A case of Yersinia pseudotuberculosis infection in canaries

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Ενδιαφέρουση Περίπτωση

Περιστατικό ψευδοφυματίωσης (υερσινίωσης) σε καναρίνια
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ΠΕΡΙΛΗΨΗ. Περιγράφεται η διαπίστωση περιστατικού ψευδοφυματίωσης σε καναρίνια και ο τρόπος αντιμετώπισης της. Η νόσος εμφανίστηκε σε κατάστημα πώλησης "πτηνών αναψυχής", όπου μαζί με τα καναρίνια υπήρχαν και άλλα είδη πτηνών, τα οποία επίσης είχαν προσβληθεί από την νόσο. Αρχικά ασθένησαν τα καναρίνια και στη συνέχεια τα υπόλοιπα πτηνά. Οι κυριότερες μακροσκοπικές αλλοιώσεις που διαπιστώθηκαν χαρακτηρίζονταν από την ύπαρξη πολυάριθμων οζιδίων μεγέθους κεφαλής καρφίτσας μέχρι κεχριού στο παρέγχυμα του ήπατος και της σπλήνας, καθώς και στο τοίχωμα του εντέρου κατά εστίες. Επίσης, παρατηρήθηκε διόγκωση του ήπατος και της σπλήνας και υπεραιμία με εξέλκωση του βλεννογόνου του εντέρου. Στην ιστολογική εξέταση διαπιστώθηκαν στο παρέγχυμα του ήπατος και της σπλήνας και στο τοίχωμα του εντέρου εκτεταμένες εστίες πηκτικής νεκρώσης μέσα στις οποίες διακρίνονταν πολυάριθμες βοτρυοειδείς αποικίες Gram-αρνητικών κοκκοβακτηριδίων. Στη μικροβιολογική εξέταση των σπλάχνων απομονώθηκε και ταυτοποιήθηκε το βακτηρίδιο Yersinia pseudotuberculosis.

Η διαπίστωση της νόσου γίνεται για πρώτη φορά στην Ελλάδα. Η θεραπευτική αγωγή με ενροφλοξασίνη στη δόση των 150 mg/L πόσιμου ύδατος αποδείχθηκε περισσότερο αποτελεσματική στα αρχικά στάδια της νόσου και κυρίως στα άλλα είδη πτηνών.

Λέξεις ευρετηρίασης: Ψευδοφυματίωση, καναρίνια

ABSTRACT. This study describes the diagnosis, treatment and sanitary procedures in canaries and other cage-birds in a pet-shop, infected by Yersinia pseudotuberculosis. Main macroscopic lesions in the liver, spleen and intestine were characterized by numerous nodules, from pin-head size up to a little larger. Also, swelling of the liver and spleen and congestion with ulceration of the mucus of the intestine were observed. Parenchyma of the liver and spleen, as well as in the intestinal wall, numerous small or larger cluster-shaped colonies of Gram-negative cocccobacteria were found with coagulative necrosis of the normal tissue in the development areas of the intestine were observed. The therapeutic schema with enrofloxacin in dose of 150 mg/L in drinking water showed itself to be more effective in the first stages of the disease in other cage-bird species, but less so in canaries.

Key words: Pseudotuberculosis, canaries

INTRODUCTION
Pseudotuberculosis is an infectious disease with a hyperacute, acute or chronic course, which is characterized by nodules in various organs. The cause of the disease is Yersinia pseudotuberculosis. This is a Gram-negative cocccobacterium, which grows at low temperatures (Dorrestein 1997). There are ten serotypes, from which the stereotypes I, II and III are found in birds (Bisping et al. 1988). Canaries, pigeons and parrots are considered to be

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Εργασία αυτή περιγράφεται περιστατικό ψευτοτούμφης σε καναρίνια.

Τα καναρίνια, μια εβδομάδα μετά την άφιξη τους, άρχισαν να εμφανίζουν ανορεξία, υπνηλια, ανοιχτόμενο

sensitive to the disease. In canaries the disease is revealed in its hyperacute and acute form, whereas in pigeons and parrots, it usually presents a chronic course (Cock et al. 1999). Mammals can also be affected by the disease (Gyles 1993). Very rarely humans are affected, causing gastroenteritis or even septicemia (Makino et al. 1994). The disease is endemic to Europe, where an unknown number of wild birds are considered to be carriers, without any symptoms. The microorganisms that have been isolated belong mainly to the serotypes I and II (Gerlach 1994). Epidemics have been reported in Denmark, Germany, UK and USA (Garpenter et al. 1997).

Transmission of the microorganism occurs by means of wild birds and rodents through their faeces, which contain the food and water of the breeds (Gylstorff et al. 1987, Harris 1991). Factors that can attribute to the dissemination of the disease include low temperatures, poor sanitary conditions and inappropriate food (Parsons 1991). Sudden deaths, without any symptoms, occur in the hyperacute form of the disease, and depression, diarrhoea, dehydration and dyspepsia in its acute form, whereas in the chronic form weakness, limping, lack of coordination in movement and paralysis are observed (Gerlach 1994). Mortality rates can reach up to 100% (Garpenter et al. 1997). In cases of the hyperacute form during autopsy the liver and the spleen are found to be swollen and bloody content in the abdominal cavity is often found. In the acute form, amber-like nodules in the liver, spleen and kidneys can be observed, whereas in the chronic form the nodules are sizeable and can be found in the intestinal serous cavities and muscles.

Diagnosis is based on pathological and microbiological examinations. The use of broad-spectrum antibiotics can reduce the losses, especially in canaries. Therapy is not effective in cases where the disease is advancing rapidly or where nodules have formed in the internal organs.

In this paper a case of pseudotuberculosis in canaries and other cage-birds, in a pet-shop trading in cage-birds, and its treatment, are described.

DESCRIPTION OF THE CASE

Case History

Ten dead canaries (Serinus canaria) and 2 zebra finches or "paradise birds" (Taenopygia guttata), originating from a pet-shop trading in cage-birds, were brought to the Clinic of Avian Medicine. The canaries belonged to a group of about 60, 1-year-old, birds and had been introduced to the pet shop 15 days previously. The zebra finches belonged to a group of 20 birds, which had resided in the shop for some time, together with about 40 budgerigars, 10 cockatiels and 12 lovebirds.

One week following their introduction to the shop, the canaries had anorexia, sleepiness, ruffled feathers and a death rate of 2-7 per day. Within a period of 5 days after the appearance of the symptoms, the disease spread to

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αποτελέσματα

τα ζωντανά πτηνά απομακρύνθηκαν από το κατάστημα, ναϊκή κοιλότητα υγρό ερυθρώπης χρωιάς. Οι νεφροί ήταν υδαρή κόπρας. Εξαίρεσην τρομακτικά μεγέθους κεφαλής καρφίτσας έως κεχριού, ήπαρ και η σπλήνα ήταν διογκωμένα και υπήρχαν αφθονιά ζωντανών δεν είχε καμία σχέση με τα προηγούμενα. Ένα γήσε ενροφλοξασινή στη δόση των 50 mg/L πόσιμου νερού για 5 ημέρες από την εμφάνιση των συμπτωμάτων, λειτουργεί χωρίς κανένα πρόβλημα.

λα είδη πτηνών του καταστήματος και ο ιδιοκτήτης χορηγήθηκε ενροφλοξασινή στη δόση των 50 mg/L πόσιμου νερού για 10 ημερές. Η εξέλιξη της νόσου παπαγαλάκια 6/40, στις ζέβρες 4/20, στα cockatiels 2/10 συγκεκριμένα, οι θάνατοι στα καναρίνια ήταν 50/60, στα budgerigars, 40 ψιττάκια. Η προέλευση των καναρινιών στο κατάστημα. Η επιχείρηση, μετά την εφαρμογή της τροποποιημένης Ziel-Neelsen (Quinn et al. 1994, 22

Στην εξέταση επιχρισμάτων από τα οζιδια και τα σπλήνα, που χρωματίστηκαν με Ziel-Neelsen, καθώς και με την τροποποιημένη Ziel-Neelsen (Quinn et al. 1994, the other bird species in the pet shop and the owner administered enrofloxacin at a dose of 50 mg/L in the drinking water for 5 days, without any positive results. Subsequently, the dead birds were brought to the Clinic. On suspicion of pseudotuberculosis, it was recommended that all pet birds should be administered enrofloxacin at a dose of 150 mg/L (threefold of the initial dose) for another 5 days. The treatment had positive results in all bird species, except canaries. In particular, the death rate in canaries was 50 out of 60, 6 out of 40 in budgerigars, 4 out of 20 in zebra finches, 2 out of 10 in cockatiels and 2 out of 12 in lovebirds. At the end of the therapy, all living birds were removed from the shop, decontamination of the premises and equipment was carried out and since the shop needed to continue functioning, new birds were introduced: 40 canaries, 40 budgerigars, 20 parrots of other species and 30 zebra finches. The origin of the canaries was totally unrelated to those of the previous birds. One month after the introduction of the new birds, the problem reappeared in a similar form to the previous case, but only in canaries. All birds were immediately removed from the shop and treatment with enrofloxacin at a dose of 150 mg/L in drinking water for 10 days was applied. In canaries the course of the disease was rapid, with significant losses, reaching 60%. No loss was observed in the other bird species, which remained healthy. Strict disinfection, control of rodents and delay to the introduction of canaries into the shop was recommended to the owner. The business has now been operating without problems for one year since the appliance of this method of treating the disease.

RESULTS

During an autopsy of the 12 birds (10 canaries and 2 zebra finches), the following findings were recorded: livers and spleens were swollen and many nodules, from pinhead up to a little larger size, were observed, elevated and...
υπόθεση ή μεγαλύτερες βοτρυοειδεις Gram-αρνητικών Arabinose ή παρέγχυμα του ήπατος και της σπλήνας, καθώς και στο λογικώς η καρδιά, το ήπαρ, η σπλήνα και το έντερο. Στην Χλαμύδια, δε βρέθηκαν οξεάντοχοι βάκιλοι ούτε στοιχειώσεις υπήρχε γύρω από τις νεκρωμένες περιοχές λεπτή και ετερόφιλων κυττάρων παρατηρήθηκαν μέσα και γύρω (Εικόνες 2,3). Μεγάλος αριθμός μακροφάγων κοκκοβακτηριδιων με πηκτική νεκρωσή του φυσιολογικού από το οίδημα των καρδιακών κυττάρων και τη μεκαρδιά δεν παρατηρήθηκαν σημαντικές αλλοιώσεις, εκτός η επώαση έγινε στους 37°C για 24 ώρες (Barrow et al. 1994, Quinn et al. 2000) για τυχόν προσβολή των πτηνών από φυματίωση ή παστεριδιαση και ακολούθησε βεβαίωση της νόσου.

Αποτελέσματα

<table>
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<tr>
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<tr>
<td>Oxidase</td>
<td>Negative</td>
</tr>
<tr>
<td>Glucose</td>
<td>Positive (without gas production)</td>
</tr>
<tr>
<td>Lactose</td>
<td>Negative</td>
</tr>
<tr>
<td>Mannitole</td>
<td>Positive</td>
</tr>
<tr>
<td>Maltose</td>
<td>Positive</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Negative</td>
</tr>
<tr>
<td>Xylose</td>
<td>Positive</td>
</tr>
<tr>
<td>Arabinose</td>
<td>Positive</td>
</tr>
<tr>
<td>Christensen (Urea)</td>
<td>Positive</td>
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<tr>
<td>Kliger (H2S)</td>
<td>Positive</td>
</tr>
<tr>
<td>Nitrato</td>
<td>Positive</td>
</tr>
<tr>
<td>Mannitale-motility</td>
<td>Negative</td>
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</tbody>
</table>

Incubation took place at 37°C for 24 hours (Barrow et al. 1994, Quinn et al. 2000)

Histopathological examinations: The heart, liver, spleen and intestines were examined histopathologically. No significant lesions were observed in the heart, except of edema of cardiac muscle cells and partial loss of transverse striation. In reverse, in the parenchyma of the liver and of the spleen, as well as in the intestinal wall, numerous small clearly circumscribed (Fig.1). The intestines were congested and their content was aqueous and had a reddish colour. In six canaries, numerous nodules were observed in their intestines with blood in the abdominal cavity. The kidneys were slightly swollen and discoloured and the nutritional condition was medium to poor.

No parasites (protozoa or worms) were found during parasitological examinations of the gastrointestinal tract.

In the examination of smears originating from the nodules and internal organs (stained with Ziel-Neelsen and modified Ziel-Neelsen) (Quinn et al. 1994, 2000), neither acid-fast organisms nor elementery bodies (LCL) were found, which are characteristic for tuberculosis and chlamydia infection, respectively.

After the examination of the lesions and the results of the above examinations, bacteriological and histological examinations were carried out, in order to confirm the suspicion of a case of pseudotuberculosis.

Bacteriological examinations: The liver, spleen and heart were used for isolating the microorganism. The cultures were made on 7% sheep blood and MacConkey agar. After incubation for 24 hours at 37°C, the presence of colonies from the liver and spleen was observed in both substrates.

Colonies in blood agar were of a small size, lubricous, grey in colour and non-haemolytic. After Gram-staining, the organisms had the form of Gram-negative cocco-bacteria. Biochemical tests were conducted and the following results were recorded:
Η περισσότερο ευρεία περιχαρακτική ζώνη από πολυάριθμα ετερόφιλα κύτταρα και μακροφάγα. Σφαιροειδείς αποικίες παρόμοιων Gram-αρνητικών κοκκοβακτηριδιών διαπιστώθηκαν και μέσα στα αιμοφόρα αγγεία του ήπατος και της σπλήνας, μεταξύ των ερυθρών αιμοσφαιρίων. Επιπλέον, στο έντερο παρατηρήθηκαν και εξελκώσεις του βλεννογόνου στις περιοχές όπου οι νεκρώσεις βρίσκονταν κοντά στον αυλό.

**ΣΥΖΗΤΗΣΗ ΚΑΙ ΣΥΜΠΕΡΑΣΜΑΤΑ**

Το υψηλό ποσοστό θνησιμότητας των καναρινιών, σε συνδυασμό με τις χαρακτηριστικές αλλοιώσεις των σπλάχνων (Rimler and Glisson, 1997), έθεσαν την υποψία της νόσου. Οι βακτηριολογικές και ιστολογικές εξετάσεις επιβεβαίωσαν την αρχική μας διάγνωση. Η άφθονη ανάπτυξη του βακτηριδίου από το ήπαρ και τη σπλήνα στο αιμάτουχο άγαρ και στο MacConkey agar, στη θερμοκρασία των 37 °C, μας απάλλαξαν από τη χρήση άλλων εκλεκτικών υποστρωμάτων.

Η μόλυνση, που αρχικά παρουσιάστηκε στα καναρίνια, πιθανόν να προήλθε από τα ποντίκια, τα οποία θεωρούνται μαζί με τα άγρια πτηνά οι κυριότερες πηγές μόλυνσης (Gylstorff et al. 1987). Η παρουσία ποντικιών στο κατάστημα δεν αποκλείστηκε από τον ιδιοκτήτη. Η πρόσφατη ταλαιπωρία μεταφοράς των καναρινιών στο κατάστημα πιθανόν να συνέβαλε στην εκδήλωση της νόσου, επειδή οι κακές συνθήκες διαβίωσης και διατροφής προδιαγράφουν στα πτηνά και ιδιαίτερα στα καναρίνια (Parsons, 1991).

Η μη αποτελεσματική θεραπεία των καναρινιών της πρώτης παρτίδας με την ενροφλοξάσινη, η οποία θεωρείται από τις πλέον δραστικές ουσίες έναντι της Yersinia pseudotuberculosis, πρέπει να αποδοθεί στην κατάλληλη θεραπεία, καθώς και στη χορήγηση μικρής δόσης (50 mg/L), ενώ η συνιστούμενη δόση είναι 150 or larger cluster-shaped colonies of Gram-negative coccobacteria were found with coagulative necrosis of the normal tissue in the developmental areas of the bacterium, as well as around them (Fig. 2,3). A large number of macrophages and heterophil cells were observed within and around the necrotic areas. In most of the necroses, a narrow or broader demarcating zone, consisting of numerous heterophil cells and macrophages, was present. Spheroid colonies of similar Gram-negative coccobacteria were also discovered in the blood vessels of the liver and spleen, between red blood cells. Furthermore, ulcerations of the mucosa were observed in the intestine, in areas where necrosis was near to the lumen.

**DISCUSSION AND CONCLUSIONS**

The high mortality of the birds, combined with the characteristic visceral lesions (Rimler and Glisson 1997), made us suspect pseudotuberculosis and bacteriological and histological examinations confirmed this initial diagnosis. The presence of heavy growth of the bacterium from the liver and spleen in blood and McConkey agar, at a temperature of 37 °C, saved the use of other selective substrates.

The initial infection appearing in the canaries must have originated from mice, which, together with wild birds, are considered a main source of contamination (Gylstorff et al. 1987). The presence of mice in the shop was not ruled out by the owner. Furthermore, manifestation of the disease may have been exacerbated by the recent stress faced by the canaries during their transportation to the shop, because unfavourable living and feeding conditions predispose birds, and especially canaries, to this disease (Parsons, 1991).

The ineffective initial therapy in canaries with
ΠΕΡΙΟΔΙΚΟ ΤΗΣ ΕΛΛΗΝΙΚΗΣ ΚΤΗΝΙΑΤΡΙΚΗΣ ΕΤΑΙΡΕΙΑΣ 2005, 56(1) 25

enrofloxacin, which is considered one of the most effective against Yersinia pseudotuberculosis, has to be attributed to the administration of a small dose (50 mg/L), whereas the recommended dose is 150 mg/L (Haesebrouck et al. 1995), as well as to the delay of the initiation of the therapy. The time of the antibiotic administration plays an important role, since the correct administration of the antibiotic during the first stages of the disease can discourage the onset of septicaemia, when its concentration in the blood is appropriate (Haesebrouck et al. 1995). On the other hand, a delay in the administration is ineffective, because the already formed nodules block the antibiotic from reaching the microorganism that is located in the centre of the necrotic tissue (Gerlach 1994).

The contamination of the second lot of canaries might have been promoted by the poor conditions during transportation and have been caused by mice, which the owner could not eradicate and even by bacteria that remained in the cages after their disinfection. The ineffectiveness of the therapy in these canaries has to be attributed to the onset of the hyperacute form. The survival rate of the other birds in both lots has to be attributed to their lower sensitivity against the disease, resulting in the manifestation of its chronic clinical course (Cork et al. 1999) and the enrofloxacin’s effectiveness. Also, the effectiveness of the therapy in the second lot was due to the early administration of the proper dose.

From the above, we conclude that pseudotuberculosis, as it is known in Europe (Gyles 1993), also exists in our country and has to be seriously considered in cases of sudden deaths in pet-shops with cage-birds and in canary breeds. The disease must be differentiated from tuberculosis, chlamidiosis, salmonellosis and pasteurellosis.

Special care must be given to treatment. Therapy is not always possible, because in the hyperacute and often in the acute form of the disease, deaths occur suddenly. Furthermore, in the chronic forms antibiotics are unable to penetrate necrotic tissues. Enrofloxacin is effective, if it is administered early after the onset of the disease.

In order to prevent the disease, the following measures should be taken:

1. Stress of the birds, like transport, overcrowding, etc. should be avoided. Strict health regulations should be applied to the cages and the water and feeding equipment.
2. Pet-shops with cage-birds should be free of mice and wild birds, which are the main sources for bacteria that contaminate their food and water.
3. Birds newly-introduced to breeds and pet-shops should be isolated and should be observed for a period of one month at least.