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# Isolation and identification of *Campylobacter* species in apparently healthy Ostriches

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## Abstract

In the present work, a total of 150 sampleswas collected from apparently healthy ostriches (102fecal samples, 36 water samples and 12food samples) at different geographical sites. Ostrich's samples were obtained from the zoo (46 samples),private farms (77 samples) and 27 samples fromgovernmental farms.All sampleswere cultured ontothioglycolate broth at 37°C under microaerophilic condition (5% $O_2$, 10% $Co_2$ and 85% $N_2$) and examined after 24 hrs under phase contrast microscope for characteristic campylobactermotility. The positive samples for isolation of *Campylobacter* species were identified biochemically. The rate of isolation of *Campylobacterjejuni* and *C. coli*was 24.67 %( 37out of 150 samples) as follows: where 21(14%) isolates were recovered fromfeces, 13(8.67%)from water samples and 3(2%) from food samples.

The prevalence of *C. jejuni* was higher among collected in ostrich's samples obtainedwith the rate of 18 %( 27 samples) .On the other hand*C. Coli*with the rate of 6.67% (10 samples).Results showedhighest rate of the isolated *Campylobacter* species from samples obtained fromthezoo18(39.13%), followed by private farms 17 (22.08%) while it was 2(7.41%) at the governmental farms.

In conclusion, the isolation of *Campylobacter* species fromostrichesgetsuseful information upon the diagnosis of bacterial diseases affecting these birds. Also, proper management should be offer for zoo birds to maintain their good health and hygienic programs must be applied.Preventive medical programs should be adaptive to the bird especially in the zoo, periodic fecal examinations and treatments ofdiseased ostrich, good nutritionand potential health care must be taken for these birds.

**Keywords**:*Campylobacter jejuni*,*Campylobacter coli*, ostriches, isolation, PCR.

## Introduction

Ostrich breeding is currently one of the best investments profitable business.Ostrich (*StruthioCamelus*) was abundant and broadly distributed in Egypt at the end of Ninth century(Davies, 2003). Campylobacteriosis is an illness caused by *Campylobacter* spp. Member of genus *Campylobacter*is "slender, curved motile Gram negative bacilli", which differed from the classical cholera and halophytic vibrios(Abid et al., 2013). Campylobacteriosis is a disease of a significant social and economic burden (Sheppard et al., 2009 and Mughiniet al., 2012).

*Campylobacter* species are commonly present in the intestinal tract of both healthy and diseased animals and birds, and are often found in their manure. The organisms are associated with infection of the gastrointestinal tract(Wagenaar, 2013).The most common cause of human infection is *Campylobacter*jejuni. It is the most commonly reported bacterial cause of foodborne infection in the USA and other countries(Patrick et al., 2013). Commonly reported symptoms of campylobacteriosis include diarrhea, abdominal pain, fever(Ternhaget al., 2005 and Mughiniet al., 2012).Genus*Campylobacter*consists of sixteen species and eight subspecies, all of which are natural inhabitants of the intestinal tract of poultry and warm-blooded domestic animals where microaerophilic conditions and the warm body temperature constitute an ideal environment for their continuous growth. The consumption of contaminated food and water by some species causes gastrointestinal illness in human (El-Jakeeet al., 2008).

The thermophiliccampylobactershave been isolated from many species of birds including gulls, puffins, owls, pigeons,blackbird's quails, turkeys, ducks gees and ostriches. Birds might be execrators of *Campylobacter* species, some serotypes associated with infections in human (Bolton, et al., 1992 andStephens et al., 1998). Wild and domestic birds act as major reservoirs of *Campylobacter species* and play a role in epidemiology of the disease(Wedderkopp, et al., 2003).*Campylobacters* generally colonize avian as a commensally organism, with the possible of ostriches. (Allen and Griffths, 2001).

The aim of the present work wasto figure out the role and contribution of *Campylobacter* spp in diarrhea causing agents in ostrich in Egypt and its impact on Ostrich stress response.

## Materials and Methods

***1- Samples collection:***

A total of a total of 150 samples was collected from apparently healthy ostriches (102 fecal samples, 36 water samples and 12 food samples) at different geographical sitesfor *Campylobacter*isolation .Ostrich's samples were obtained from the zoo (46 samples), private farms (77 samples) and 27 samples from governmental farms(**Table, 1**).

***2- Bacteriological* isolation *of Campylobacter*spp:**

All ostrich's samples were cultured ontothioglycolate broth at 37°C under microaerophilic condition (5% $O_2$, 10% $Co_2$ and 85% $N_2$). All samples were examined after 24 hrs under phase contrast microscope for characteristic *campylobacter* motility (Ledergerberet al., 2003).

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### *3- Biochemical identification ofCampylobacter isolates:*

Positive *Campylobacter* isolates were identified biochemicallyaccording toAchaet al., (2004)using hippurate hydrolysis test to distinguish between *Campylobacterjejuni* and *Campylobacter coli*. The development of deep purple colour indicated positive results of *Campylobacter jejuni*

## Result

**Table (1): Samples collected from different farms.**

| Source of isolates | Examined number | Types of examined samples | | |
|---|---|---|---|---|
| | | Feces | Water | Food |
| Private Farms | 77 | 58 | 16 | 3 |
| Governmental Farms | 27 | 18 | 6 | 3 |
| The zoo | 46 | 26 | 14 | 6 |
| Total | 150 | 102 | 36 | 12 |

**Table (2):The incidence rate of *Campylobacter* species isolated from apparently healthy Ostrich.**

| Examined sites | Examined number | No. of positive isolates | The examined samples | | |
|---|---|---|---|---|---|
| | | | Feces | Water | Food |
| Private Farms | 77 | 17 (22.08%) | 11 (14.29%) | 5 (6.49%) | 1 (1.30%) |
| Governmental Farms | 27 | 2 (7.41%) | 2 (7.41%) | 0 | 0 |
| The zoo | 46 | 18 (39.13%) | 8 (17.39%) | 8 (17.39%) | 2 (4.35%) |
| Total | 150 | 37 (24.67%) | 21 (14%) | 13 (8.67%) | 3 (2%) |

Table (3):The incidence rate of positive *Campylobacter* species in ostrich from the examined samples of the examined sites:

| The examined samples | Examined number | No. of positive isolates * | *Campylobacter* species isolates | |
|---|---|---|---|---|
| | | | *Campylobacter jejuni* | *Campylobacter coli* |
| Feces | 102 | 21 (20.59%) | 15 (14.71%) | 6 (5.88%) |
| Water | 36 | 13 (36.11%) | 9 (25%) | 4 (11.11%) |
| Food | 12 | 3 (25%) | 3 (25%) | 0 |
| Total | 150 | 37 (24.67%) | 27 (18%) | 10 (6.67%) |

*% was calculated according to the total number of examined samples

Table (4):The incidence rate of *Campylobacter* species in ostrich in all examined sites:-

| Source of isolates | Examined number | Number of positive isolates * | *Campylobacter* species isolates | |
|---|---|---|---|---|
| | | | *Campylobacterjejuni* | *Campylobactercoli* |
| Private farms | 77 | 17 (22.08%) | 8 (10.39%) | 9 (11.69%) |
| Governmental farms | 27 | 2 (7.41%) | 2 (7.41%) | 0 |
| The zoo | 46 | 18 (39.13%) | 17 (36.96%) | 1 (2.17%) |
| Total | 150 | 37 (24%) | 27 (18%) | 10 (6.67%) |

*% was calculated according to the total number of examined samples

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## Discussion

Campylobacteriosis is the most reported foodborne gastroenteritis disease and poses a serious health burden in industrialized countries, poultry are a natural host for *Campylobacter jejuni* and also for *Campylobacter* species in general, and that colonized broiler chicks are the primary vector for transmitting this pathogen to humans(Hermanset al., 2012).

As showed in Table (2)37different ostrich samples were found to be bacteriologically positive for *Campylobacter*isolationwith incidence of (24.67%). It was clear that the highest isolation of *Campylobacter* species was (14%) in fecal samples followed by water samples (8.67%) then food samples which were (2%).

The highest rate of the isolated *Campylobacter* specie was recorded from the water samples were collected from the zoo 8 (17.39%), followed by private farms 5 (6.49%)whereas it was negative at the governmental farms. Out of 36 examined water samples 13(8.67%)of positive *Campylobacter*is nearly matchedwith the results recorded by Agatha et al., (2013).

Twenty one ostrichfecal samples (14%)were positive forisolation of *Campylobacter*species, with the highest rate was recordedat the Zoo(17.39%)followed by private farms (14.29%)then Governmental farms (7.41%) (Table,2).These results agreed with the finding reported by Masoodet al., (2011) and Ioannaet al., (2012).

From the results presented in Table (3),it was elucidatedthat, the more prevalent *Campylobacter* species recovered from collected samples was *Campylobacter jejuni*(18%)while *Campylobacter coli* wasisolated with anincidence of (6.67%).The highest percent of positive *Campylobacter jejuni* isolates were identified in water samples the same as foodsamples (25%)followed by fecal samples(14.71%).While the highest percent of *C. coli* isolates were identified in water samples (11.11%)followed by fecal samples (5.88%).These results agreed withAlessandraet al., (2007)andSalihuet al., (2009).

It is clear from Table (4) highest rate of isolation of positive *Campylobacter* species were recovered from the zoo(39.13%)followed by private Farms (22.08%)then governmental farms (7.41%).These results were agreedwithAgatha et al.,(2013).

The highest percent of *Campylobacter jejuni* isolates were observed at zoo (36.96%)followed by private farms (10.39%) then governmental farms (7.41%),while the highest *Campylobacter coli* isolates percent were observed in Private farms (11.69%)followed by zoo (2.17%)while it was negative at governmental Farms.

## Conclusion

Finally,we concluded that*C. jejuni*were the most prominent bacterial species causing enteritis in ostrich. Preventive medical programs should be adaptive to the bird especially in the zoo, periodic fecal examinations and treatments for diseased ostrich, good nutritionand potential health care will be taken for these birds.Further research is also needed to better understand the relationship between Campylobacters and Ostrich and theirbacterial resistance.

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# عزل وتصنيف ميكروبات الكامبيلوباكتر من النعام السليم ظاهريا

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فى هذه الدراسة ، تم جمع عدد ١٥٠ عينة من طيور النعام سليمة ظاهريا " ١٠٢ عينة براز، ٣٦ عينة مياه ، ١٢ عينة من الغذاء" من مواقع مختلفة جغرافيا. تم أخذ عدد ٤٦ عينة من نعام حديقة الحيوان ، عدد ٧٧ عينة من المزارع الخاصة وعدد ٢٧ عينة من المزارع الحكومية. تم زرع جميع العينات فى شوربة الثيوجليكوليت الخاصة بزرع الكامبيلوباكتر فى درجة حرارة ٣٧°م فى جو يحتوى على ٥% اوكسجين ، ١٠% ثانى أكسيد الكربون ، ٨٥% نيتروجين.وتم الفحص بعد مرور ٢٤ ساعة من الزرع بأستخدام ميكروسكوب الفيز كونترست لفحص ميكروبات الكامبيلوباكتر عن طريق حركته المميزة . العينات الايجابية يتم تصنيفها بيوكيميائيا.

تم عزل ميكروبات الكامبيلوباكتر جيوجناى والكامبيلوباكتر كولاى ٣٦ عينة أيجابية من أجمالى ١٥٠ عينة بنسبة ٢٤.٦٧% بياتها كالتالى: ٢١ عينة ايجابية بنسبة ١٤% من عينات البراز ، ١٣ عينة ايجابية بنسبة ٨ر٦٧% من عينات المياه و٣ عينات أيجابية بنسبة ٢% من عينات الغذاء. وكانت نسبة عزل ميكروب الكامبيلوباكتر جيوجوناى أعلى فى العينات عدد ٢٧ عينة أيجابية بنسبة ١٨% من ميكروب الكامبيلوباكتر كولاى عدد ١٠ عينات أيجابية بنسبة ٦٧ر٦% . وأثبتت النتائج ان أعلى نسبة عزل كانت من عينات حديقة الحيوان عدد ١٨ عينة أيجابية لميكروبات الكامبيلوباكتر بنسبة ١٣ر٣٩% تتبعها المزارع الخاصة كانت نتيجة العزل ١٧ عينة أيجابية بنسبة ٠٨ر٢٢% بينما كانت نتيجة العزل أيجابية لعدد ٢ عينة بنسبة ٤١ر٧% من المزارع الحكومية.

ونستخلص من هذه الدراسة أن عزل ميكروبات الكامبيلوباكتر من النعام يعطى معلومات تفيد فى عزل وتشخيص الامراض البكتيرية التى تصيب الطيور وكذلك طرق التربية المستخدمة فى حديقة الحيوان للمحافظة عليها بصحة جيدة وتطبيق برامج رعاية سليمة صحيا. ولابد من أتباع برامج طبية للطيور خاصة فى حديقة الحيوان مع الكشف الدورى للبراز وعلاج النعام المريض مع أعطاء الغذاء الجيد والرعاية الصحيى المناسبة.