseases as described previously (19).

Body condition scoring (BCS scale: 1 = thin, 5 = obese) was recorded before dry-off, at the routine post-partum examination (5-12 days after calving) and between 40 to 60 days after calving (peak yield BCS).

Ovsynch and treatment groups

The Ovsynch group (OS) included cows treated with the Ovsynch protocol. Hormonal synchronization was used on the farms as part of a study evaluating the efficacy of Ovsynch in comparison to pedometry, or for re-synchronization of animals detected in heat during Saturdays or holydays (the farms did not inseminate cows during Saturdays for religious reasons). OS Cows were treated with IM injection of 150 μg Gonadorelin Acetate (Gonabreed, Parnell Laboratories, New Zealand) followed seven days later by IM injection of 500 μg Cloprostenol Sodium (Estrumate, Schering-Plough, Germany), followed two days later with another IM injection of 150 μg Gonadorelin Acetate. OS cows were insemination 16-24 hours after the last GnRH injection regardless of heat signs.

Cows from the OS group were individually matched to the control cows by farm, calving year and parity. When possible, the calving season, calving diseases and calving to first AI interval (voluntary waiting period) of the matched control cow were also similar.

In the second part of the study the cows were divided into six groups:

1. **Control (CO)** – cows inseminated at the detected heat.
2. **Anestrus (AN)** – cows inseminated after treatment with Ovsynch protocol with no detected estrus in the previous 24 days.
3. **Ovsynch at days 0-4 (OS4)** – cows inseminated after treatment with Ovsynch protocol 0-4 days after detected estrus.
4. **Ovsynch at days 5-12 (OS12)** – cows inseminated after treatment with Ovsynch protocol 5-12 days after detected estrus.
5. **Ovsynch at days 13-17 (OS17)** – cows inseminated after treatment with Ovsynch protocol 13-17 days after detected estrus.
6. **Ovsynch at days 18-24 (OS24)** – cows inseminated after treatment with Ovsynch protocol 18-24 days after detected estrus.

Statistical analysis

Computerized data were retrieved from the farm computers and ICBA central computer and analyzed using Excel (version 2010, Microsoft, Redmond, WA) and SPSS 17.0.1 (SPSS Inc., Chicago IL, USA). Lactation Incidence Risk (LIR) for all recorded diseases and reproductive performance parameters were evaluated for Control and Ovsynch cows. The same evaluation was done for cows in the different Ovsynch timing groups.

Reproductive performance parameters included first artificial insemination conception rate (1st AI CR), the interval from calving to pregnancy (Days Open), time from 1st AI to conception (Waste days) and the proportion of cows not pregnant > 150 days after calving (Empty at 150 DIM ie. Days in Milk = Days from Calving).

The effect of Ovsynch on dichotomous outcome variables was evaluated using conditional multivariable logistic regression modeling.

Separate analysis was done for Ovsynch versus Control cow and for Ovsynch timing groups as an independent variable. Various parameters and disease conditions were tested in the models as possible confounders. These included: farm, calving season (May to September = Summer; October to April = Winter), twin calving, retained placenta (RP, retained fetal membranes ≥ 24 hrs. post calving), clinical metritis (CM), ketosis, displaced abomasum, body condition loss ≥0.5 between calving and peak milk yield, mastitis at calving, mammary edema at calving, stillborn calves and induction of calving. Crude bivariate associations of outcome and potential confounding variables with treatment were initially assesses by use of Pearson χ^2 asymptotic 2-sided tests of significance for reproductive dichotomous parameters and student's T test for waiting period and days empty. To build the models, the Control versus Ovsynch timing group variables were forced into the model and subsequent covariates with significance of $P \leq 0.25$ were included in primary multivariate models. The final models were built with entry criteria set at $P < 0.05$ and exit criteria set at $P > 0.10$. For all analysis, values of $P < 0.05$ were considered significant. The Odds Ratios (OR) were calculated for all variables in the model.

The conditional logistic multivariate analysis was done using the Cox regression procedure for dichotomous outcome variables with pairing as strata, and linear regression procedure was used for scale outcome variables. The effects of Ovsynch treatment and its timing in the estrus cycle on days open were evaluated using Kaplan-Meier survival analysis. As the segmentation of the waste days is not equal, there is an association between specific period of times and elevated risk for conception. Also the risk of conception is higher on the first AI and declining later. To compensate for these facts, the effect of the treatment was analyzed using a Proportional Odds model as proposed by Cox.

For all survival analyses, days open were limited to 200 days, cows not pregnant at 200 DIM were considered as empty.

RESULTS

Descriptive statistics

During the study period 267 cows were treated by Ovsynch in the study farms. Control cows were matched and differed from the Ovsynch cows only in waiting days (Table 1).

Reproductive performance

The Ovsynch protocol and the timing of its beginning in the estrus cycle did not affect significantly the 1st AI CR (Table 2).

Table 1: Descriptive statistic of the calving diseases LIR and waiting period of the Ovsynch and Control cows.

Parameter	Ovsynch	Control	Overall	P value
Farm:				
A	197	197	394 (73.8%)	1
B	70	70	140 (26.2%)	1
Parity :				
1	95	95	130 (35.6%)	1
2	61	61	122 (22.8%)	1
3+	111	111	222 (41.6%)	1
Summer Calving's	37.1%	35.2%	36.1%	0.652
RP[1]	13.9%	17.2%	15.5%	0.282
CM[2]	37.1%	36.3%	36.7%	0.857
Ketosis	19.1%	16.5%	17.8%	0.428
Waiting period[3]	100.7	95.9	98.3	0.007

1 – Retained placenta, retained fetal membranes ≥ 24 hrs.
2 – CM – Clinical/Puerperal metritis.
3 – Waiting period – calving to 1st service interval.

Table 2: Descriptive statistics of different reproductive parameters in the control (Inseminated at estrus) and the different study groups.

		Ovsynch Group*					Over all**
	Control	2	3	4	5	6	
N	267	93	32	98	25	19	267
1st AI CR[1]	39.7%	35.5%	46.9%	34.7%	40%	31.6%	36.7%
Empty >150 DIM[2]	36.7%	54.8%	46.9%	36.7%	48%	31.6%	44.9%
Days empty[3]	144.9	169.4	163.1	147.5	161.5	145	158.1
Waste days[4]	27.1	28.9	33.6	23.5	30.5	24.1	27.3

* group 2 – Ovsynch protocol started ≥25 days from estrus or no estrus before Ovsynch, group 3 – Ovsynch protocol started 0-4 days after estrus, group 4 – Ovsynch protocol started 5-12 days after estrus, group 5 – Ovsynch protocol started 13-17 days after estrus and group 6 – Ovsynch protocol started – 18-24 days after estrus.
**Over all – all Ovsynch cows.
1 1st AI CR – the proportion of cows conceived from 1st artificial insemination.
2 Empty >150 DIM – the proportion of cows not pregnant > 150 d after calving.
3 Days empty – Mean calving to conception interval. Maximum value is 250 days.
4 Waste days – Mean 1st AI to conception interval.

Table 3: Logistic regression model summaries of the relationship between the cyclic status of the cows when Ovsynch is initiated and the Empty at 150 DIM in cows inseminated by pedometry or by Ovsynch.

Variable	β	S.E.	df	Sig.	O.R.	95% CI for O.R	
						Lower	Higher
Group: Control			2	0.047	Reference		
Anestrus Ovsynch[1]	0.634	0.256	1	0.013	1.885	1.141	3.113
Cyclic Ovsynch[2]	0.170	0.209	1	0.416	1.185	.787	1.786
Parity :1			2	<0.001	Reference		
2	-0.045	0.278	1	0.871	1.885	1.141	3.113
3+	0.861	0.232	1	<0.001	1.185	0.787	1.786
Calving Diseases[3]	0.353	0.202	1	0.081	1.423	0.957	2.117
Long Waiting period[4]	1.073	0.222	1	<0.001	2.923	1.890	4.520
Constant	-1.403	0.266	1	<0.001	0.246		

1 – Cows inseminated after treatment with Ovsynch protocol with no detected estrus in the previous 24 days.
2 – Groups 3-6 combined. The cows had a detected estrus 24 days or less prior to the beginning of the Ovsynch protocol.
3 – Retained placenta, clinical metritis and ketosis.
4 – Calving to 1st AI interval ≥108 days (higher quartile).

The control cows Empty at 150 DIM rate was lower than in the Ovsynch cows (36.7% and 44.9 respectively; OR = 0.817, $P = 0.0321$, table 2). The timing of the Ovsynch protocol in the estrus cycle affected the probability of groups 1 and 5 to be Empty at 150 DIM.

Only group 1 (Anestrus group) differed from the other Ovsynch groups. For further analysis of cows Empty at 150 DIM, the cyclic Ovsynch groups (3-6) were united. The cyclic status of the cows at the initiation of the Ovsynch protocol affected significantly the Empty at 150 DIM rates. Cows inseminated by Ovsynch protocol without a detected estrous 24 days before the initiation of the protocol had higher Empty at 150 DIM rate in comparison to the control group (OR to be empty at 150 DIM = 1.885, $P = 0.013$, table 3). Cyclic cows inseminated by Ovsynch protocol did not differ from cows inseminated in pedometry detected estrus (Table 3).

Cows inseminated by Ovsynch had longer open periods than control cows (mean days open = 146.1 and 135.7 respectively, $P = 0.017$). This difference was mainly due to the cyclic status of the cows. Cyclic cows inseminated by the Ovsynch protocol did not differ from the control (mean days empty = 141.2, Figure 1), while the anestrus cows had significantly longer empty period (mean days empty = 155.4, Figure 1).

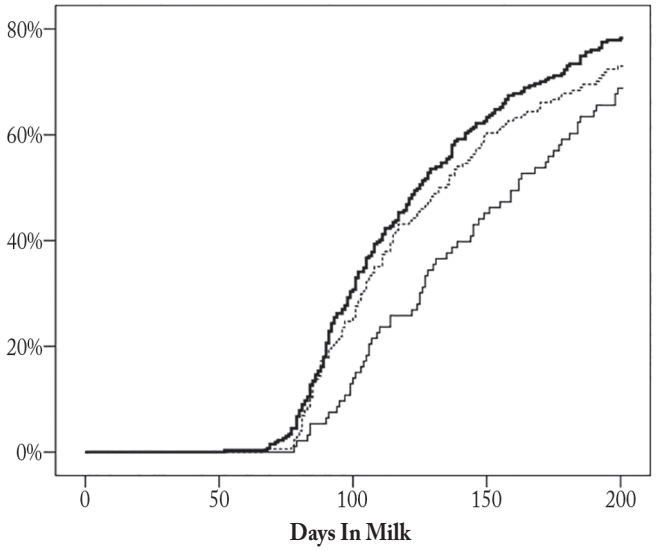

Figure 1: Survival plot of pregnancy rate in Control cows inseminated at estrus detected by pedometry (—), Cyclic cows inseminated by Ovsynch protocol (····) and Anestrus cows inseminated by Ovsynch (—). The mean survival time of the Anestrus cows differed significantly from the Control cows in the Log Rank test ($P = 0.018$, mean survival time of 155.4 and 135.7 respectively). The Cyclic cows did not differ from the Control group (mean survival time of 141.2 and 135.7 respectively).

Higher lactation numbers and lower body condition scores at peak yield were associated with reduced pregnancy rates. Post-partum endometritis was associated with reduced pregnancy rates, but the effect declined as DIM increased. Summer calving tended to reduce the pregnancy risk (OR = 1.079, P = 0.0607). The OR to become pregnant of cows inseminated by Ovsynch at 0-4 or 13-17 days after estrus was reduced (OR = 0.706, $P < 0.0001$ and OR = 0.881, $P < 0.0001$ respectively).

DISCUSSION

The novelty of this study is in combining the TAI with automated estrus detection systems, and by comparing the conception rate of cows inseminated by Ovsynch protocol started at different times of the estrus cycle. The main finding in this study was that the timing of the beginning of the Ovsynch protocol in the estrus cycle had no effect on 1st AI conception rate. This finding was unexpected as Presynch protocols are widely used for CR improvement (7, 8, 20, 21). The purpose of the Presynch protocols is to lower the proportion of anovular cows (22), and to time the beginning of the Ovsynch protocol to the period of time where maximal reproductive efficacy can be achieved at days 5-12 of the estrus cycle (20, 21). The start of the Ovsynch protocol at this timing is supposed to ensure the ovulation of smaller follicles at the timed AI, with higher CR (20). Modification of the Ovsynch protocol by adding presynchronization had improved pregnancy rates in previous studies (7, 9, 10, 23). However, the results of this study did not demonstrate advantage (as reflected in the measured reproductive parameter) to the starting of synchronization protocol at days 5-12 of the estrus cycle or to any other different timing. The beneficial effects of presynchronization with prostaglandin might be due to effects on pyometra and subclinical endometritis (24), hence not present in this study.

The anestrus cows that were treated by Ovsynch did not differ significantly from the other study groups in 1st AI CR. The Anestrus cow group was actually composed of two different populations of cows: cyclic cows which the pedometry system failed to detect in estrus and acyclic cows. In some of previous studies, cows with anovular follicles had low CR when inseminated by Ovsynch (13, 25). As the anestrus cows group included the cyclic cows not detected by pedometry system, this sub-population may confound the effect of the acyclic cow population on 1st AI conception.

This confounding effect was limited to the 1st AI, as anestrus cows treated by Ovsynch, had longer calving to conception interval (Figure 1), a fact that can be explained by the continues effect of the acyclic cows sub-population on pregnancy rate of the anestrus cows. The longer calving to conception interval demonstrates a low efficacy of Ovsynch as a treatment for ovarian inactivity as was documented before (13, 26). However, as the control group was composed of cyclic cows detected in estrus by pedometry, the efficacy of Ovsynch as a treatment for ovarian inactivity should be compared to other treatment options.

Over all, cows inseminated based on Ovsynch or by pedometry did not differ in the 1st AI conception rate. This finding is similar to findings in previous studies in Israel (1) and worldwide (5, 6, 10, 17), suggesting that Ovsynch can replace pedometry in farms without pedometers or activity meters, or in farms where management conditions limits the efficacy of estrus detection by electronic aids. Recently, Neves *et al.* (5) found that in north American conditions automated estrus detection systems are at least equal to TAI in the reproduction efficacy, despite the fact that this study was conducted in free stall commercial dairy herds (as opposed to the loose shade Israeli system). They also found

that herd level management may confound the effects of the reproductive management system.

CONCLUSIONS

Timing Ovsynch with estrus detection did not improve the reproductive performance of treated cows. In farms with conditions allowing for high efficacy of estrus detection systems, and where such systems are available, Ovsynch protocols may be redundant as a reproduction management tool for all cows.

The reproductive performance of anestrus cows treated by Ovsynch is not comparable to the cyclic cows; however the efficacy of Ovsynch as a treatment of anestrus should be compared to other treatment options.

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Detection of *Toxoplasma gondii* and *Neospora caninum* antibodies in Wild Boars *(Sus scrofa)* in Eastern Turkey

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ABSTRACT

The aim of this study was to examine for the presence of anti-*Toxoplasma gondii* and *Neospora caninum* antibodies in wild boars and to study the impact of infection between the sylvatic and domestic life cycles of these apicomplexan parasites. For this purpose, sera were collected from hunter-killed wild boars (*Sus scrofa*) during the winter period of 2011 from Erzurum province of Turkey. Collected sera were examined for antibodies against *T. gondii* and *N. caninum* by Sabin Feldman dye test (SFDT) and competitive-enzyme-linked immunosorbent assay (c-ELISA), respectively. Out of 12 collected samples, 4 (33.3%) of sera were found to be seropositive at the dilution of 1:16 for *T. gondii* however no seropositivity in any of the samples was detected against *N. caninum*. To the best knowledge of the authors, this is the first serologic study to detect anti-*T. gondii* antibodies in wild boars in Turkey.

Keywords: Wild Boar; *Sus scrofa*; Eastern Turkey; *Toxoplasma gondii*; *Neospora caninum*; Sabin-Feldman; c-ELISA.

INTRODUCTION

Toxoplasma gondii is one of the most important zoonotic protozoa that infect a variety of birds and mammals, including humans. Felids are the definitive hosts and many animal species are the intermediate hosts. Among these intermediate hosts, feral pigs are infected with *T .gondii* have been found to be infected at different rates between 4.4 to 45.9% (1). Antibodies to *T. gondii* have been reported in wild boars from Austria, Brazil, Czech Republic, France, Germany, Italy, Japan, Slovak Republic, Spain, United States (Georgia and South Carolina) (1, 2). The meat of wild boars with tissue cysts of *T. gondii* may be an important source for human toxoplasmosis when people consume these meats undercooked or without control (3).

Neospora caninum, an important parasitic disease of cattle, causes reproductive failure, especially abortion (4). Neosporosis is primarily a disease of cattle and dogs but antibodies have been detected a series of species, including domestic, wildlife and zoo animals (5). Dogs can acquire infection by ingestion of infected tissues; and intermediate hosts can be infected either by horizontal, postnatal or by vertical transmission (6). Due to the economic impact of neosporosis, existing studies are concentrated on farm animals and there is limited serological data about wild life (4-6). Anti-*N. caninum* antibodies have been reported in wild boars from Spain and the Czech Republic (7, 8).

For both *T. gondii* and *N. caninum* wild pigs may be important indicators for following environmental contamina-

tion since they are omnivorous animals obtaining infection from their local environment (9). Serologic tests (IFA, MAT, LAT, DT and c-ELISA) are used to monitor anti-*T. gondii* and *N. caninum* antibodies in wild pigs (1, 8, 9).

The aim of this study was to investigate the presence of anti-*T. gondii* and *N. caninum* antibodies in wild boars and to examine the impact between the sylvatic and domestic life of pigs in relation to these apicomplexan parasites.

MATERIAL AND METHODS

Sample collection

Blood samples were collected from 12 wild boars that were provided by certified hunters from Horasan and Pasinler regions of Erzurum province in Turkey in the year 2011. From information provided by hunters, the majority of these wild boars were adult in the age range 1-3 years, and all of them were female. The blood samples were collected for serological studies from the heart, following the death of animals after shooting. The blood samples were transferred into vacuum tubes, allowed to clot and then centrifuged at 4000 rpm for 10 minutes at room temperature. Subsequently they were placed into eppendorf tubes and stored at -20°C until use.

Serologic examination

All sera were examined for *T. gondii* antibodies using the SF dye test (SFDT) as described (10). The examinations were carried out at the National Reference Laboratory for Parasitology, Public Health Institution of Turkey. The procedure included two steps in preparation for performing the test. Healthy 3-4 week old white Swiss albino mice were injected with the virulent RH strain of *T. gondii*. *T. gondii* RH antigen was collected from the peritoneal fluid of mice after 48 hrs post injection. As an activator serum, human serum seronegative for *T. gondii* was used including factors such as magnesium, properdin, C_2, C_3, C_4. Alkaline methylene blue dye was prepared with 9.73 ml of 0.53 % Na_2CO_3 (Sigma, Seelze, Germany), 0.27 ml of 1.91 % $Na_2B4O_7.10H_2O$ (Merck, NJ, USA) and 25 mg of methylene blue (Difco, Detroit, MI, USA). Following inactivation of complement at 56°C for 30 minutes, 25 µl of test sera were prepared with normal saline in dilutions of 1:4, 1:16, 1:64, 1:256 and 1:1024. The antigen was added to the sera preparations of 25 µl activator serum at approximately 25 *T. gondii* tachyzoites observed in a microscopic field of 40X magnification,. The mixture was incubated in a water-bath at 37°C for 50 minutes. 25 µl of alkaline methylene blue was added to the mixture and kept in 4°C for 10 minutes. Examination was carried out using light microscopy with 40X objective to gauge whether *T. gondii* tachyzoite were stained. If more than 50 % of tachyzoites on one microscopic field were not stained, this dilution step was accepted as positive. Titers of 1:16 and greater were considered as positive (11). Positive and negative controls, which were confirmed by IFAT method, were included in the above procedure.

Serologic examination (c-ELISA)

Antibodies to *N. caninum* were detected by using a commercially available competitive enzyme-linked immunosorbent assay (c-ELISA) kit (VMRD, USA). The test was done by following the instructions of manufacturer. The mean optical density (OD) at 630 nm was determined for all wells using a microplate reader (ELx 800 UV, Universal Microplate Reader, Bio-Tek Instruments, Invc., Winooski, VT, USA). The percent inhibition for each test sample was determined using the below mentioned formula:

$$\text{Inhibition (\%)} = 100 - [(\text{Sample O.D.} \times 100) \div (\text{Mean Negative Control O.D.})]$$

The samples with values of ≥ 30% inhibition were regarded as positive and those with the values < 30% inhibition were regarded as negative (12).

RESULTS

Anti-*T. gondii* antibodies in SFDT test were detected in 4 of 12 (33.3%) wild boar sera to be seropositive at 1:16 dilution and, no seropositive cases (0%) were detected for *N. caninum* antibodies.

DISCUSSION

The wild boar is the only member of the suidae family present in Turkey and bioecological data about this animal are limited (13). Except in touristic regions, pork meat consumption is restricted in Turkey; however, wild boars are important game species and have the potential to cause damage to agricultural crops in Turkey (13). When the wild boar population increases, hunting organizations are arranged to cull wild boars and in so doing decrease the crop damage. Due to restricted

meat consumption, hunted animals are often left behind, close to the farm lands and consumed by domestic and wild carnivores. This scenario leads wild boars into importance in the sylvatic and domestic life cycles of *T. gondii* and *N. caninum*.

Studies concerning *N. caninum* infections in wild boars is limited (8, 14). Almería *et al*. studied 298 wild boars in Spain and found a prevalence of 0.3% (14). Bártova *et al*. studied on 565 wild boars and they detected the prevalence as 18.1% in Czech Republic (8). Researchers used c-ELISA for monitoring and IFAT for confirmation. In our study we could not detect any seropositivity with c-ELISA. In domestic pigs, experimental and natural infections have shown a low *N. caninum* incidence that suggests that environmental exposure to *N. caninum* is rare. This opinion may explain why we could not detect seropositivity for wild boars in our survey (14). Therefore, it appears that wild boar may not play an important role between the sylvatic and domestic life cycle of *N. caninum* infection.

Ranucci *et al.* studied 400 wild boars in Italy and found the prevalence of *T. gondii* as 14% using IFAT (2). Gauss *et al*. (15) studied on 507 wild boars and found the prevalence of *T. gondii* 38.4% with MAT in Spain. Richommeet *et al.* studied 148 wild boars and found the prevalence 17.6% with MAT in France (16). Hejlícek *et al.* (17) studied on 124 wild boars and found a prevalence of 15% with SFDT in the Czech Republic. Furthermore the prevalence rates of *T.gondii* were found as 18.2% (n=170) and 34.4% (n=257) in Georgia and South Carolina provinces of United States respectively with MAT, 4.4% (n=90) and 5.6% (n=108) in Kukamoto and Iriomote Island of Japan respectively with LAT (1).

In our study we detected *T. gondii* antibodies in 4 out of 12 (33.3%) animals with SFDT. The differences between the results may be associated with different climatic conditions, sample sizes, serological tests used and different species of final hosts, their population density and their feeding behaviors. Although our sample size is small, 33.3% prevalence may be important for the transition between sylvatic to domestic life cycles by domestic and wild felids.

Limitations of this study include the small sample size and the fact that only female wild pigs were tested. In this regard this study only gives an indication of the distribution of these diseases among wild pigs may be considered a preliminary study to further larger and wider ranging studies in this area of Turkey. Differences in infection rates between the sexes has not been extensively studied for either *Toxoplasma gondii* and *Neospora caninum* although a study in dogs has demonstrated a greater prevalence *of N. caninum* antibodies in female dogs than in males (18, 19).

In conclusion, it is hoped that the results of this preliminary study will contribute to the understanding of these apicomplexan parasites' epidemiology in the wild life. Further studies are required for developing effective control programs and a clear understanding of these diseases both in Turkey and worldwide.

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Retrospective Study of Disease Occurrence in Captive African Pygmy Hedgehogs *(Atelerix albiventris)*

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ABSTRACT

African Pygmy hedgehogs (*Atelerix albiventris*) are commonly kept in zoological collections and as pets. The aim of this study was to present disease occurrence in captive African Pygmy hedgehogs. Medical records of 106 male and female African Pygmy hedgehogs, both privately owned and from zoological collections, presented to a veterinary teaching hospital from 1994 to 2013 were reviewed. The most common diseases found in captive African Pygmy hedgehogs in this study were dermatological diseases (66.04%), gastrointestinal diseases (33.02%), and skeletal diseases (15.09%). Other identified disorders included neurologic disease, obesity, and renal disease. This is the first retrospective study to describe occurrence of diseases observed in captive African Pygmy hedgehogs. The most common clinical finding in captive hedgehogs was dermatological disease, specifically acariasis. Based on this study, it is recommended that all captive hedgehogs be examined regularly for timely diagnosis and treatment of common diseases of this species.

Keywords: Captive African Pygmy Hedgehogs; *Atelerix albiventris*; Common Diseases; Dermatology; Gastroenterology; Orthopedics.

INTRODUCTION

The African Pygmy hedgehog (*Atelerix albiventris)* is a member of the family Erinaceidae, order Insectivora (1). African hedgehogs are native to the savannah and steppe regions of central and eastern Africa (1). In the wild, African hedgehogs are nocturnal and spend the daytime in burrows (1). They are insectivores and their diet consists mainly of invertebrate predators such as earthworms, slugs, and snails (1, 2). Wild African hedgehogs reach sexual maturity at 1 year of age; however, there are reports of hedgehogs reproductively active prior to this age, particularly in captivity (3). Hedgehogs are popular as pets and are also common in zoological collections (1, 4).

Due to the popularity of this captive species, several retrospective studies have been previously reported in hedgehogs but focused mainly on neoplasia. Other diseases have only been briefly or anecdotally described in the literature (1, 4-7).

The goal of this review is to present disease occurrence in captive African Pygmy hedgehogs presented to the veterinary teaching hospital at the Kansas State University.

MATERIALS AND METHODS

Animals

Medical records of privately owned and zoologically kept captive African Pygmy hedgehogs were reviewed. These animals were presented for varying signs of disease or regular health checks and were examined by the Exotics and Zoological Medicine Service, College of Veterinary Medicine, Kansas State University, between 1994 and 2013.

History and Clinical Examination

Each African Pygmy hedgehog's history, including any previous diagnoses or treatments, was obtained from its medical record. Depending on the nature of the hedgehog, most of the physical examinations were performed under general anesthesia.

Laboratory Testing

In some cases, blood was obtained from the jugular vein under short-term isoflurane gas anesthesia. Occasionally, when only a very small amount of blood was required, the peripheral vessels (cephalic vein, lateral saphenous vein) were used. Blood samples were then submitted for complete blood count and plasma biochemical analysis. When indicated, tissue samples were submitted for cytology, bacteriology, mycology and/or histology tests. Fecal parasitological testing was performed in the cases of diarrhea, progressive weight loss, and some annual health examinations.

Imaging Methods

When clinically indicated, abdominal ultrasonography and two-view (dorsoventral and lateral) whole body radiographs were obtained under general isoflurane gas anesthesia. Computed tomography scan was performed in one case with skull pathology. Echocardiography was performed in one case with suspected cardiac disease.

RESULTS AND DISCUSSION

A total of 106 individual cases were reviewed, out of which 47 animals were intact females and 59 were intact males. The mean age was 26 months (range 1 month to 9.0 years) and mean weight was 348 g (range 112 g to 786 g). The goal of this study was to determine disease occurrence in captive African Pygmy hedgehogs by retrospectively reviewing medical records of cases admitted to a veterinary teaching hospital for clinical evaluation over a time period of 9 years. Description of the observed disease conditions in African Pygmy hedgehogs revealed in this study are summarized in Table 1.

In this study, the oldest animal was 9 years old and 9 other hedgehogs were older than 4 years of age. The expected life span of wild African Pygmy hedgehogs is reported to be up to 4 years, though it has been noted they can live up to 10 years in captivity due to advances in veterinary care (2, 6). In this study, adult hedgehogs weighed 361 g mean for males (range 112 g-770 g) and 369 g mean for females (range 124 g-670 g). The weight of adult hedgehogs has been

Table 1: Prevalence of health disorders in 106 captive African pygmy hedgehogs from 1994-2013 presented to the College of Veterinary Medicine, Kansas State University.[a]

Organ/tissue disease	All hedgehogs % (N=106)
Dermatological disease	66.04
Ectoparasites	46.22
Pododermatitis	3.77
Dermatophytosis	5.66
Otitis externa	2.83
Neoplasia	4.72
Gastrointestinal disease	33.02
Oral squamous cell carcinoma	9.43
Tooth root abscess	4.72
Dental calculus/gingivitis/periodontitis	4.72
Liver disease	3.77
Musculoskeletal disease	15.09
Degenerative joint disease	1.89
Spondylosis	2.83
Annular pedal constriction	4.72
Neurologic disease	11.32
Wobbly hedgehog disease	8.49
Obesity	10.38
Renal disease	9.43
Hematuria	4.72
Interstitial nephritis	0.94
Glomerulopathy	0.94
Multifocal interstitial fibrosis	1.89
Reproductive disorders	8.49
Mammary carcinoma	1.89
Uterine neoplasia	3.77
Endometrial polyps	0.94
Pyometra	0.94
Respiratory disease	7.55
Pneumonia	1.89
Ocular disease	5.66
Cataracts	3.77
Globe proptosis	1.89
Cardiac disease	1.89
Congestive heart failure	1.89
Endocrine disease	1.89
Thyroid gland hypertrophy	0.94
Thyroid carcinoma	0.94
Status ante finem (diagnosis not known)	8.49
Healthy animals	11.32

[a] Some animals displayed more than one symptom or disease and were included in more than one group.

reported to be 400-600 g for males and 300-400 g for females (1).

Dermatologic disease was the most common health disorder seen in hedgehogs, being recorded in 66.04% (70 of 106) of all hedgehogs examined in this study. The most common dermatological disease seen was mite infestation (acariasis) identified in 65.71% (46 of 70) of the dermatological cases. The species of mites was not recorded in every case, but when recorded (5 of 46), the species observed were *Chorioptes*, 60% (3 of 5) and *Caparinia*, 40% (2 of 5). Two cases with fleas and one case with lice were also found.

External parasites (fleas, ticks, and mites), as well as a variety of dermatophytes were commonly seen in hedgehogs in this study and have been previously reported in the literature (8). Acariasis is commonly reported in pet hedgehogs (1). Mites are normal inhabitants of hedgehogs in the wild, but infestation can become severe in ill or immunocompromised animals (7). Similar to what was observed in this study, the most common mite of hedgehogs is *Caparinia spp*. (non-burrowing mite), with reports of *Chorioptes spp.* (burrowing mite) also being implicated (1). However, in many of the cases of mites that were identified in this study, the species was not recorded.

Dermatophytosis was found in hedgehogs in this study with all cases diagnosed as *Trichophyton spp.* based on fungal culture. The most commonly identified organisms in the literature include *Trichophyton spp.* and *Microsporum spp.* (1, 9).

In 4.72% of cases in this study, dental disease, including calculus, gingivitis, and periodontitis was noted, which has commonly been reported in the literature in hedgehogs (1).

Various skeletal diseases have been recorded in hedgehogs (10). Spondylosis was noted on radiographs of three hedgehogs in this study. A case series (n = 4) of intervertebral disc disease in hedgehogs noted spondylosis as a radiographic finding in three hedgehogs, hypothesized to be a result of abnormal movement between vertebrae, degeneration of the annulus fibrosis and inflammation with bony proliferation from the protruding disc (10). Annular pedal constriction, as noted in this study in five hedgehogs, causes an interference with the blood supply leading to local congestion, edema, hypoxia, and/or avascular necrosis (11). These injuries can be caused by cage wire, fabric, string, hair, or other fibers (11). Hedgehogs appear to be particularly prone to this injury of a leg, foot or digit, often requiring amputation (11). In this study, removal of the foreign material resulted in return to normal function of the limb in all but one case which required amputation.

A common presenting complaint of neurologic hedgehogs in this study was ataxia. Neurological disease is reported commonly in hedgehogs in the literature (1). Common causes of ataxia in hedgehogs include torpor, Wobbly Hedgehog Syndrome (WHS), intervertebral disc disease, trauma, toxins, infarcts, malnutrition, and neoplasia (1, 12-13). WHS was the most common cause of ataxia found in this study (8.49%). WHS (as a result of demyelination) is a progressive paralysis in hedgehogs that begins with ataxia and ends in complete paralysis within 15 months of the onset of clinical signs (12, 14). However, intervertebral disc disease (IVDD) can also present with similar signs as WHS (1, 13). Brain tumors have also been reported to present with similar signs as WHS and IVDD with reports of astrocytomas, gemistocytic astrocytomas and microgliomas (12). One report of anaplastic astrocytoma in the spinal cord of a hedgehog has also been reported (15). Additionally, circling, as seen in hedgehogs in this study, may be caused by otitis media/interna or primary neurologic disease (1).

Obesity was found in 10.38% of cases in this study, which is reflective of a previous report (16). Obesity is commonly reported in pet hedgehogs as a result of improper husbandry, including overeating, incorrect diet, lack of exercise, and an ambient temperature that is too cold (16).

Renal diseases in this study were found in 9.43% of cases, but many of the hedgehogs with suspected renal disease were not necropsied to allow a definitive diagnosis. A retrospective study of post mortem findings in 14 African hedgehogs revealed renal disease in 50% of the hedgehogs including tubulointerstitial nephritis, chronic renal infarcts, glomerulopathy, and tubular nephrosis (17). When necropsy was performed in hedgehogs in this study, similar histopathological findings were also described.

In this study, cardiac disease was clinically diagnosed in just 1.89% of cases and necropsy findings from 13.21% of cases revealed no evidence of cardiomyopathy. In a study published in 2000, 38% (16 of 42) of hedgehogs examined on necropsy, were noted to have cardiomyopathy (18).

Cases of leiomyoma and endometrial polyps were found in this study, however some of the suspected cases of uterine disease in this study did not have necropsy or other diagnostic tests performed. Uterine disease in African hedgehogs has been reported in the literature (5, 13, 19-20). The types of

tumors of the uterus reported in hedgehogs in the literature includes adenoleiomyosarcoma, adenosarcoma, endometrial stromal cell sarcoma, endometrial polyps, adenoleiomyoma, uterine adenocarcinoma, carcinosarcoma and uterine spindle cell tumor (5, 13, 18-19). One case of pyometra was found in this study. The predominant cause of abnormal vaginal discharge is reported to be neoplasia but pyometra and metritis have been reported (1, 20, 21).

Neoplasia was the third most common general pathology in hedgehogs observed in 20.75% (22 of 106) of cases in this study, when incorporating all body systems. The most common neoplastic process observed in this study was oral squamous cell carcinoma 45.45% (10 of 22 neoplasias). Mammary gland adenocarcinoma was seen in 9.09% (2 of 22 neoplasias) of cases. Dermal neoplasia was reported in 22.73% (5 of 22) of neoplasia cases.

Oral squamous cell carcinoma was commonly diagnosed in hedgehogs ranging from 1-6 years of age. Oral neoplasms, particularly squamous cell carcinomas are commonly reported in hedgehogs in the literature (1). Oral squamous cell carcinoma was reported as the third most common tumor of the hedgehog preceded by mammary gland adenocarcinoma and lymphoma in a retrospective study, in hedgehogs ranging from 2-5.5 years (4).

In this study, oral squamous cell carcinoma was the most commonly reported neoplasm in 45.45% (10 of 22 neoplasias) of cases, mammary gland adenocarcinoma was seen in 9.09% (2 of 22 neoplasias) of cases, and lymphosarcoma was not reported. Dermal neoplasia was reported in 22.73% (5 of 22) of neoplasia cases, and is also considered relatively common (4). Neoplasia is reported commonly in African hedgehogs in the literature (1, 4-6). There are various reports of tumors and disseminated neoplastic processes in hedgehogs, affecting many body systems (4-6). Several retrospective studies reported various neoplasms in African hedgehogs (1, 4-6). In one retrospective study of 66 hedgehogs, the most common tumors included mammary gland adenocarcinoma, lymphosarcoma, and oral squamous cell carcinoma (4).

Limitations of this study include the accuracy of information available in the record, which is inherent in any retrospective study. The age of the animals was reported by the owners and may not be accurate. The reproductive status was also reported by the owners and verified by the attending clinician.

The population of hedgehogs used in this study is a good representation of the captive hedgehog population, from both zoologically kept and privately owned hedgehogs, since both primary care and secondary referral cases are included. Additionally, given the large time scale, there was a decreased chance of disease grouping. However, the localized geographical area may represent a localized gene pool which may result in different disease prevalence in other areas of the world.

CONCLUSION

The most common disease in hedgehogs found in this study is dermatological disease, particularly ectoparasites. Gastrointestinal disease, skeletal disease, and neurologic disease were all relatively common presentations. This retrospective study may assist in extending our clinical knowledge of disease prevalence in captive African Pygmy hedgehogs.

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Diaphragmatic Hernia in Horses in Israel: A Case Series

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ABSTRACT

Diaphragmatic hernia (DH) is possibly more frequent in the horse than typically reported in the literature. Since DH is not as rare as perceived, reporting on its occurrence is critical in order to increase awareness of this problem. DH typically presents as an emergency situation, whose prompt treatment influences the prognosis. Thus, it should be included in the differential diagnosis of horses presenting with signs of colic or of respiratory distress, or with a combination of the two. The aims of this study were to determine the prevalence of DH cases at the Veterinary Teaching Hospital of the Koret School of Veterinary Medicine and to describe the clinical signs, case management and outcome of these cases. The equine medical records of our hospital for January 2008 – August 2012 were reviewed. The information retrieved included chief complaints, mode of diagnosis, physical characteristics of the hernia, organs found in the thorax, treatment, results and, where relevant and pathology report. The number of DHs was compared to the hospital's caseload and colic surgeries during the reference period. Four horses were presented with DH in the reference period constituting 0.46% of all surgeries and 1.29 % of all colic surgeries in the reference period, and 0.14% of the annual referral figure. Three of the four cases showed signs of colic with respiratory symptoms, whereas the fourth presented only acute respiratory distress. The survival rate was 25%, one patient being successfully treated. The prevalence of DH was found to be more frequent at the KVSM-VTH than previously reported. Early diagnosis and a suitable surgical approach proved essential to the successful surgical repair of DH, but the overall survival rate remained low.

Keywords: Diaphragmatic Hernia; Equine; Prevalence; Respiratory Distress; Colic.

INTRODUCTION

Diaphragmatic hernias (DH) are classified as true, having a hernia sac, or false where this is a lack of a hernia sac. False hernias can probably be better defined as a diaphragmatic defect, rupture or tear (1). Nevertheless, in order to conform to the current literature the term DH will be used throughout the manuscript for consistency. A further classification takes into account the etiology of DH, namely congenital and acquired. The congenital form is mostly associated with an abnormal development of the various parts of the diaphragm, which do not fuse together, while the acquired form is usually caused by trauma or increased intra-abdominal pressure (e.g. parturition (1, 2)). Congenital as well as acquired DH are typically left-sided (1, 3), though a right sided congenital, Morgagni type DH has been also reported (4). Interestingly, there is an in-between form that can be defined both as traumatic-acquired and as congenital. This form of DH occurs upon parturition in which the foal's ribs fracture and tear its diaphragm upon passing through the birth canal (5).

The location of the defect in the diaphragm varies, but it most commonly occurs at the junction between the tendineous and the muscular parts of the diaphragm. The location of

the lesion is probably affected by the nature of the etiologic factor (1). The organs that are usually incarcerated in DH are the small and large intestines (1, 2, 6), but the spleen, the stomach and the liver can also be involved (2).

All types of DH can remain subclinical for prolonged periods and clinical signs typically appear acutely upon incarceration or strangulation of intestine. Clinical signs are typically signs of colic, but may include and limited to respiratory distress (7). Symptoms however may be mild, such as lethargy and exercise intolerance alone, up to a stage when organs migrate into the thoracic cavity. Initial suspicion of DH can be established following physical examination, when signs of colic are accompanied by respiratory distress. Other, inconsistent, non-specific, signs include resistance upon nasogastric intubation and a sensation of emptiness upon rectal palpation. Useful diagnostic techniques include thoracic radiographs and ultrasonographic evaluation (1). Notwithstanding, diagnosis is unfortunately often reached only by exploratory celiotomy or post-mortem examination (2).

Treatment is exclusively surgical and aims at removing the herniated bowel from the thorax, with or without resection, and repairing the diaphragmatic defect (8). Post-operative treatment should comprise management of pneumothorax and pleuritis in addition to the typical post-operative colic treatment regimen.

This report describes the diagnostic procedures and the treatment of four cases of DH which were recorded at the Veterinary Teaching Hospital of the Koret School of Veterinary Medicine (KVSM-VTH) during January 2008 – August 2012. The study was carried out in order to better understand DH prevalence and possibly assist improve future treatment of similar cases.

MATERIALS AND METHODS

This article is a retrospective cohort study that was carried out in the KVSM-VTH between January 2008 and August 2012. The clinical and clinical pathology records of the DH cases recorded in the reference period were reviewed. The clinicians involved in the cases also assisted in the retrieval of information regarding the clinical history, signalment, medical and surgical treatment, and outcome for their respective cases. Records were found for two fillies, a mare and a stallion, aged 10 days, 7 months, 18 years and 17 years respectively.

The main equine population seen in the hospital comprised riding and breeding horses. The total number of cases for each year was retrieved and an average case load per year was computed. The same calculation was performed for the number of surgeries and colic surgeries at the hospital. The prevalence of DH was calculated in percentage terms, along with the percentage of DH in total surgeries and colic surgeries.

RESULTS

During the reference period, the KSVM-VTH received an average of 67 surgical colics per year, and DH comprised 1.29% of our surgical colic caseload. The KVSM-VTH, with an average of 637 referrals per year over the reference period (January 2008 –August 2012), received four horses with DH during this time which amounted to 0.14% of the total referrals. Only one of the four cases with DH survived (25%) to discharge with a good long-term outcome.

CLINICAL CASES

CASE 1

Clinical History

A 10-day-old Arabian filly was presented to the KVSM-VTH, after uneventful pregnancy and parturition. The owners reported having noticed signs of weakness and carpal swelling on the right forelimb the following day. On arrival to the hospital the complete blood count (CBC) revealed, WBC of $6.3x10^9$/L, Reference Range (RI): 5.6-12.1x10^9/L), packed cell volume (PCV 37%, (RI): 27-43%) and total solids (TS) of 6g/dl, (RI): 6-8g/dl) were within the reference ranges. Sample of synovial fluid taken from the right inter-carpal joint was consistent with synovial infection (TS 7g/dL, (RI): 2-3.5g/dl; lactate 8 mmol/L, (RI): 0-2 mmol/L; glucose 19 mg/dL, (RI): 76-130 mg/dL).

Case Management

Amikacin (20 mg/kg s.i.d., Vetmarket, Shoaham, Israel), ampicillin (20 mg/kg q.i.d., Penibrin, Sandoz GmbH, Kundl, Austria), flunixin meglumine (0.5 mg/kg b.i.d., Norbrook laboratories Ltd, Newry, N .Ireland) and LRS (Teva Medical, Petah Tikva, Israel) were administered intravenously and ranitidine (7 mg/kg t.i.d., Dexcel Pharma, Jerusalem, Israel) was administered orally. The next day the filly underwent

arthroscopic surgery under general anaesthesia. In both the inter-carpal and the radio-carpal joints, pannus, synovial edema and discoloration were found, consistent with synovial sepsis. In both joints, pannus was removed, partial synovectomy was performed, followed by generous lavage (12 liters per joint) and amikacin (1g) was injected. The joints were lavaged daily under heavy sedation (butorphanol (3 mg, Morphasol, Animedica GmbH, Senden, Germany), diazepam (10 mg, Teva Medical, Petah Tikva, Israel) and xylazine (10 mg, Sedazine, AST Farma, Oudewater, The Netherlands)) using teat cannulas, for 10 days. Since clinical signs persisted despite the aggressive management, joint lavage was replaced by regional limb perfusion (RLP) with imipenem (Merck Sharp & Dohme, Chibret, France) alternating with intra-articular injections with imipenem. The same day the filly exhibited difficulty in expiration subsequent to tranquilization. Dyspnea recurred the following day and lateral thoracic radiographs taken in recumbent position showed no evidence of a respiratory pathology.

After another RLP procedure carried out the same week, the patient was kept under observation, with daily bandage changes, as there was improvement in the condition of carpus. During the third week of hospitalization, the filly's lameness and general condition improved, whereas acute respiratory distress continued to flare up occasionally.

On day 23 of hospitalization, the filly developed severe respiratory distress accompanied by tachycardia and tachypnea (heart rate 120 beats/min and respiratory rate 56 breaths/min). At that stage, the filly had a severe bout of coughing and then collapsed in agonal breathing. An attempt was made to insert an endotracheal tube, but the filly collapsed and stopped breathing. Intubation was successfully performed but resuscitation efforts were attempted to no avail, until death ensued.

Post-mortem examination

The post-mortem examination revealed: yellowish liquid filling the thoracic cavity, the lung lobes were collapsed, and discoloured, with consolidation in ~30% of the lung field. In addition, there was a 15 cm long defect in the right crus of the mid-diaphragm at the border of the muscular and fibrous portions of the diaphragm. The margins of the opening were thick. Multiple loops of the small intestine were found in the thoracic cavity. The dorsal loops appeared thickened and compromised (Figure 1).

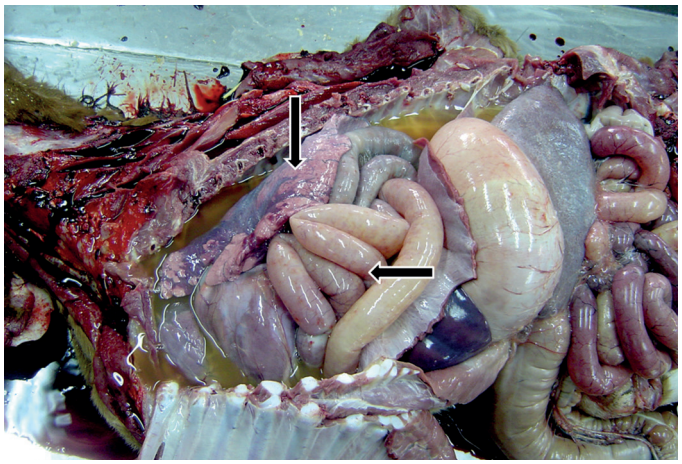

Figure 1: Necropsy photograph of case 1, depicting diaphragmatic hernia in a 10 days old Arabian filly. Vertical white arrow points to the herniated small intestine while the horizontal white arrow points to the torn diaphragm. Vertical black arrow points to the stomach while the horizontal black arrow points to the collapsed lungs.

CASE 2

Clinical History

A 7 months old Arabian filly had had a history of severe abdominal pain. Two days earlier the owner called the referring veterinarian to treat the filly for a ventral abdominal swelling. The veterinarian detected that the swelling included the right-side thorax, and administered anti-inflammatory medication. Several hours before arriving to the hospital, the filly showed severe signs of abdominal pain, which were unresponsive to analgesics, and was referred to the hospital.

On arrival to the hospital The filly was severely painful and required potent analgesia in order to tolerate the initial evaluation. A swelling was detected on the right hemi-thorax and on the ventral part of the abdomen. Blood tests included CBC: (WBC of $4.2x10^9$/L, Reference Range (RI): 5.6-12.1x10^9/L), packed cell volume (PCV 45%, (RI): 27-43%) and total solids (TS 8.2g/dl, (RI): 6-8g/dl) were consisted with mild dehydration and leukopenia. On ultrasound examination, a portion of the small intestine with peristaltic movement, and fractured ribs were visible in the swelling on the right hemi-thorax. At that stage, the filly was submitted for urgent abdominal exploration under general anaesthesia.

Case Management

Preoperative medication was administered intravenously as follows: benzylpenicillin sodium (20,000 IU/kg, Norbrook laboratories Ltd, Newry, N. Ireland), gentamicin (6.6 mg/kg,

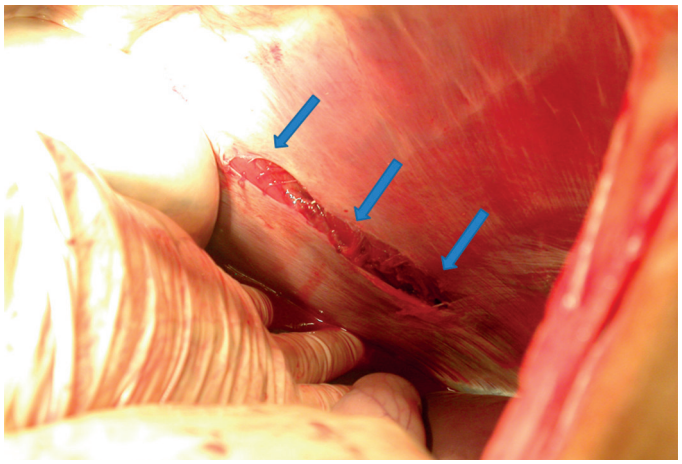

Figure 2: Intra-operative photograph of case 2, depicting a diaphragmatic tear in a 7 months old Arabian filly. The image is viewed through an abdominal approach for exploratory celiotomy.

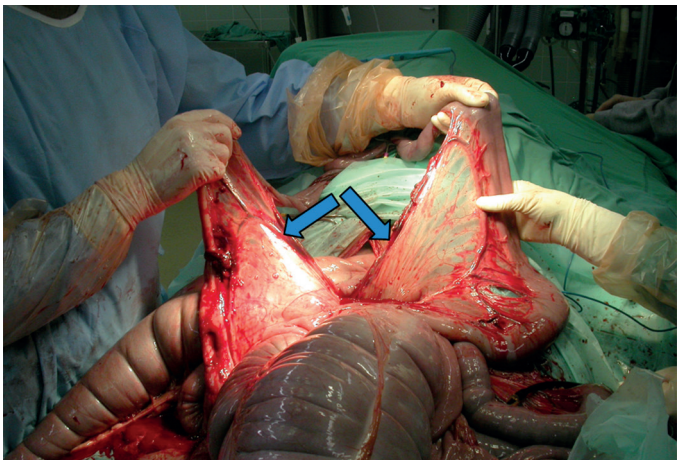

Figure 3: Intra-operative photograph of case 2, depicting torn mesentery and damaged small intestine in a 7 months old Arabian filly suffering from diaphragmatic hernia. The arrows points to the extensive tear in the small intestine mesentery.

Gentaveto, Eurovet, Netherlands) and flunixin meglumine (1.1 mg/kg). The patient was then premedicated intravenously with 100 mg xylazine, and induction was performed with 220 mg of ketamine (Clorketam, Vetquinol, Paris, France) and 10 mg of diazepam. The filly was positioned in dorsal recumbency and isoflurane (Piramal Critical Care, Inc, Bethlehem, Pennsylvania, USA) was used to maintain anaesthesia.

A ventral abdominal midline approach was performed, and small intestine entrapped in a diaphragmatic tear, were observed. The diaphragmatic tear was about 10 cm long, located on the right ventral muscular portion of the diaphragm (Figure 2). After carefully reducing the incarcerated intestine into the abdominal cavity, it was found to be non-viable. Over 70% of small intestine was discolored and had no pulse or motility with several tears in the mesentery (Figure 3). In addition, the right hemi-thorax had a large defect that contained some of the damaged intestine, and four sharp-edged fractured ribs 12-15. The ribs were fractured at the costo-chondral junction. There was no external wound, neither at the thorax nor caudally, but the ribs lacerated the thoracic wall allowing several loops of small intestine to migrate subcutaneously. Due to poor prognosis the filly was euthanized at the owner's consent.

CASE 3

Clinical history

An 18 year-old mixed breed mare suffered from colic which had begun about twelve hours earlier and had responded well to the initial analgesic therapy in the field. Nevertheless, a few hours later the mare showed severe signs of colic and was referred to the hospital. On arrival at the hospital the mare had severely compromised cardiovascular status with marked tachycardia (100 beats per minute), hyperemic mucous membranes and cold extremities. Auscultation revealed decreased borborygmus and nasogastric intubation produced 13 liters of reflux. On rectal examination, dry feces and a gas-distended large colon were felt. A CBC revealed a high WBC count ($15.4x10^9$/L, RI: 5.6-12.1$x10^9$/L) and elevated PCV, indicative of hemoconcentration (PCV 48%, RI: 27-43%), while TS was low (4.7 mg/dL, RI: 6-8mg/dL), and lactate concentration was elevated (5.1 mmol/L, RI: < 2 mmol/L). During examination, the mare showed signs of uncontrollable pain and immediate exploratory celiotomy was therefore uninitiated.

Case Management

Preoperatively the mare was given sodium penicillin G (20,000 IU/kg) and gentamicin (6.6 mg/kg) intravenously. The mare underwent induction and isoflurane and anaesthesia. With the mare in dorsal recumbency, a ventral midline abdominal approach was performed. Exploration revealed a defect in the diaphragm, about 22-cm long, situated in the left dorsal part of the diaphragm, at the musculo-tendinous junction. The stomach, several meters of the small intestine, the pelvic flexure and a left lobe of the liver were incarcerated in the thorax. Furthermore, a large colon volvulus was found (360° counter-clockwise). The cecum and the large colon were edematous and purple. Part of the omentum was adhered

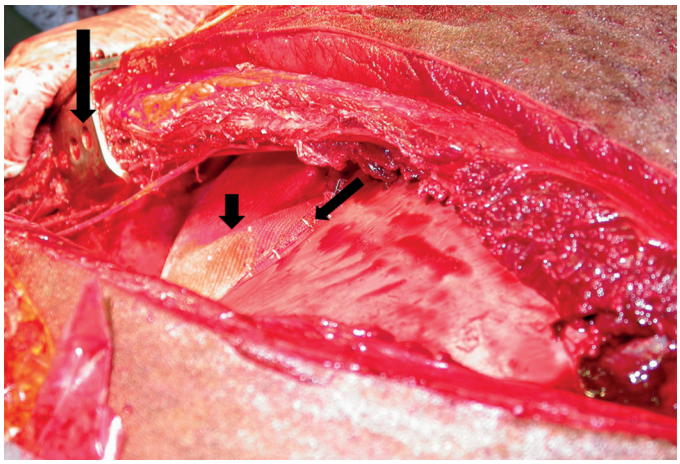

Figure 4: Intra-operative photograph of case 3, depicting a polypropylene mesh stapled to a tear in the diaphragm in an 18 year-old mixed breed mare. Long vertical arrow points to the finochietto rib retractors, short vertical arrow points to the polypropylene mesh and the oblique arrow depicts the skin staples used to attach the mesh to the diaphragm.

to the lateral border of the hernia. The abdominal organs were retrieved and returned in their normal position in the abdominal cavity. The volvulus was manually corrected, which led to an improvement in color and motility of the large colon. The omentum was ligated and resected. A blood gas analysis performed an hour after induction showed respiratory acidosis (with values of 102.9 mm Hg for PaO_2, (RI: 100-500 mm Hg) 50 mm Hg for $PaCO_2$ (RI: 30-45 mm Hg) and 7.26 for blood pH (RI: 7.35-7.45), which was resolved by having the mare tilted 30 degrees head up (reverse trendelenburg position) for an hour in order to decrease the pressure exerted on the lungs by the abdominal organs. Attempts to correct the hernia through the abdominal cavity failed. The deep dorsal location and considerable tension on the sutures, due to the large gap in the diaphragm; combined with the friable diaphragmatic muscular tissue; hindered the repair. The abdomen was lavaged and closed in a routine fashion. At that stage the mare was positioned in right lateral recumbency as the left hemithorax was prepared for surgery. An incision was made in the left hemithorax over the 11th rib, approximately 30cm length of rib was removed using a gigli wire saw (Narang Medical Limited, Delhi, India) and the space between the 10th and the 12th rib was expanded using a Finochietto rib spreader. The tear was located and an atelectatic left lung lobe was detected. Suturing proved difficult using this approach. A polypropylene mesh was attached to cover the diaphragmatic defect using surgical skin staples (Figure 4). At termination of the procedure two 32 French drains (Well Lead Medical, Panyu district, Guangzhou city, China) were inserted, one dorsally at the 13th intercostals space and one ventrally at the 8th intercostal space.

Postoperatively the mare received intensive treatment including: intravenous fluid therapy consisting of crystalloids (LRS) and colloids (Hetastarch, Fresenius Kabi AG, Badhomburg, Germany)), fresh frozen plasma, antibiotic therapy (penicillin 22.000 IU/kg q.i.d. IV and gentamicin 6.6 mg/kg s.i.d. IV), analgesics (flunixin 1 mg/kg b.i.d. IV) and anti-endotoxin therapy (polymyxin B 6000 IU/kg b.i.d., IV, X-GEN Pharmaceuticals Inc, Northport, NY). Ice therapy applied on all four extremities to prevent for laminitis. Initially the horse received a nasal oxygen supply (15 liters/min).

Post-operative radiographs confirmed good lung inflation and verified that the mesh was intact at its location. The drains were removed the day after surgery following a significant decrease in the amount of blood and air that was drained. Two days after surgery significant edema developed at both incisions but both remained dry. At that stage, oral antimicrobials (enrofloxacin (7.5 mg/kg s.i.d., Phibro Animal Health Corporation, Petah Tikva, Isreal) and metronidazole (25 mg/kg t.i.d., Vetmarket, Shoham, Israel)) and gastric mucosal protectants (omeprazole 4 mg/kg s.i.d., Nature Vet Pty Ltd., Glenorie, NSW, Australia) and ranitidine 6.6 mg/kg q.i.d.) were added. The mare recovered smoothly and was discharged 12 days after surgery. The mare resumed her career as an endurance horse, with no further respiratory or colic problems in a five-year post-operative follow-up.

CASE 4

Clinical History

A 17-year-old male Quarter horse was referred to the KVSM-VTH with clinical signs of acute colic, which lasted for 12 hours. He was unresponsive to analgesics or sedatives and was referred to the hospital. The owners reported weight loss and occasional breathing difficulties in the last few months.

The horse presented to the hospital with a heart rate of 40 beats/minute, a respiratory rate of 30 breaths/minute with the presence of a heart murmur. On rectal examination cecal impaction containing hard fecal material was palpated. The horse was treated with mineral oil (Vetmarket, Shoaham,

Israel) and water through the nasogastric tube and LRS intravenously. Though the horse received potent analgesia several times, pain recurred and emergency celiotomy was recommended.

Case management

Preoperative medication was administered intravenously as follows: benzylpenicillin sodium (20,000 IU/kg), gentamicin (6.6 mg/kg), and flunixin meglumine (1.1 mg/kg). The horse was placed under general anesthesia, positioned in dorsal recumbency and isoflurane anesthesia was used. On exploratory celiotomy a 6 cm x 6 cm right ventral diaphragmatic tear was found. The cecum was incarcerated in the hernia and it was adhered to the pleura in several places. During manipulation of the cecum significant bleeding occurred in the thorax. The hernia was closed by direct suturing, and a drain was inserted into the mid-height, right hemi-thorax at the 12th inter-costal space. Following recovery the horse had respiratory distress and thoracic radiographs revealed large intestine in the thorax, along with an old fracture in the right fourteen rib. The horse was taken to surgery in an attempt to repeat the repair of the diaphragm, however death occurred during induction.

DISCUSSION

Diaphragmatic hernia is commonly referred to as a rare lesion in the horse (9), however it is probably more common than previously considered (1). In one recent study, DH made up to 1% of all colic surgeries performed between 1998 and 2005 at a university referral hospital (1). This figure is comparable with the percentage of surgeries for ileocecal intussusception or gastrosplenic entrapment (1.3%, 0.3%, respectively) – two conditions that are not considered rare (10). The incidence of DH reported in the KVSM-VTH in this study (1.3 %) is similar to the results mentioned above. In another retrospective study from a single center, over two cases of DH per year were recorded (6). Thus, according to this and to our experience, DH is quite uncommon but definitely not rare.

The four cases making up the current series presented exclusively diaphragmatic ruptures or defects, none of which had a hernia sac. This is in accordance with previous studies, confirming that true DHs, containing a hernia sac, are indeed rare (1). With regard to etiology, it is reasonable to assume that all our cases had a traumatic origin, in two cases, the trauma was recent and in the other two, it occurred months prior to the acute presenting episode. In several reported cases, there was a history of trauma months to years prior to an acute episode of colic. It is likely that the damage to the mare's diaphragm occurred at the time of the trauma/parturition, however the acute episode was probably triggered by the sudden incarceration of viscera in the thoracic cavity.

In case 1 (the 10-day old foal) the respiratory clinical signs were mild but evident since arrival and most likely had existed since parturition. It is likely that during parturition, while passing in the birth canal, the filly fractured her ribs, which tore the diaphragm. This case falls into the mixed category of congenital DH with a traumatic cause. In congenital DH, the defect is located mainly on the left side, owing to the incomplete fusion of the pleuroperiontal folds that is secondary to the slower development of the left lung (1). Acquired DH also seems to be predominantly left-sided, probably because the liver acts as a protection on the right side (1, 2). In contrast to the predominance of left-sided DH in the literature (1, 2, 6), in three of the four cases that make up our series, the lesion was located on the right side of the diaphragm. In three of the presented cases, the DH appeared on the ventral aspect, which is consistent with their traumatic history combined with the evidence of ipsilateral rib fractures. Overall, two cases in this series are presumed to be related to parturition associated trauma (cases # 1 and #3), while two were likely due to direct external thoracic trauma (cases # 2 and #4).

The diagnosis of DH is typically difficult to make even during pre-operative evaluation. The clinical manifestations of DH typically include acute abdominal signs or colic; colic and dyspneic signs may be consecutive (11) or simultaneous, and only seldom do respiratory signs occur alone. Our first case presented with exclusive respiratory signs for several days prior to a fatal abdominal crisis episode. The respiratory signs can be attributed to the defect in the diaphragm leading to a loss of negative pressure in the thoracic cavity resulting in atelectatic lungs. This may be supported by a report describing a mare suffering from a diaphragmatic rent without any herniation and showing respiratory signs only (12). The fatal episode could be attributed to the migration of viscera into the thoracic cavity. It is interesting that case 4 had history of respiratory problems, demonstrated as episodes of dyspnea, months prior to the abdominal crises that led to the referral. Cases #2, #3 in our series did show signs of abdominal

pain, involving respiratory distress as well, however, at least initially, respiratory distress was interpreted as part of the abdominal crisis. This is consistent with the reported concern that respiratory signs in foals with DH, are often masked by the acute abdominal crises and attributed to abdominal pain and stress (11).

Most commonly, DH is diagnosed either at abdominal exploration or at necropsy. Radiographs and ultrasonography are considered the most useful diagnostic aids for attaining ante-mortem or pre-operative diagnosis of DH (1). In the current series, ultrasonographic evaluation was useful though not definitive in the diagnosis of DH in one horse; in two cases DH was diagnosed by abdominal exploration, and in one case only at necropsy. Ultrasonographic evaluation can nevertheless easily lead to false positive diagnosis due to the bell-shaped diaphragm and the cranial migration of the small intestine. In our experience, when used judiciously, both ultrasound and radiograph can be helpful in the diagnosis of DH. An effort should be made to incorporate these modalities in the workup of any colic case where clinical signs are indicative of respiratory compromise, or where the history or initial evaluation may lead the clinician to suspect DH (1, 12). In case #1 in this series clinical signs were exclusively of a respiratory nature, radiographs however revealed no DH. The difficulty in sometimes detecting DH should be borne in mind and one should not rely on any single negative diagnostic test to rule out diagnosis of DH.

The only definitive treatment for DH is surgical repair. The prognosis in general is not good and it largely depends on early diagnosis, on the location and length of the tear, and on the degree of intestinal damage (2, 6, 8). Romero and Rodgerson (2) reported that out of 25 horses which were operated on, only 6 survived more than three months following surgery, while 11 were euthanized during surgery. Similarly, 7 out of 44 survived to discharge in a recent retrospective study by Hart and Brown (6). Nevertheless, long term results were positive in the same study with 71 % of horses discharged surviving for over a year. Recently a DH diagnosed during abdominal exploration was successfully repaired, three weeks later, in the standing horse, using thoracoscopy (13).

Although the thoracic approach to DH repair is described in the literature, the conventional approach is typically abdominal. Hart and Brown (6) described repairing nine DHs via an abdominal incision, and Romero and Rodgerson (2) reported repairing DH in 14 horses using the same approach. In that study, five of the horses that underwent surgery had DH corrected by means of a polypropylene mesh applied by direct suturing or using a hand stapling device. Four of these horses did not survive to discharge. This may indicate low chances of success in correcting DH using a mesh through an abdominal approach. Case #4 in our series was treated by direct suturing through the abdomen and failed immediately after surgery. This defect was neither large nor dorsal but still failed. It may further support the inferiority of the abdominal approach for repairing DH.

The thoracoscopy technique used by Röcken *et al.* (13) agrees with our elected thoracic approach, although the techniques used and the type of lesion was different. In that report, an endoscope was used, while in case #3; in the current report; an open approach was used performed by rib resection. Furthermore, in that study the defect was corrected using interrupted sutures; conversely, in our case the defect was corrected with mesh and staples. All the above supports our clinical impression that approaching the DH through the thorax is preferable. Thoracoscopy may prove the method of choice for repairing DH in the future since it provides the best access to the lesion and is minimally invasive.

By and large the surgical success rate for equine DH repair remains low. In Romero and Rodgerson's report (2), 25 horses with DH underwent surgery, but only eight survived to discharge. Similar results were reported by Hart and Brown (6), in a population of 26 horses that underwent surgery, seven were discharged from hospital, and two of them died within the first year. The same study showed that horses with dorsally located rents, which were 10 cm or more in diameter, had the worst prognosis with 92% of them being euthanized; while rents located ventrally and less than 10 cm in diameter were associated with a far better success rate (63%). In the current study, however the only survivor had a large and dorsal tear. No conclusions, with regards to the effect of lesion location and size on prognosis, can be drawn from this single case. Nevertheless, thoracic approach may enable repair of tears that are irreparable by the traditional abdominal approach and thus improve the overall survival rate.

In this study, 25% of the horses with DH survived, which is a similar survival rate to other reports (2). Two cases were related to parturition, one in the dam and the other in the neonate emphasizing that Mares and foals should be monitored for clinical signs related to DH. The thoracic approach proved

useful in the current report as well as in a recent study. In conclusion, earlier diagnosis and improved surgical techniques may improve the survival rate of horses suffering from DH.

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Pharmacokinetics of Enrofloxacin and its Metabolite Ciprofloxacin after Intracoelomic administration in Tortoises *(Testudo hermanni)*

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ABSTRACT

Enrofloxacin belongs to the fluoroquinolone class of antibiotics. It is commonly used in a variety of reptile species due to its wide spectrum of efficacy, partly due to its formation of an active metabolite ciprofloxacin. Enrofloxacin shows wide disposition variability among all species resulting in large differences in the plasma concentrations of both enrofloxacin and ciprofloxacin. The aim of this study was to evaluate the pharmacokinetics of enrofloxacin and ciprofloxacin after a single intracoelomic injection of 10 mg/kg of enrofloxacin in 9 tortoises (*Testudo hermanni*). Blood samples were collected at 0, 0.5, 2, 4, 10, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240 and 264 h and analyzed using a validated high performance liquid chromatography (HPLC) florescence method. Plasma concentrations of enrofloxacin were quantifiable in all subjects for up to 240 h, while ciprofloxacin was detected in all subjects up to 120 h. The C_{max} (s) of enrofloxacin and ciprofloxacin were 8614 ± 1116 ηg/mL obtained at 2.19 h and 605 ± 43 ηg/mL obtained at 4.23 h, respectively. The values of C_{max}/MIC ratio and $AUC_{0\text{-}24}$/MIC ratio of enrofloxacin with a MIC value of 0.5 μg/mL were 17.23 and 132.78, respectively. In conclusion, an administration of 10 mg/kg of enrofloxacin via the intracoelomic route in *Hermann's* tortoises produced optimal pharmacodynamic parameters.

Keywords: Enrofloxacin; Ciprofloxacin; Pharmacokinetics; Intracoelomic Administration; *Testudo hermanni* Tortoises.

SHORT COMMUNICATION

Fluoroquinolone antibiotics belong to a group of synthetic antimicrobials that are widely used in veterinary medicine. Their spectrum of activity includes Gram positive, Gram negative and Mycoplasma species responsible for a vast array of pulmonary, urinary and digestive infections (1). Enrofloxacin is a prototypical fluoroquinolone, showing treatment efficacy for the major bacterial conditions in several animal species (2, 3). The enhanced efficacy demonstrated by enrofloxacin is due to the formation of an active metabolite, ciprofloxacin which exhibits potency and spectrum of activity similar to that of the parental drug (1). The pharmacokinetics of enrofloxacin following various routes of administration have been investigated in different species of turtles and tortoises with plasma concentrations of enrofloxacin and ciprofloxacin showing wide disposition variability among the species (3-5). Considering tortoise species are more closely related to one another than to other orders of animals, this underlines the importance of conducting pharmacokinetic studies for individual species rather than extrapolating doses from data generated in other reptile species (6).

The treatment of bacterial infections should be based on

a rational scientific approach. The most common pharmacokinetic/pharmacodynamic (PK/PD) approach for antimicrobial agents uses plasma concentration as the PK input value and minimum inhibitory concentration (MIC) as the PD input value (7). Fluoroquinolones are considered to be well-tolerated drugs in both humans and animals, however, their intensive use has led to a significant increase in antimicrobial resistance (1). Therefore, several PK/PD indices such as C_{max}/MIC and $AUC_{0\text{-}24}$/MIC have been included in the present study to evaluate the clinical efficacy of enrofloxacin.

The aim of this study was to evaluate the PK of enrofloxacin and its metabolite ciprofloxacin in *Testudo hermanni* after a single intracoelomic injection of 10 mg/kg enrofloxacin, and to establish if 10 mg/kg is an optimal dosage for treatment of different bacterial infections.

Nine tortoises of undetermined age, including both sexes (five males and four females), with a body weight range from 0.4 to 2.95 kg, were used. The tortoises were housed indoors, divided equally into three glass containers, with access to indirect sun light and heat lamps (UVB 5%). Animals were maintained at 30 to 33˚C with 250-W infrared heat lamps suspended 0.5 m above the floor to allow turtles to regulate their own body temperature. Tortoises were conditioned for a 2-week period prior the commencement of the study. Tortoises were judged to be in good health based on physical examinations, normal activity, and routine acceptance of food. These observations were made by specialized veterinary personnel. All the tortoises were fed a mixture of vegetables, and given access to fresh water *ad libitum*.

Animal care and handling was performed according to the provision of the EC council Directive 86/609 EEC. The study protocol was approved by the University of Pisa's ethics committee for animal welfare (CEASA) and transmitted to the Italian Ministry of Health.

Enrofloxacin as the commercial injectable solution (Enrovet® 25mg/mL, Bio98, Milan Italy), was diluted with saline to 10 mg/mL and given as a 10 mg/kg bolus by intracoelomic injection in the left prefemoral fossa using a sterile 22-gauge, 3.75-cm needle. The dose used in the present study was selected based on previous studies in turtles (3, 5, 8). The drug was diluted because a previous study demonstrated that a 10 mg/kg intracoelomic injection of 10 mg/mL in yellow bellied slider turtles did not cause local irritation and soft tissue necrosis (5). These changes did occur when the same dose was used at higher concentrations (25 mg/mL) (9-10). Blood samples (0.5 mL or 0.25 mL in subjects greater than or less than 0.5 kg body weight, respectively) were collected from the subcarapacial venipuncture site at 0, 0.5, 2, 4, 10, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240 and 264 h after enrofloxacin administration. Although subcarapacial blood collection could be considered suboptimal because of potential lymph contamination, enrofloxacin has been reported to be equally distributed in blood and lymph (7), and thus the pharmacokinetic data were not expected to be affected by sampling method. The blood samples were immediately transferred to tubes containing heparin, centrifuged and stored at -20˚C until they were analyzed. Sample analysis was completed within 30 days of collection. The analytical method was based on a previous method using high performance liquid chromatography (HPLC) with a fluorescence detector (5). Pharmacokinetic analysis of enrofloxacin and ciprofloxacin was performed using WinNonlin 5.3.1 software program according to a non-compartmental model.

No adverse effects at the point of injection and no behavioural or health alterations were observed in the animals during or after the study. Some transient, self-resolving side effects such as uncoordinated movements were noticed in an earlier study (5) in yellow bellied slider turtles. Species differences might have triggered this distinction in side effects.

Blood levels of enrofloxacin were quantified in all subjects up to 240 h following injection. Blood levels of ciprofloxacin were detected in all subjects up to 120 h. The semi-logarithmic blood concentration *vs.* time average curves for enrofloxacin and ciprofloxacin are reported in Figure 1. The pharmacokinetic parameters are reported in Table 1.

The mean maximum blood concentration of enrofloxacin (C_{max} 8614.64 ± 1116.36 ηg/mL) was reached at 2.19 h. This value, if normalized for the dose, was within the range of peak concentrations shown in previous studies on intramuscular injection of 5 mg/kg enrofloxacin in Gopher tortoises (2.4 μg/mL) (4) and Indian star tortoises (3.59 μg/mL) (11). Similar trends were noticed also in the ciprofloxacin concentration. The C_{max} of ciprofloxacin was 605.16 ± 43.04 ηg/mL attained at 4.23 h. This value was comparable to that shown in Indian star tortoises (0.35 μg/mL) after intramuscular injection of 5 mg/kg enrofloxacin, if normalized for the dose (11), but higher than that shown in yellow-bellied slider turtles (0.32 μg/mL) following an intracoelomic injection of 10 mg/kg enrofloxacin (5). The values of apparent terminal half-life ($T_{1/2}\lambda z$) of enrofloxacin and ciprofloxacin were 37.00 ± 11.97 h

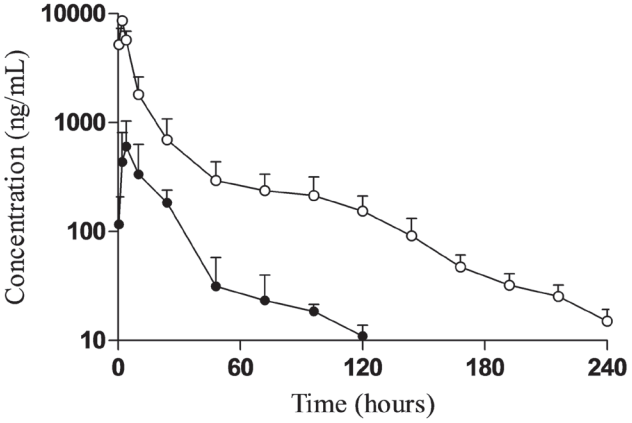

Figure 1: Mean semi-logarithm plasma concentrations of enrofloxacin (-○-) and ciprofloxacin (-●-) *vs* time curves following intracoelomic injection of enrofloxacin (10 mg/kg) in tortoises (n=9). Bars represent the standard deviations.

Table 1: Pharmacokinetic parameters of enrofloxacin and ciprofloxacin after 10 mg/kg enrofloxacin intracoelomic injection in tortoises (*Testudo hermani*) (*n*=9)

		Enrofloxacin			Ciprofloxacin		
Parameter	**Units**	**Mean**		**SD**	**Mean**		**SD**
r^2		0.99	±	0.01	0.97	±	0.01
λz	1/hr	0.02	±	0.03	0.01	±	0.01
$T_{1/2}\lambda z$	hr	37.00	±	11.97	49.06	±	5.82
T_{max}	hr	2.19	±	0.58	4.23	±	0.93
C_{max}	ng/mL	8614	±	1116	605.16	±	43.04
AUC_{0-24}	hr*ng/mL	66388	±	4647	7952	±	318
$AUC_{0-\infty}$	hr*ng/mL	102123	±	9476	12835	±	1244
Vz/F	mL/kg	5227	±	926	55140	±	443
CL/F	mL/hr/kg	97.92	±	19.98	779.08	±	88.17
$AUMC_{0-\infty}$	hr*hr*ng/mL	3383764	±	42011	412688	±	58959
$MRT_{0-\infty}$	hr	33.13	±	1.69	32.15	±	1.28

r^2 = correlation coefficient.
λz = terminal phase rate constant.
$T_{1/2}\lambda z$ = terminal half-life.
T_{max} = time of peak.
C_{max} = peak plasma concentration.
Vz/F = apparent volume of distribution.
CL/F = apparent clearance.
AUC_{0-24} = area under the plasma concentration-time from 0-24 h curve.
$AUC_{0-\infty}$ = area under the plasma concentration-time from 0 h to infinity curve.
$AUMC_{0-\infty}$ = area under the first moment curve.
$MRT_{0-\infty}$ = mean resident time.

and 49.06 ± 5.82 h, respectively. $T_{1/2}\lambda z$ of enrofloxacin was longer than that reported in earlier studies: Gopher tortoises (23.1 h) (4) and Indian star tortoises (5.1 h) (11). This difference might be due to the different routes of administration (intracoelomic *vs.* intramuscular) and the previously mentioned wide variability in pharmacokinetic parameters among tortoise species. In agreement with this speculation, a recent study involving a 10 mg/kg intracoelomic injection of enrofloxacin in yellow-bellied slider turtles (5) has shown that intracoelomic administration significantly increased the drug $T_{1/2}\lambda z$ compared to the same parameter after intramuscular and oral administration in red-eared slider turtles (8) and after oral administration in Loggerhead sea turtles (3).

The reported MICs of enrofloxacin for most susceptible Gram negative, Gram positive bacteria and Mycoplasma isolated from domestic animals were < 0.1 μg/mL, with some additional moderately susceptible isolates having MICs of 0.125-0.5 μg/mL (4). C_{max}/MIC ratio > 10 and AUC_{0-24}/MIC ratio of 100 and 125, are required for fluoroquinolones to have antibiotic activity and to limit the development of bacterial resistance, respectively (7, 12-13). In the present study, considering a bacterium with a MIC value of 0.5 μg/mL, the C_{max}/MIC ratio of enrofloxacin was 17.23 and the average AUC_{0-24}/MIC ratio was higher (132.78) than the required safety value. In contrast, C_{max}/MIC ratio and AUC_{0-24}/MIC ratio of ciprofloxacin were below the target ranges. These results could be due to the limited extent to which ciprofloxacin is produced in reptiles (<15%), as compared to mammals (35%) (14). This finding is in line with the low contribution of ciprofloxacin shown in reptiles (5,8). It has been postulated that this minimal presence of ciprofloxacin could be due to the slow metabolism of turtles and tortoises. In fact, cytochrome P450 3A, the enzyme that metabolizes enrofloxacin to ciprofloxacin, has been found to be poorly expressed in reptiles and fish (15-16).

In conclusion, the plasma concentrations of enrofloxacin achieved in this study after intracoelomic administration of 10 mg/kg enrofloxacin are adequate to reach the target end points associated with efficacy of fluoroquinolones in tortoises (*Testudo hermanni*).

CONFLICT OF INTEREST STATEMENT

None of the authors of this paper have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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Acute Pancreatitis in a Horse – a Case Report

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ABSTRACT

This report presents a case of acute pancreatitis in a 30 year old local breed horse. The horse was diagnosed clinically with severe acute abdominal pain, distended small intestine, a left dorsal large colon displacement and large colon impaction. On post mortem examination pathological changes in the pancreas were observed without intestinal impaction. Histopathologically, the pancreatic lesions were diagnosed as acute pancreatitis with peripancreatic fat necrosis. In addition to these findings, multifocal necrotizing hepatitis was identified as well as a mild interstitial nephritis and tubular nephrosis. This case demonstrates the difficulty in making a clinical diagnosis of pancreatitis in a horse and the importance of a thorough macroscopic and histological evaluation of the pancreas in horses with a history of abdominal pain.

Keywords: Horse; Colic; Abdomen; Impaction; Pancreas; Pancreatitis; Hepatitis.

INTRODUCTION

Acute pancreatitis is rarely diagnosed in horses and the true prevalence of the disease is probably under estimated appearing less commonly than in other species (1, 2, 3). It appears to be much less commonly reported in horses than in other species (1). Both acute pancreatitis and chronic pancreatitis have been documented. Acute pancreatitis is usually associated with severe acute colic, often characterized as an acute small intestinal obstruction with significant reflux and peritonitis. Chronic pancreatitis tends to cause inappetance, weight loss, lethargy and mild recurrent signs of colic (1, 2). Previous studies have identified horses with gastric distention and/or rupture with acute pancreatitis (1, 3, 4, 5).

Pancreatitis has been reported to occur in adult horses although cases of pancreatitis have been described in foals at post mortem (4, 5, 6, 7). Ante-mortem diagnosis is difficult on the basis of clinical and laboratory findings. No specific diagnostic tests are available and although reference values for serum and peritoneal fluid amylase and lipase activities have been published, their diagnostic accuracy has not been established (4, 8). Furthermore, trypsin is produced in small amounts by the equine pancreas (4).

Clinical signs are non-specific; the most important are signs of colic, gastrointestinal reflux and shock (2-5). The abdominal pain originates from gastric distention, peritonitis and hemoperitoneum (9). Thus the lack of specific clinical pathological parameters and clinical signs makes the clinical diagnosis of equine pancreatitis a challenge in the live horse.

In dogs, cats and humans pancreatitis is associated with nutritional imbalance, abdominal trauma, hypercalcemia, hyperlipidemia, drug induced, bacterial and viral infection, vascular impairment, cholecystitis, small intestinal obstruction and duodenal reflux (9, 10). Etiology in horses includes partial or complete destruction of the pancreatic duct (cholangiohepatitis and cholethiasis), migration of *Strongylus equinus* and *Parascaris equorum*, duodenitis, duodenal ulcers and possible vasculitis in foals and associated with other disorders, commonly those involving the gastrointestinal tract or liver (4, 10, 11). Migration of *Strongylus equinus* and *Parascaris equorum* to the pancreas can produce pancreatic tis-

sue destruction and extensive fibrosis (12). Histopathology of acute pancreatitis includes large numbers of neutrophils that infiltrate the pancreatic parenchyma and percolate between the intralobular septae and acini, the peripancreatic fat shows evidence of necrosis. Histopathology of chronic pancreatitis is indicated by marked fibrosis between acinar lobuli and surrounding the pancreatic ducts. Interstitial mononuclear cell infiltrate may be present (4).

This case study describes an adult horse with pancreatitis diagnosed at post mortem examination while clinically as a case of severe acute colic that did not respond to analgesic medication and required abdominal emergency surgery. The case illustrates the difficulty in the diagnosis of equine pancreatitis in the clinical situation and proposes the consideration of pancreatitis as a differential diagnosis under conditions of colic of unknown origin.

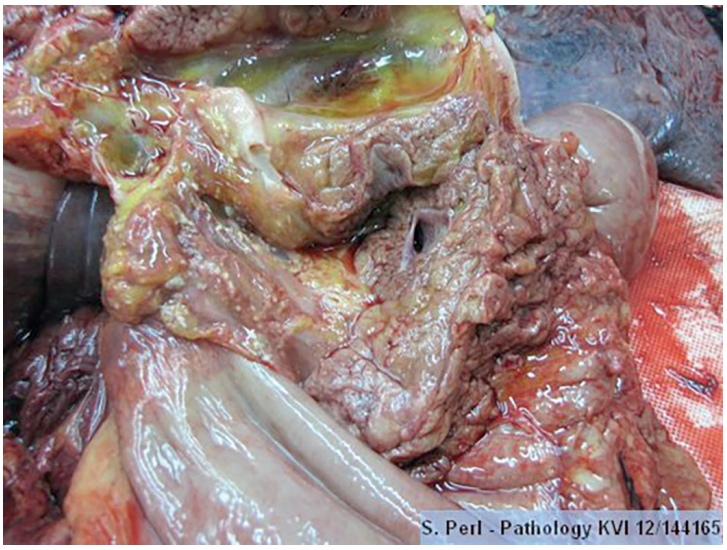

Figure 1: Pancreas: Edema and multiple white yellowish foci of necrosis.

CASE HISTORY

Clinical history

A 30-year-old local breed horse with an acute abdomen was admitted to the Koret School of Veterinary Medicine - Veterinary Teaching Hospital (KSVM-VTH). On physical examination before referral, the horses demonstrated severe abdominal pain even after NSAID (flumixin meglumine) administration and had elevated heart rate (60 beats/minute). Nasogastric intubation was performed by the referring veterinarian and resulted in spontaneous reflux of approximately 25 L of fluid.

Rectal examination revealed swollen small intestines, large colon displacement and large colon impaction. Prior to referral the horse received butorphanol, medetomidine and xylazine. On arrival the horse was dehydrated (dry mucus membranes and decreased skin turgor). Heart rate was 72/minute. Passage of a nasogastric tube resulted in 4 liter reflux. Rectal examination at the hospital diagnosed a left dorsal displacement of the colon and large colon impaction.

The packed cell volume was 53% (Reference interval (RI) 32-52%), total solids were 8.6 g/dL (RI 5.3-7.9 g/dL). creatinine 4.54 mg/dL (RI 0.9-2.0mg/dL) and lactate 7.6 mmol/L (RI up to 2mmol/L). The horse was prepared for exploratory laparotomy, while showing continuous and unrelenting pain. It was therefore treated with xylazine (Vetmarket, Shoham, Israel) and butorphanol (Alvegesic, Dachra Veterianary ptroducts, Shropshire, UK). While preparing the horse, signs of shock were evident: mean arterial pressure of 20 mm Hg, weak pulse, disrupted ECG and cyanotic mucous membranes. Prior to induction after consulting with the owners, the horse was euthanized at the request of the owners due to the poor prognosis.

Post mortem examination

The horse was sent for a full post mortem examination. Post mortem examination revealed slightly collapsed lungs and fibrous tags on the abdominal surface of the diaphragm. The stomach contained sparse fluid content. The pancreas was edematous and with multiple white yellowish foci (Figure 1). The small intestine showed multifocal serosal hemorrhage for almost its entire length. The large intestine contained dry content without pathological lesions in the mucosa. The liver and kidney showed no macroscopic pathological changes. There was no evidence of an impaction.

Histopathology

Histopathological examination of the intestines showed engorgement of blood vessels and multifocal serosal hemorrhage of the small intestine with diffuse submucosal edema. In the pancreatic tissue, multifocal to diffuse infiltrations by inflammatory cell mainly neutrophils and histiocytes were present, as well areas of peripancreatic fat necrosis (Figure 2 and 3). In some areas intralesinal bacterial colonies could be seen (Figure 4). A few of the kidney's tubules were distended and contained an amorphus eosinophilic material. Numerous interstitial

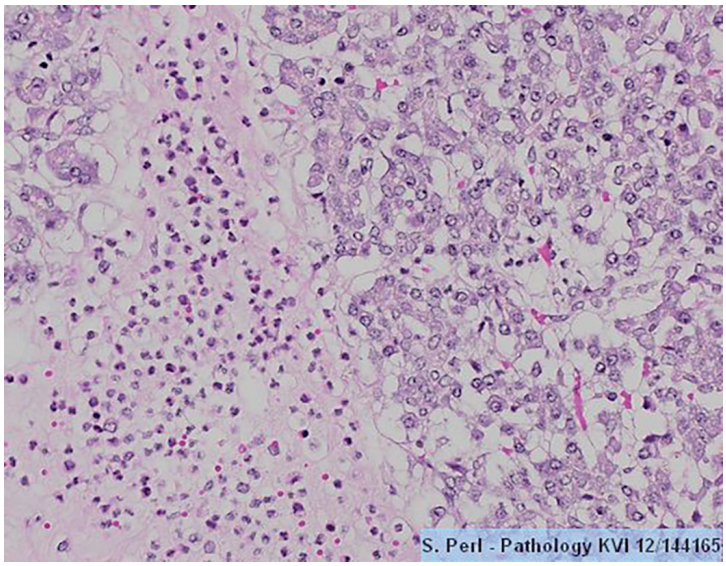

Figure 2: Pancreas: Interlobular edema and infiltration with mainly neutrophils and histiocytes. x20

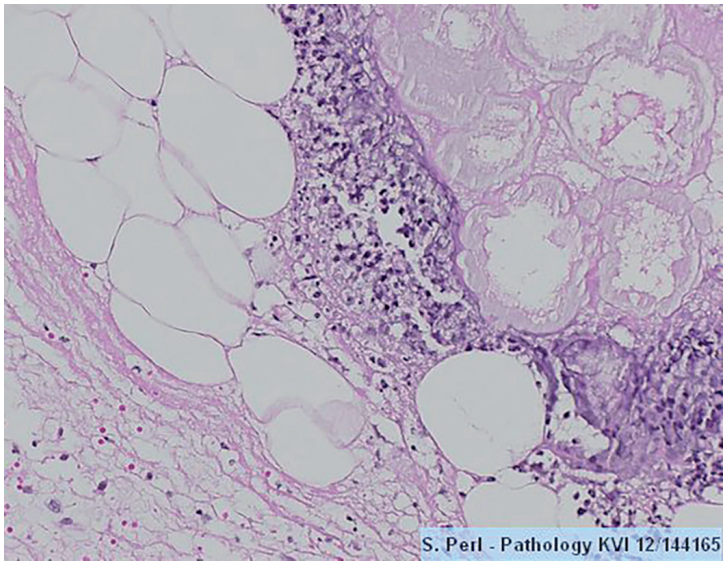

Figure 3: Pancreas: Peripancreatic fat necrosis and multifocal infiltration with inflammatory cells. x20

foci of inflammatory mononuclear cells were evident in the parenchyma. Diffuse congestion was present mostly in cortex. The liver showed multifocal areas of necrosis and infiltrations with mainly neutrophils. Diffuse congestion was also present.

DISCUSSION

Pancreatitis in horses can be a primary condition or may be associated with other disorders, commonly those involving the gastrointestinal tract or liver (4). Pancreatitis may be under diagnosed owing to the presence of nonspecific abdominal pain.

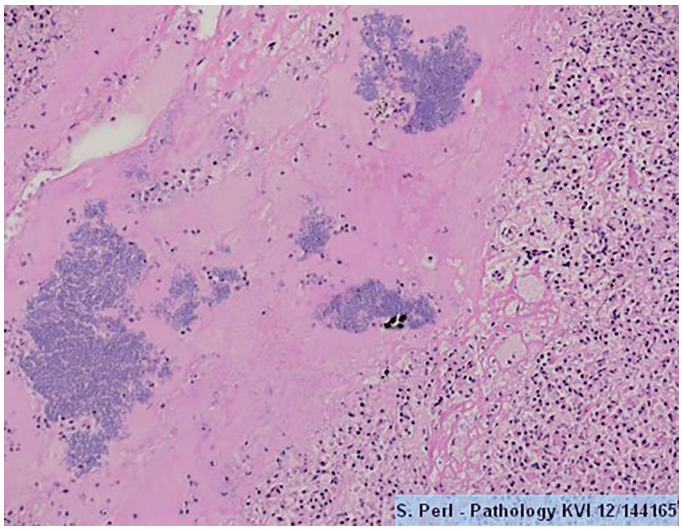

Figure 4: Pancreatic necrosis, infiltration with inflammatory cells and bacterial colonies. x10

In this case report, it is unclear and difficult to determine whether the horse suffered from primary pancreatitis or secondary pancreatitis associated with hepatitis. The case history (pain duration, other symptoms, appetite, etc.) is lacking and in addition, blood tests including complete blood count and chemistry are also absent. Laboratory abnormalities associated with acute pancreatitis include increased activities of serum (and sometimes peritoneal) amylase and lipase (6).

The histopathological finding of multifocal necrotizing hepatitis has many etiologies: Causes of hepatitis in horses include serum hepatitis, cholangiohepatitis and chronic active hepatitis with occasional cases of hematogenous bacterial hepatitis, abscesses, viral hepatitis, parasitism and chronic infiltrative inflammatory disease (7).

It has previously been hypothesized that horses with enteritis or strangulated small intestinal lesions may develop acute pancreatitis and hepatitis as a result of ascending influx of intestinal fluid through the pancreatic and bile duct, with subsequent activation of pancreatic enzymes (4, 6, 10, 11). Once activated, these enzymes are responsible for autodigestion of pancreatic tissue, resulting in necrosis of the acini and pancreatic islets with interstitial fat necrosis and necrotizing vasculitis. The release of pancreatic enzymes stimulates the production of inflammatory cytokines, which, in turn triggers an inflammatory cascade which leads to a systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), shock and death (4, 8).

Large colon disorders such as volvulus and displace-

ments could cause reduced blood flow to the pancreas and induce pancreatitis (4). In this case report, the rectal examination diagnosed large colon displacement and obstruction, but this was not verified in post mortem examination (5). Similarly to previous cases, this horse had distended small intestines and large amount of gastric reflux before referral, signs that are sometimes encountered in acute cases of pancreatitis (1, 4, 5).

In conclusion, pancreatitis should be considered in horses with unexplained moderate to severe abdominal pain with or without gastric reflux. Although the ante-mortem diagnosis of acute pancreatitis in the horse is difficult, it should be included as a differential diagnosis. Results from this case study emphasize the importance of a pathological and histopathological evaluation of the pancreas in horses with a history of abdominal pain.

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Concurrent Neosporosis and Hepatozoonosis in a Litter of Pups

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ABSTRACT

This case report describes a concomitant infection in a litter of puppies with two apicomplexan protozoa *Neospora caninum* and *Hepatazoon canis*. The different potential routes of infection are discussed along with a description of the course of the disease. A private practitioner in the South of Israel (Beer Sheva) submitted blood samples for serological testing for toxoplasmosis and neosporosis from a pup of 6 weeks of age (from a liter of 6 pups) showing neurological symptoms. The results were seropostive for *N. caninum* and seronegative for toxoplasmosis. After 3 weeks another pup also started to develop neurological signs. Samples including blood smears, serum samples for serology for *Neospora* and *Toxoplasma* and fecal samples from all the pups. Out of the 6 pups tested four were found seropositive for *Neospora* while all pups were seronegative for *Toxoplasma*. Of the six pups, four were found to be infected with *Heptaozoon canis*, three of which were co-infected with *N. caninum*. One pup was infected with only *H. canis* and another pup with only *N. caninum* and a third pup was found to be negative for both *H. canis* and *N. caninum.* Treatment consisted of trimethoprim sulfadiazine and clindamycin which were administered for six weeks. The pups recovered completely, with the exception of paresis of the left hind limb in the first diagnosed puppy. An important conclusion from this case report is the need to test all pups in a litter for *N. caninum* where even only one pup shows clinical signs. Furthermore treatment at an early age and for a prolonged period of time appeared to be successful in preventing the progression of the clinical signs. To the best knowledge of the authors this is the first natural concomitant infection case of *N. caninum* and *H. canis* in a litter of puppies.

Keywords: *Neospora caninum*; *Hepatazoon canis*; Concurrent Infection; Dog; Pups.

INTRODUCTION

Neospora caninum, an apicomplexa protozoan from the family Sarcocystidae is the causative agent of abortion in cattle and neuromuscular diseases in dogs (1). The definitive hosts of *N. caninum* are domestic dogs and wild canids. Dogs may be infected by *N. caninum* horizontally by consumption or vertically by *in utero* transmission of tachyzoites during pregnancy (1, 2). In naturally infected dogs and cattle the predominant route of infection is considered to be transplacental (1, 2).

Hepatozoon canis is an arthropod-borne infectious agent of the apicomplexan protozoa from the family *Hepatozoidae* in the suborder *Adeleorin*. *H. canis* transmission takes place by ingestion by the intermediate host, the dog, of the brown dog tick *Rhipicephalus sanguineus*, the definitive host that contains mature oocysts (3). Most domestic dogs probably become infected with *H. canis* through grooming ticks from their hair coat. Also like other apicomplexan parasites horizontal transmission through the uterus from dam to its offspring has been demonstrated to take place (3).

Concurrent infections in dogs are reported to occur with a variety of pathogens (3). Often they may be transmitted by a common vector such as the tick. However co-infecting pathogens seemingly unrelated may be involved, resulting in clinical manifestations which may make the clinical diagnosis more complex. The relationship between the concurrent infections in this case involves two diseases of puppies, neosporosis and hepatozoonosis, both of which can be transmitted *in utero*. On the other hand, infection by one agent may influence the susceptibility to a new infection which may influence the progression of the existing condition.

This case report describes a concomitant infection in a litter of puppies with two apicomplexan protozoa *N. caninum* and *H. canis*. The different potential routes of infection are discussed along with a description of the course of the disease. To the best knowledge of the authors this is the first natural concomitant infection case of *N. caninum* and *H. canis* in a litter of puppies.

CASE REPORT

A sample of serum was received from a private practitioner from a pup named "Fistuk" of 6 weeks of age from a liter of 6 pups born in the city of Beer Sheva in the South of Israel. The pup showed neurological symptoms which in the opinion of the practitioner resembled either that of toxoplasmosis or neosporosis. The clinical signs in the pup were that of paresis of the left hind limb which appeared to be progressing to the right hind limb. In addition the pup appeared to be both fecally and urinarily incontinent.

The serum sample was tested by the immunofluorescence antibody test (IFAT) for both *Toxoplasma gondii* and *Neospora caninum*. A pronounced high titer for were found for *N. caninum* (1:12,800) while no reaction to *T. gondii* were observed. The pup was treated orally with trimethoprim sulfadiazine (Resprim, Teva Medical, Petah Tikva, Israel) and clindamycin (Dalacin C, Pfizer Pharmaceutical, Israel). Two weeks later the pup showed an improvement in the right hind limb but not in the left hind limb.

When the pups reached 9 weeks of age, another pup, named Pufi, also started to develop neurological signs of paresis in the left hind limb and furthermore had difficulty in rising. Samples including blood smears, serum samples for serology for *Neospora* and *Toxoplasma* and fecal samples were collected from all the pups. Out of the 6 pups tested four were found seropositive for *Neospora* with high antibody titers (Table 1) while all pups were seronegative for *Toxoplasma*. Of the six pups, four were found to be infected with *Heptaozoon canis*, diagnosed from examination of their blood smears and three of which were co-infected with *N. caninum*. One pup was infected with only *H. canis* and another pup with only *N. caninum* and a third pup was found to be negative for both *H. canis* and *N. caninum.* No oocysts, or other parasites were found in fecal samples.

Clinical signs resembling those of neosporosis were only seen in the two pups described above. The other two pups with hepatozoonosis and/or neosporosis did not appear to exhibit any detectable clinical signs of disease.

Positive pups for either *N. caninum* or *H. canis* parasites were treated *per os* with trimethoprim sulfadiazine (15mg/kg q12h) (Resprim, Teva, Petach Tikva, Israel) and clindamycin (10mg/kg q12h) (Dalacin, Pfizer) for six weeks (2). All pups were found to be healthy after 6 weeks after treatment. The incontinent pup Fistuk improved although the left hind limb remained paralytic. The pup named Pufi recovered completely.

No *H. canis* parasites were observed in the blood smears

Table 1: Antibody titer for *Neospora caninum* and the detection of *Hepatozoon canis* gamonts in a litter of 6 pups

Date	10-Oct-12	5-Nov-12		11-June-13	
Name	Ab titers *N. caninum*	Ab titers *N. caninum*	Blood smear	Ab titers *N. caninum*	Blood smear
Fanta	n.d.	1:12800	Hepatozoon	1:12800	Negative
Pupa	n.d.	1:12800	Negative	1:12800	Negative
Lipstick	n.d.	Negative	Hepatozoon	Not done	Negative
Popcorn	n.d.	Negative	Negative	Not done	Negative
Pufi	n.d.	1:12800	Hepatozoon	1:12800	Negative
Fistuk	1:12800	1:12800	Hepatozoon	1:12800	Negative

n.d. = Not done

examined at the end of the six week treatment period. The four *Neospora* seropositive puppies continued with high antibody titers.

DISCUSSION

The predominant route of natural infection in dogs by *Neospora caninum* is considered to be transplacental (1, 2). The bitch from this report from the south of Israel was adopted by the current owner as a stray dog. It is suggested that free roaming dogs in urban areas have a greater seroprevalence for *N. caninum* than companion pets (4).

It has been reported that a variable number, but not all pups in a litter from a dam infected with *N. caninum* may have clinical manifestations (4, 5, 11). Indeed, in this litter four of the six pups presented as infected, judged by serological investigation (IFAT) and of them only two showed clinical signs at different intervals after birth. An important conclusion from this study is the need to test all puppies in a litter for *N. caninum* where even only one pup shows clinical signs. From the frequency of the disease among the pups in this litter this conclusion appears to be applicable to both *N. caninum* and *H. canis*.

The clinical signs presented by the pups were typical of neosporosis for their age: Congenitally infected pups tends to exhibit a more severe form of the disease (2). Pups of less than 6 months of age exhibit a severe disseminated form showing ascending paralysis of the limbs with the pelvic limbs affected more severely than the thoracic limbs (1, 6). Muscle atrophy, contracture and fibrosis occur in many pups (2). Gradual, progressive, ascending paralysis with hyperextension of the hind limbs in congenitally infected puppies is the most common clinical manifestation of the disease in dogs less than 6 months of age (2).

H. canis infection causes disease ranging in severity from an incidental hematologic finding in an apparently healthy dog to a debilitating and life threatening illness (3). Even so, when high parasitemia is present *H. canis* may also induce severe clinical manifestations (e.g. lethargy, fever, anorexia, weight loss, lymphadenomegaly and anemia) associated with a high parasite load (3, 7). The possibility that the clinical signs were exacerbated due to the concurrent infection with *H. canis* cannot be completely discounted, however *H. canis* is found primarily in the hemolymphatic tissues and not directly related to the neurological system (3).

Concurrent infections with *H. canis* in dogs have been described with *Ehrlichia canis* and *Babesia canis* where all three infections are transmitted by the same tick vector, *R. sanguineus.* Other pathogens reported to be involved in concurrent infections include parvovirus, canine distemper, *Anaplasma phagocytophilum*, *Anaplasma platys*, *Toxoplasma gondii* (8) and *Leishmania infantum*. To the best knowledge of the authors' coinfection of *Heptozoon canis* with *Neospora caninum* has not been previously described.

The seroprevalence of *H. canis* in dogs in Israel has been reported to be 33% (9). The incidence of clinical disease for *H. canis* is considered to be much lower than the exposure rate with most dogs probably undergoing a subclinical form of the disease. In fact in the litter described in this study one pup was infected with *H. canis* only and was not reported to show clinical signs. Although the route of infection cannot be definitely ascertained, it seems likely that the pups were infected *in utero.* Horizontal transmission through in the uterus from the dam to its offspring has been demonstrated in *H. canis*. In a study of naturally infected pregnant bitches meronts were found in the spleen of a pup that died 16 days after birth and blood gamonts were detectable as early as 21 days in other pups. The possibility of these pups being naturally infected by ingestion of the *Rhipicepahalus sanguineus* ticks cannot be completely discounted, however taking into account the young age of the pups and the relatively high rate of infection within the litter this seems unlikely. Furthermore it would have been necessary for the young pups to ingest the ticks and this makes the possibility of infection after birth as even more unlikely.

Symptoms seen in the two pups were strongly suggestive of infection with *N. caninum* (1). Treatment with trimethoprim sulfadiazine and clindamycin for six weeks was successful in treating the two affects pups, one pup had recovered completely, and the other remained with paresis of the left hind limb whereas the other signs including urinary and fecal incontinenence were alleviated. Although the current treatment protocol for *H. canis* is imidocarb dipropionate alone or in combination with doxycycline, a similar treatment used in this study has been recommended for several years in the treatment of *H. canis* (10). This treatment regime was not applied in the treatment of two puppies in this study indicating that *H. canis* may not have been involved in the symptomology of this concurrent infectious condition.

The exact interaction of *N. caninum* and *H. canis* in the

pathogenesis of the symptoms is unknown but from the treatment outcome it does appear that *N. caninum* did play a prominent or maybe even a singular role.

In conclusion, this case study is to the best knowledge of the authors the first report of a concomitant infection of neosporosis and hepatozoononosis in a litter of puppies. Treatment at an early age and for a prolonged period of time appeared to be successful in preventing the progression of the clinical signs and possibly preventing clinical signs in other litter mates which were infected. Our study is in agreement with others who shown that in naturally infected dogs, treatment can improve clinical signs but may not eliminate the infection, as the puppies remained seropositive (11).

The clinical signs were strongly indicative of those caused by *N. caninum* although the role of *H. canis* in the development of the symptomology could not be established.

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Bartonellosis and Ehrlichiosis in Dogs and Cats

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Bartonelloses are a group of diseases caused by *Bartonella* species. The latter are small pleomorphic Gram-negative bacilli, belonging to the α-2 subdivision of *Proteobacteria*. They are intracellular bacteria, parasitizing RBCs and endothelial cells, transmitted by arthropod vectors. They cause persistent/cyclic bacteremia. More than 30 species exist in the genus (with many more variants). Over than 15 species are associated with human diseases. Six species have been reported in cats, including *Bartonella henselae*, *Bartonella clarridgeiae*, *Bartonella koehlerae*, *Bartonella quintana*, *Bartonella bovis* and *Bartonella vinsonii* subsp. *berkhoffii.* Most cats naturally or experimentally infected with *Bartonella* spp., exhibit no clinical signs. Their CBC, biochemistry and urinalysis results are usually within normal reference intervals. In Israel, a molecular survey indicated that 25.1% of the general cat population (31% of stray cats and 19% of indoor cats) were positive for *Bartonella* spp. Three species were detected, including *B. henselae* (15.6 & 15.5%), *B. clarridgeiae* (12.3 & 3.2%) and *B. koehlerae* (5.6 & 1.3%) in stray and indoor cats, respectively. Cat scratch disease (CSD) is the best known disease caused by *Bartonella* species. The etiologic agent of CSD is *B. henselae*, causing several syndromes in humans. Immunocompetent human individuals present usually more local signs, while immunocompromised individuals may present systemic signs, which may be fatal. Veterinarians and veterinary staff are at greater risk for CSD. Transmission of *B. henselae* from cats to humans probably occurs through contamination of cat scratches with flea excrement. Transmission may also occur through cat bites (blood, saliva, flea-excrement). Dogs can be infected with several *Bartonella* spp. including B. *vinsonii* subsp. *berkhoffii*, *B. henselae*, *B. clarridgeiae*, *B. washoensis*, *B. elizabethae*, and *B. quintana*. Domestic dogs are more likely to be accidental hosts. They are excellent sentinels for human infections because a similar disease spectrum develops in dogs.

Canine monocytic ehrlichiosis (CME) is an important canine disease worldwide. It is caused by the obligate intracellular rickettsia *Ehrlichia canis* and is transmitted by the brown dog-tick of the *Rhipicephalus sanguineus* complex. Recent molecular studies detected *E. canis*-DNA in cats from Portugal and Brazil. However, to date *E. canis* was not isolated and cultured from suspected cats. Diagnosis of the disease is challenging due to its variable presentations. Molecular techniques are becoming the definitive diagnostic methods of CME. Polymerase chain reaction (PCR) and sequencing are sensitive methods for detecting and characterizing *E. canis*-DNA, respectively. Detection of *E. canis* DNA can be achieved as early as 4 to 10 days post-inoculation. Several assays are based on different target genes, however the 16S rRNA and the p30-based PCR assays are commonly used. The p30-based assay is more sensitive than the 16S rRNA-based assay, probably because the fact that each *E. canis* contains one gene copy of the 16S rRNA while multiple copies of the p30 gene. Splenic samples are considered more sensitive samples for the evaluation of ehrlichial elimination by PCR. Real time PCR is a more sensitive assay than conventional PCR, allowing quantitative analysis of specific DNA. It is less prone to contaminations than conventional methods, and therefore is rapidly becoming the preferred method for diagnosis of *E. canis*. Recently, loop-mediated isothermal amplification (LAMP) is becoming an important assay for the in clinic diagnosis of infectious diseases. LAMP-based assays have been developed for the diagnosis of *E. canis*. They will probably become important assays in future diagnosis of CME.

MERS-CoV: A Humped Zoonosis?

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In June 2012, a 60-year-old male citizen of Bisha, southeast Saudi Arabia, presenting with pneumonia associated with acute renal failure and was hospitalized in Jeddah. A novel beta corona virus, later coined Middle East Respiratory Syndrome Coronavirus or MERS-CoV, was isolated from his sputum and sequenced. The patient died and became to be known as the "index case" of a hitherto unknown corona infection.

Since then, the epidemic has spread, mainly in Saudi Arabia and neighbouring countries with sporadic cases elsewhere. As of 16 Feb 2015, a total of 1016 cases of MERS-CoVhave been reported by local health authorities worldwide, including 406 deaths (case fatality rate 39.96%). The numberof reported cases in the most affected countries were (in brackets, fatal): Saudi Arabia 886 (372), United Arab Emirates (UAE) 71 (10), Qatar 11 (5) and Jordan 10 (4). Retrospectively it was found that thevery first clinical cases of MERS-CoVoccurred, in fact, in April 2012 in Jordan, involving acluster of patients in a public hospital in Zarqa, about two months before the detection of the "index" case in Saudi Arabia. Clinical cases have been reported, so far, in 10 countries in the Middle East (Saudia, UAE, Jordan, Qatar, Oman, Kuwait, Egypt, Yemen, Lebanon and Iran),and in 13 countries elsewhere. In Europe (Austria, UK, France, Germany, Italy, Netherlands, Greece), Asia (Turkey, Malaysia, Philippines), Africa (Algeria, Tunisia) and America (USA). Most of the infections, in the Middle East have been shown to occur in healthcare settings. All cases which have been reported outside the Middle East, first developed infection within the region and were then exported. These travel-related cases have, so far, rarely infected others in the destination countries.

Several international research centers have invested efforts to unravel theepidemiology of MERS-CoV with partial success. In particular, they tried to identify the animal reservoir of the virus and its mode of transmission.

The SARS ("Severe acute respiratory syndrome") experience in 2003, namely a novel, zoonotic coronavirus disease casing seriousdisease in humans (but with lower fatality rates and higher inter-human infectivity), became the benchmark. Bats were found to be the primary reservoir of SARS virus. Hence, bats became main suspects as the primary reservoir of MERS-CoV. So far, there is just one single report on the detection of MERS coronavirus genetic material in a bat (Taphozousperforatus, the Egyptian tomb bat, Saudia,October 2012). No viable, whole virus could be obtained from this or anyother bat-derived sample to present (February 2015).

The initial suspicion of dromedary (single humped) camels as possibleintermediate hosts of MERS-CoV was raised in March 2013, when it became apparent that a MERS patientfrom the UAE who died in a hospital in Munich, Germany, had kept 4 camels as pets in his yard and had beenexposed to a sick camel shortly before falling ill. That animal was not tested. These observations encouraged researchersto focus attention to camels. Between October 2013 and December 2014, 4 countries reported to the World Organisation of Animal Health (OIE) the irrespective detections of MERS-CoV or its genetic material in sub-clinically infected camels. They were Kuwait and Oman (5 each), Qatar (9), and Iran (14 camels, reportedly illegally imported from Pakistan). In Qatar, the virus was detected in raw camel milk samples. Infections in Saudi camels have been described in scientific papers, but – so far – not reported to the OIE.

Evidence from MERS-CoV infections in camels suggests virus shedding for a limited period. During an experimental infection trial in Colorado, USA, 3 adult dromedary camels were inoculated with a human isolate of MERS-CoV. Transient, primarily upper respiratory tract infection developed in each of the 3 animals. Clinical signs were benign. Each camel shed large virus quantities from the upper respiratory tract. The infectious virus was detected in nasal secretions through 7 days postinoculation, and viral RNA was detectable up to 35 days postinoculation.

The possibility for reinfection of camels cannot at this stage be excluded since immunity to infection is poorly understood. MERS-CoV has been identified in camels which have antibodies against the virus. There are still many MERS-CoV cases in humans of which the source of infection remains obscure. The implications of these findings for management and control recommendations need further investigation.

Serological studies suggest that antibodies to MERS-CoV have been detected with a prevalence range of 0-100% (varying within countries and between countries) in populations of camels in Middle East and African countries. This range of prevalence indicates the need to assess risk factors for infection between and within herds. The results are suggestive of MERS-CoV circulating since decades; the earliest positive camels in Saudi Arabia date back to 1993, corresponding with the start of increased camel imports, in particular from Somalia and Sudan. International trade may have contributed to the virus' diversity and persistence, but its epidemiology, and particularly its late emergence as cause of human disease, is still in need of considerable research effort. There does not seem to be at present room for animal culling nor practicality of trade restrictions within the affected regions.

According to published literature, other species of animals, including sheep, goats, cattle, water buffalo and wild birds, have tested negative for the presence of antibodies to MERS-CoV. However owing to the relatively small sample sizes the results of these studies cannot exclude infection in other animal species. Based on receptor studies other animal species have been identified as potential hosts.

Although genetically related viruses have already been detected in bat species around the world, and a fragment of viral genetic material matching the MERS-CoV was found in one bat from Saudi Arabia, current evidence does not (yet) indicate a direct link between bats and MERS-CoV in humans. More evidence is needed to directly link the MERS-CoV to bats or other animal species.

In Jordan, 10 human cases were reported in 2012. A serological investigation in domesticanimals was applied there between June and September 2013; neutralising MERS-CoV antibodies were found in all (11) camel sera tested, while sera from goats and cattle tested negative. In view of the fact that Israel borders Jordan, Israeli investigators are encouraged to take notice of these data and include MERS-CoV within their scientific objectives. This may include serology in camels (Israel's population estimated at 3000-4000, most of them in the south). The scarce information on MERS-CoV in bats calls for enhanced research.

The "Egyptian tomb bat" is known to be present in the Jordan valley, which separates Jordan and Israel and is shared by both. According toa recent report of Israel's nature protection agencies, there are at least 4 active colonies of T. perforatus in Israel. The largest cave, found to include hundreds of breeding bats, is situated on the northern shore of the Dead Sea, very close to the Jordanian border. Sampling and testing these bats deserves to become a prioritised "One Health" project, encouraged and supported by local and international public health, animal health, andnature protection agencies, preferably combining regional cooperative efforts.

Severe Subcutaneous Mast Cell and Eosinophilic Infiltration Associated with Presence of Multiple *Dirofilaria repens* Microfilariae in Three Dogs

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Dirofilaria repens is a parasitic nematode in the subcutaneous tissue of carnivores, including dogs and cats, transmitted by mosquitos. It is a zoonosis, since humans might serve as accidental hosts. Infection of a dog with *D. repens* was first reported in the Palestine in 1934, and two additional cases were reported in dogs in Israel to date. This report describes *D. repens* infection in 3 dogs from Ramat Hasharon, Israel that presented subcutaneous masses, which were cytologically characterized by severe mast cell and eosinophil infiltration, with numerous microfilariae in 2/3 cases; rare microfilariae were present in the third case. In all three dogs, polymerase chain reaction of fine needle aspirates was positive for *D. repens*. The mast cells observed in all lesions were uniform

and highly granular, and with the presence of the microfilariae, mast cell tumor was considered unlikely. This report suggests that *D. repens* infection-associated subcutaneous lesions, characterized cytologically by massive mast cell and eosinophil infiltration, should be considered a differential diagnosis for mast cell tumor, especially in geographic locations endemic for this nematode. These dogs were infected with *D. repens* despite a routine preventative doramectin therapy against *Spirocerca lupi* administered every three months, probably due to the relatively long time-interval between treatments. It seems that Ramat HaSsharon is endemic for the nematode.

Hemostatic Abnormalities in Dogs with Naturally-Occurring Heatstroke

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Heatstroke in dogs induces both procoagulant and anticoagulant states, characterized by several hemostatic disturbances, often leading to DIC and death. This prospective observational study aimed to characterize hemostatic analytes and their association with mortality in dogs with naturally-occurring heatstroke. Citrated and ETDA blood samples were collected at presentation and at 4, 12, 24, 36 and 48 hours post-presentation (PP) from 30 client-owned dogs with naturally-occurring heatstroke. Hemostatic tests performed included platelet count, prothrombin and activated partial thromboplastin times (PT and aPTT, respectively), antithrombin activity (ATA), total protein C activity (tPCA) and fibrinogen and D-dimer concentrations. The survival rate was 60% (18/30 dogs). Older age, higher heart-rate and rectal temperature at presentation and time from onset of clinical signs to presentation were significantly associated with mortality. Hemostatic analyte results at presentation were not associated with mortality; however prolonged PT and aPTT at 12-24 hours PP, lower tPCA at 12 hr PP and hypofibrinogenemia at 24 hours PP were significantly ($P \leq 0.05$) associated with mortality. Increased D-dimer concentration and low ATA were common at all time-points, but were not associated with mortality. The frequency of DIC increased in non-survivors throughout hospitalization, but DIC was not associated with mortality. The number of abnormal hemostatic abnormalities during the first 24 hours was significantly ($P=0.04$) higher in non-survivors. All but one dog received fresh frozen plasma (FFP), with no difference in FFP dose between survivors and non-survivors (4.4 vs. 4.5 units per dog, respectively, $P=0.45$). In conclusion, hemostatic derangements are very common in dogs with naturally-occurring heatstroke. PT, aPTT, tPCA and fibrinogen concentrations became associated with the outcome at 12-24 hours PP, exemplifying the need for serial sequential measurement of multiple laboratory hemostatic tests during hospitalization, even if results are within reference interval at presentation. In this cohort, DIC was not associated with outcome, possibly because FFP was used extensively compared to previous studies of heatstroke in dogs.

Canine Protothecosis in Israel: A Case-Series of Four Dogs

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Protothecosis is caused by *Prototheca* spp., a saprophytic achlorophyllous algae. The disease has been identified in people and animals in Europe, Asia, Africa, Australia, Pacific Ocean islands and North America. The most frequently reported clinical sign in dogs is intermittent, protracted bloody diarrhea. Dogs generally develop

a disseminated disease, and the type and severity of the clinical signs vary, depending on the tissue involved. Between 2013 and 2014, four dogs were diagnosed with protothecosis in Israel, based on identifying *Prototheca* organisms in cytological smears or histopathology of biopsies. Three of the four dogs had a history of protracted bloody diarrhea. Two dogs had disseminated disease, while two had localized infection, one of which with recurrent subcutaneous lesions, while in the other, clinical signs were limited to the gastrointestinal tract. Shortly after the diagnosis, one dog with systemic disease died, while the other was euthanized. The two remaining dogs were both treated orally with itraconazol, while the dog with gastrointestinal infection was treated additionally with oral nystatin. This dog was euthanized six months after the diagnosis. The other dog, treated with itraconazol, showed no signs of disseminated infection 14 months after diagnosis, although reoccurrence of the subcutaneous infection occurred.To the best of our knowledge, this is the first report of protothecosis in dogs in Israel. Although an uncommon disease, care should be taken to diagnose such infected dogs early, to facilitate early specific treatment. However, the prognosis is poor and although treatment might prolong life, it usually will not result in disease resolution.

Sleeping and Resting Respiratory Rates in Dogs with Subclinical Left-Sided Heart Disease

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The objective of this study was to characterize sleeping respiratory rates (SRRs) and resting respiratory rates (RRRs) measured in the home environment, of dogs with subclinical heart disease that did result in left-sided congestive heart failure. The study was a prospective cross-sectional study and included 190 adult dogs with subclinical left-sided heart disease. Most (n=174) dogs had mitral valve disease of various severities. Clients collected ten 1-minute SRRs or RRRs during a period ranging from 5 to 178 days (median 15 days). Clinicians provided echocardiographic and medical data on each patient.The within-dog mean SRR (SRRmean; 16 breaths/min, range: 9-33) was significantly (P<0.001) lower than the within-dog mean RRR (RRRmean; 21 breaths/min, range 10-43). Seven dogs had SRRmean and 33 dogs had RRRmean > 25 breaths/min; 1 dog had SRRmean and 12 dogs had RRRmean > 30 breaths/min; these dogs mostly had a left atrial (LA)-to-aortic ratio > 1.8. Dogs with moderate LA enlargement had a significantly higher SRRmean than did other dogs (P = 0.003). However, median SRRmean for each of 4 levels of LA enlargement was < 20 breaths/min; median RRRmean for each of 4 levels of LA enlargement was < 25 breaths/min. Both within-dog SRR and RRR remained stable for 10 consecutive measurements (P = 0.08-0.4). Treatment with cardiac medications and presence of pulmonary hypertension were not associated with SRRmean or RRRmean. It was concluded that dogs with confirmed subclinical left-sided heart disease of various severities generally had SRRmean < 25 breaths/min, which was infrequently exceeded at any time. SRR and RRR remained stable, regardless of individual within-dog SRRmean or RRRmean.

Septic Lens Implantation Syndrome in a Dog Following a Lens' Cat Claw Injury with Positive Medical Outcome

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Septic implantation syndrome (SIS) refers to traumatic inoculation of bacteria into the lens cortex and development of lenticular abscess following capsule rupture. This results in a delayed-onset severe endopthalmitis. A squela of glaucoma frequently leads to enucleation. A 3-year old male, Labrador retriever dog was referred to Eyecare Clinic two days after a cat claw injury to the right eye. On presentation, examination revealed corneal perforation, a 3-mm lens capsule rupture and mild anterior uveitis. Due to good response to medical treatment, the owner declined lens removal and pursuit medical treatment. During the following month, the corneal ulcer healed and the anterior uveitis resolved, and on bimonthly rechecks the eye remained quiet. Eight months post presentation, the dog showed acute severe blepharospasm. Examination revealed whitish swollen lesion in the lens and severe uveitis. Cytology from aqueous humor aspirate reveled neutrophils, and a tentative diagnosis of SIS was made. Following aggressive medical treatment for two days, the uveitis has improved but the lens lesion remained unchanged. Since lenticular abscess was suspected, the dog underwent cataract surgery. Lens capsule histopatology revealed severe supportive inflammation and Gram stain was positive for bacteria, confirming the diagnosis of SIS. Following surgery, the eye improved dramatically. Six months post-surgery, the dog was visual, pain free, with no clinical sign of uveitis. The few SIS published cases were all diagnosed based on globe's histopathology following enucleation. To our knowledge, this is the first documented clinical case of SIS with a positive outcome.

Evaluation of Intratestiscular and Insicional Line Infiltration with Ropivacaine for Castration in Dogs under Sedation with Medetomidine-Butorphanol-Midazolam

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Administration of local anesthetics can decrease general anesthesia requirements and reduce post-operative pain. The goal of this study was to evaluate whether intratesticular and incisional ropivacaine infiltration produces sufficient intra- and post-operative analgesia in castration of dogs under deep sedation. Twenty-three healthy male dogs presented for castration were sedated with medetomidine 0.01 mg/kg, butorphanol 0.2 mg/kg and midazolam 0.2 mg/kg IM, and were randomly assigned to receive 0.2-0.4 mL/kg 0.5% ropivacaine (group R) or an equivalent volume of saline (group S) injected intratesticularly and along the incision line. Gross movement during the procedure was treated with diazepam 0.5 mg kg^{-1} and if it recurred this was considered a failure, and general anesthesia was induced. Post-operatively, all dogs received 2.2 mg/kg carprofen SC and atipamezole IM to reverse sedation. Pain was evaluated before sedation and 1, 2, 4, 8 and 24 hours post-operatively using a visual analog scale (VAS; 0-100), the Glasgow composite pain scale (GCPS; 0-24) and a mechanical algometer. Methadone was used for rescue. The anesthetist and pain assessors were blinded to the treatment. There was no significant difference in the frequency of general anesthesia induction, however, the median (range) time to failure in group S was significantly shorter than in group R (6 [3-25], and 56 [36-76] minutes, respectively). VAS at 8 hours was significantly higher in group S (13.87±10.02) than in group R (5.5±4.01). In conclusion, intratesticular and incisional ropivacaine infiltration reduces anesthetic requirements, and produces analgesia after castration in dogs.

Laboratory Findings in Cats with Naturally-Occurring Sepsis

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Sepsis is a severe, often fatal syndrome, resulting from systemic inflammatory response to infection. The aims of this prospective study were to characterize the laboratory findings in cats with naturally-occurring sepsis. The study group included 31 client-owned cats that met set criteria for sepsis, and 34 healthy staff-owned control cats. Blood samples were collected within 24 hours from presentation for CBC, serum chemistry, prothrombin time, activated partial thromboplastin time (PT and aPTT, respectively), D-dimer and fibrinogen concentrations and activities of protein C (PCA) and antithrombin (ATA). Septic cats were significantly more anemic, had a higher nucleated red blood cell count, lower serum albumin, total protein and total calcium concentrations, and higher serum bilirubin and triglycerides concentration, as well as aspartate transaminase and creatinekinase activities ($P < 0.008$ for all) compared to the controls. The septic cats had significantly higher aPTT, and D-dimer, and lower PCA and ATA ($P \leq 0.001$ for all), and had a lower platelet count compared to the controls ($P = 0.037$). Neutrophil cytoplasmic toxicity was common in septic cats. The 10-day survival rate of the septic cats was 65%. Serum urea concentration was significantly higher ($P = 0.04$), and ATA and PCA tended to be lower ($P = 0.06$) in non-survivors compared to survivors. Naturally-occurring sepsis in cats is characterized by anemia, neutrophil toxicity, hypoalbuminemia, hypertriglyceridemia, increased muscle enzymes activity, hyperbilirubinemia and hemostatic derangement, manifested by prolonged aPTT, low PCA and ATA, increased plasma D-dimer and thrombocytopenia.

Minimally Invasive Unilateral Arytenoid Lateralization in Dogs: A Cadaveric Study

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The aims of this anatomic study were to develop a percutaneous thyroarytenoid lateralization (PTAL) technique, and to evaluate its application in dog cadavers. Twenty-two unilateral thyroarytenoid lateralization procedures were performed on 11 dog cadavers weighing 15-40 kg. Dogs were placed in ventral recumbency with the neck extended and the mouth open. A rigid endoscope was used to visualize the rima glottides. The larynx was palpated, and two hypodermic needles were passed through the skin into the lumen of the larynx, penetrating both the thyroid and arytenoid cartilages. Nylon suture material was passed through the needles to lateralize the arytenoid cartilage. A key-hole approach to the larynx was performed, and the suture material was knotted adjacent to the thyroid cartilage. The procedure was performed bilaterally; however, each side was assessed as a standalone/unilateral procedure. The change in the rima glottidis area was recorded as were the duration of the procedure and complications encountered. The landmarks for needle insertion were easily palpated in all dogs. The time required to place the suture material and to perform a unilateral PTAL was 4:30-20:19 and 9:30-16:00 min, respectively. A significant increase in the area of the rima glottidis was documented after performing unilateral PTAL. In conclusion, unilateral PTAL is a quick, minimally invasive procedure, which increases the area of the rima glottidis in cadaveric dogs. Further evaluation is needed to assess whether this technique can serve as an alternative for conventional surgical options in clinical cases.

Polycystic Kidney Disease in Four British Shorthair Cats and Successful Treatment of Bacterial Cyst Infection

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Feline polycystic kidney disease (PKD) is the most common inherited disorder in cats. Renal cysts progressively increase in size and number, resulting in gradual decrease in kidney function. An autosomal dominant mutation in exon 29 of the *polycystin-1* gene has been identified, mostly in Persian and Persian-related breeds. The same gene is involved in autosomal dominant PKD in humans. This case-series describes PKD, for the first time, in four British Shorthair cats, in Israel, of which in 2/4 cats, the same mutation reported in Persian and Persian-related cats was identified by polymerase-chain reaction testing. This mutation was introduced to the British Shorthair breeding line in past outcrossing with Persian cats. We also describe the diagnosis and successful treatment of an *Escherichia coli*-infected renal cyst in one of these cats, a common complication occurring in human PKD, unreported previously in cats with PKD. Treatment in this case included ultrasound cyst drainage, intra-cystic enrofloxacin injection and systemic antibiotic therapy.

Intraoral Approach for Sialoliths Removal in 15 Horses

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Sialolithiasis is uncommon in horses, and mainly affects horses in arid environment, such as Israel. The definitive treatment of sialolithiasis is surgical removal. Although direct and easily accessed, the transcutaneous approach is frequently associated with significant complications, mainly fistula formation. The objective of the current study was to describe intraoral approach for sialolith removal in horses and the results of the stones chemical analysis. The study design was a prospective case series, which included 15 client-owned horses diagnosed with sialolithiasis. The diagnosis of sialolithiasis was based on clinical presentation and palpation. Sialoliths were removed using the intraoral approach under general anesthesia (n=4) or in standing position with sedation and local analgesia (n=11). A mouth gag was placed, and an incision in the oral mucosa along the entire length of the sialolith was performed, followed by manual extraction of the calculi. Complications, success rate and chemical analysis of the sialoliths were recorded. Results of the study showed that sialoliths were removed successfully, and all horses resumed their previous level of work shortly after the surgery. Three horses had mild complications, including seroma (n=2) and post-operative weakness (n=1), all if which resolved with no intervention. Sialoliths were composed of 80-95% calcium-carbonate and 5-20% apatite. The vast majority contained a nidus of plant material in their center. Thus, sialoliths in the current horse population had a uniform mineral composition, consisting mainly of calcium carbonate. This study confirms that removal of sialoliths via the intraoral approach results in a high a success rate, with minimal complications.

Case Presentation: Metastatic Melanoma Causing Hind-Limb Neurogenic Paresis in an Arabian Mare

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Melanomas occur in over 80% of geriatric grey horses. These dermal tumors are usually benign, however, may metastasize. Therefore, any melanotic tumor should be considered potentially metastatic. Tentative diagnosis of melanoma often arrived at by assessing the signalment and the clinical presentation. A 12-year old Arabian mare presented to the Hebrew University Teaching Hospital with a 2-month history of progressive weight loss, poor condition, and a gradually worsening left hind-limb lameness. Historically, the mare had a mass under the tail and a mass on the neck for unknown durations, both assumed to be discrete dermal melanomas. On presentation to the hospital, the mare was weak and reluctant to move, with very poor body condition and severe atrophy of the left hind-limb and pelvis musculature. The mare suffered from severe ataxia, and a non-weight bearing lameness on the left hind-limb. There was no response to cutaneous stimulation. On rectal examination, a very large mass was palpated on the left dorsum within the pelvic area. Due to the poor prognosis, the mare was euthanized and sent for necropsy. Post mortem examination revealed a large mass (~40x30cm) in the pelvic area, with extensive metastasis to most internal organs, as well as invasion into the pelvic bones and the spinal column. These findings support the diagnosis of neurogenic paresis of the left hind-limb, caused by metastatic melanoma. Benign neglect of melanotic tumors is often recommended and practiced, but a more proactive clinical approach may be warranted to delay or prevent metastasis.

Risk Factors Associated with Mortality, and the Use of Antivenom to Treat Horses Following *Vipera palaestinae* Envenomation

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Snakebites are a common problem in human and veterinary medicine. The most common venomous snake in Israel and the Middle East is *Vipera palaestinae,* which causes most of the envenomations in humans and animals in Israel. *V. palaestinae* venom consists of several components, which adversely affect coagulation, the heart and nervous system. The clinical presentation and the hematological effects of the envenomation were investigated in humans and dog, while little is known of its effect in horses. In human medicine, treatment often includes administration of antivenom–hyperimmune serum produced from equine blood. Its use has decreased the mortality rate in humans from 10% to 1%. Horses are considered susceptible to the venom, and envenomations led to complications such as cardiotoxicity and coagulation disorders. Nevertheless, the extent of these phenomena is unclear. The effects of antivenom and its therapeutic dose in envenomed horses have not been determined. The aim of this study was to examine the risk factors associated with mortality in horses envenomed by *V. palaestinae*, and to access if antivenom administration decreases mortality. Data of 45 envenomations in horses, occurring between April and November 2013 were collected from veterinarians using questionnaires. Six horses died following the envenomation (13.3%), three (7%) presented with signs of shock, and eight (18%) had local bleeding. The risk factors that were significantly associated with mortality included clinical signs of shock (P=0.002) and the response to treatment (P=0.005). Twenty nine horses were treated

with antivenom (64%), of which only one died (3.5%), whereas five horses of 16 untreated horses (31%) died. Administration of antivenom was significantly associated with decreased mortality (*P*=0.017). In conclusion, *V. palaestinae* envenomation occurs commonly in horses in Israel during the summer, and is life-threatening. Signs of shock and response to treatment were associated with the prognosis. Administration of antivenom decreased the mortality, and should be recommended in *V. palaestinae*-envenomed horses. Further research is needed to evaluate the appropriate its timing and dosage.

Evaluation of the Pharmacokinetic Properties of a Combination of Marbofloxacin and Imipenem Administered by Regional Limb Perfusion to Standing Horses

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Combining antimicrobials of different classes may aid in broadening the antimicrobial spectrum, as well as decreasing the emergence of resistant bacteria. Our goal was to evaluate the pharmacokinetics, feasibility and safety of using a combination of marbofloxacin/imipenem in regional limb perfusion (RLP) in standing horses. Six healthy adult horses participated in the study. After being sedated, a tourniquet was applied on randomly selected front leg, approximately 10 cm proximal to the site of injection. A perfusate of 1 g marbofloxacin and 500 mg imipenem, diluted to 100 mL was injected, using 22g butterfly catheter. Synovial samples were collected from the metacarpophalengeal (MCP) joint, and blood samples were collected from the jugular vein at times: 0, 0.5, 2, 6, 12, 24 and 36 hours after injection. All samples were analyzed for marbofloxacin and imipenem concentration using liquid chromatography tandem mass spectrometry. Maximum concentration (Cmax) of imipenem and marbofloxacin in the MCP joint was 71 and 73 μg/mL, respectively. The area under the concentration curve (AUC) of imipenem and marbofloxacin in the MCP joint was 148 and 186 μg-hr/mL, respectively. The results indicate that using RLP with the combination of marbofloxacin and imipenem is safe, offers a broad spectrum of antimicrobial coverage, and has no negative pharmacokinetic effect on each single antibiotic effect.

Immobilization of Captive Nubian Ibexes (*Capra Nubiana*) with Butorphanol-Midazolam-Medetomidine or Butorphanol-Azaperone-Medetomidine and Atipamezole Reversal

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Seventeen captive Nubian ibex (*Capra nubiana*) were immobilized for transportation and/or hoof trimming, deworming, and vaccinations. Of these, 11 were immobilized with a mean ± SD combination of butorphanol (0.13 ± 0.03 mg/kg), midazolam (0.13 ± 0.03 mg/kg), and medetomidine (0.13 ± 0.03 mg/kg) (BMM), and 6 were immobilized with a combination of butorphanol (0.11 ± 0.03 mg/kg), azaperone (0.22 ± 0.06 mg/kg), and medetomidine (0.11 ± 0.03 mg/kg) (BAM) by IM injection. Induction and recovery times were recorded. Heart rate, respiratory rate, rectal temperature, blood pressure, and oxygen saturation were measured. The quality of induction, immobilization, and recovery were scored (scale 1-5; 1 = poor, 5 = excellent). Mean induction time was

significantly shorter in the BMM group compared to the BAM group (8.8 ± 2.7 vs. 20.1 ± 7.8 min, respectively). Median induction score and median immobilization score were significantly higher (i.e., better) in the BMM group versus the BAM group (5 vs. 2.5, and 4 vs. 3, respectively). The mean and diastolic blood pressures were significantly higher in the BMM group at the 25 minutes time point. Atipamezole was administered at the end of procedure, and all ibexes recovered smoothly. Mean recovery time was significantly longer in the BMM group versus the BAM group (9.5 ± 4.3 and 3.3 ± 2.2, respectively). In conclusion, at the doses used, the combination of BMM was superior to BAM for short-term immobilization of captive Nubian ibexes.

Bilateral Corneal Squamous Cell Carcinoma in a 9-Year Old Haflinger Mare

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A 9-year old Haflinger mare was referred to the Hebrew University Veterinary Teaching Hospital due to bilateral recurrent keratoconjuctivis. Over the past year, the mare had left eye corneal edema and opacity, and was treated with topical antibiotics and non-steroid anti-inflammatory drugs. Two weeks prior to referral, a right eye lesion appeared. Ophthalmic examination at the hospital revealed bilateral blepharospasm and corneal opacity, composed of infiltration, edema, vascularization and white-yellowish granules in the limbus. Corneal cytology was unremarkable. Differential diagnoses included eosinophilic keratitis, squamous cell carcinoma (SCC), equine herpes virus infection and corneal lipid dystrophy. Initial treatment included ophthalmic cyclosporine and dexamethasone.Bacterial culture was positive for *Mycoplasma bovis*, and therefore, dexamethasone was discontinued, and ophthalmic tetracycline was added. With no improvement, the mare was rechecked two months later. Corneal cytology revealed a few hyperbasophilic keratinocytes with moderate anisocytosis. Cyclosporine and dexamethasone were prescribed. Two months later, due to deterioration, bilateral keratectomy and cryotherapy were performed. Corneal cytology revealed keratinocytes in different maturation stages. Some of the highly cornified cells demonstrated lacy nuclei. Histopathological examination of corneal biopsies showed bilateral SCC. SCC is the most common equine corneal neoplasm. However, the bilateral clinical presentation was atypical. To the best of our knowledge, this is the first report of bilateral corneal SCC in the horse. Corneal tumors may be misdiagnosed, and a deep corneal biopsy is necessary for a definitive diagnosis. Interestingly, a recent study in a Haflinger breeding farm suggested a possible inherited basis for SCC in the breed.

Bovine Ephemeral Fever: Past, Present and Future

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Bovine ephemeral fever (BEF) is a unique disease affecting cattle and buffalo, caused by a virus of the family *Rhabdoviridea,* probably transmitted by Mosquitos (*Culicidae*) or by biting midges (*Ceratopogonidae*). Therefore, BEF is most prevalent in tropical and subtropical Australia, Africa and Asia. BEF outbreaks occur mainly in the warm seasons or during seasonal transitions. BEF is characterized by a short acute phase, manifested by 2-3 fever cycles, lameness, stiff gait, and commonly by sternal or lateral recumbency. Herd morbidity rates may be very high (up to 80%), but the mortality rate is low (up to 1-2%). In Israel, the disease was described already as early as 1931. Since then, several outbreaks were documented, of which

the last ones occurred in 2004, 2008-2010, and 2014. In addition, BEF occurs sporadically in different parts of Israel, but its extent is unclear. In this lecture, the epidemiological characteristics of BEF and its outbreaks were reviewed, including the risk factors, its damage, changes in morbidity characteristics, and methods of control and prevention of BEF in Israel. New research and supplementary information were presented to deepen the understanding of BEF.

Chlamydia Psittaci: A Primary Pathogen Not Just of Parrots

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Chlamydia psittaci is an intracellular obligatory bacteria that may cause endemic avian chlamydiosis, epizootic outbreaks in mammals, and psittacosis in humans. Chlamydiosis in birds is considered an ubiquitous subclinical pathogen, however, latent infections may transform the infection to an overt disease with shedding of bacteria and infection of other organisms. In commercial poultry establishments, chlamydiosis appears to occur mostly in turkeys and ducks, but commercial flocks having clinical chlamydiosis as a primary or unique pathogen are considered uncommon. There is a question concerning the significance of *C. psittaci* as a primary pathogen of the domesticated avian host, or, the importance of these species only as reservoir of *C. psittaci* infections in mammals. Samples of domesticated birds, pet birds, zoo and wild birds (cloacal, tracheal, eye swabs and blood) or carcasses of dead birds, were submitted routinely or as part of surveillance studies to the Laboratory of Avian Diseases in Kimron Veterinary Institute, Bet Dagan, Israel, to be tested for *C. psittaci* (n=395 submissions annually; average over the last 10 years). The laboratory methods in determination of *C. psittaci* included direct immunofluorescence with anti-LPS-fluorescent antibodies, regular and real-time PCR, and serological determination of *Chlamydial* antibodies by ELISA. Genotypic classification of *C. psittaci* was made by using the gene encoding the major outer membrane protein of the bacterial envelope (ompA), and further nucleotide sequencing. In some cases the bacterium could be diagnosed only following isolation in embryonated chicken eggs, by yolk-sac inoculation of 5-7 days-old specific pathogen-free eggs. The prevalence of *C. psittaci* positive cases over the last 10 years was 22.0 ± 3.7% (mean ± standard error of the mean). Genotype E was the predominant (69% of positive cases), then genotypes B (21%) and A (10%). Typical respiratory and gastrointestinal signs of *C. psittaci* such as sinusitis or conjunctivitis, and diarrhea, were noted in all groups of birds. Clinical signs that are considered atypical for this pathogen, such as neurological signs, were interestingly found in most groups (excluding psittacines). However, locomotor signs, such as difficulties in walking and limb paresis associated with leg joint inflammation, were noted only in chickens and turkeys. Additionally, the reperoductive system signs (embryonic mortality, non-hatching) were seen only in commercial poultry, including chickens and turkeys. A syndrome of ischemia and necrosis of the non-feathered head and neck skin, was seen only in turkeys. In some of these, we could reproduce the signs in artificial inoculation studies. To summarize, *C. psittaci* is a multi-systemic pathogen that may injure many organ systems in the bird, including domesticated birds, chickens and turkeys, is involved in pathologies unknown so far. Differences between bird groups exist in clinico-pathological manifestations of *C. psittaci*, and most important: the interaction between *C. psittaci*. Most importantly, the avian host is not only as an avian reservoir but it can be defined also as host-parasite interaction with clinico-pathological manifestations.

A Case Report of *Bacillus cereus* Poisoning from Minced Beef

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On March 2013, Jerusalem District Health Office team investigated a notification of gastrointestinal symptoms among five family members after eating in a local restaurant. Symptoms, which included vomiting and, in one case, fainting, occurred within several hours of consuming food at the restaurant, and were consistent with toxin poisoning. Environmental and epidemiological investigations were initiated, aiming to determine the cause of this food poisoning. This report summarizes the results of the investigation. After environmental inspection and food sampling in the restaurant, the suspected dish causing the food poisoning included minced meat. The cooked dish was not kept under temperature control, and reached the danger zone for over 2 hours (5-60 °C). The suspected pathogen was identified as *Bacillus cereus*. *B. cereus* is a well-known food poisoning organism. It can causes two types of food poisonings, known as the emetic and the diarrheal types. The emetic type is caused by a heat-stable toxin, named cereulide, produced in the food. The symptoms are usually mild, but can be severe, up to fatal sometimes. In this case, all five ill family members recovered. Control measures were recommended in order to prevent such future events.

Sustained Release Injection of Doxycycline in Chicks Based on Thermo-Sensible Polymer

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Avian chlamydiosis, is caused by the bacterium *Chlamydia psittaci*, affecting birds in general, and particularly psittaciformes that are most susceptible. The disease is a zoonotic, posing a public threat, and leads to major economic loss. The drug of choice to treat the disease is doxycycline, administered intramuscularly every 5 days or orally every 24 hours, for 45 days. This treatment induces prolonged stress for the bird, and frustrates the caretakers, therefore resulting in low level of owners' compliance. In this study, we evaluated in chicks a sustained-release formulation of doxycycline dissolved in-situ gelation system, based on Poloxamer, a thermo-sensible polymer. The aim was to maintain a serum minimal inhibitory concentration (MIC) of 1μg/mL against *Chlamydia,* for at least 21 days, following a single dose. Thirty-four light-breed chicks were injected subcutaneously once, and serum doxycycline levels were measured for 42 days at predetermined time-points. The average doxycycline concentration was maintained > MIC for 21 days. An erosive reaction developed at the injection site, however, it resolved with no medical intervention. Future studies are needed in order to improve the formulation, while keeping the duration > MIC for at least 21 days, with minimizing the local reaction at the injection site.