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Research Article

**BACTERIOLOGICAL STUDIES ON EGGS,  
GASTROINTESTINAL AND REPRODUCTIVE TRACT  
SECRETIONS OF OSTRICHES**

Running Article Title: Bacterial infection in Ostriches of Quetta Balochistan  
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**Abstract:**

*Ostrich birds are less sensitive to infectious agents because ostrich need additional factors and circumstances to manifest clinical diseases. Most ostrich diseases are multi-factorial, such as, yolk-sac retention infection, poor hygiene during egg handling and problem during or soon after naval of hatching that is initially gut colonization due to pathogenic bacteria. A total of 150 samples were processed and different types of bacterial microorganisms identified through biochemical tests. Among the egg shell E. coli (12%), Citrobacter (6%), Staph. aureus (2%) and Klebsiella (4%) were the main incriminating organisms. In egg yolk isolated bacteria were E. coli (6%) and Citrobacter (2%) while in egg albumin E.coli (4%) and Klebsiella (2%) respectively. The bacteria isolated from reproductive tract were E.coli (2%), Klebsiella (2%), and Serratia (2%) while from gastrointestinal tract secretion samples revealed E.coli (4%), Citrobacter (2%) and Klebsiella (2%). Bacterial pathogens could tolerate rapidly in harsh environmental fluctuations and exposure to various toxic chemicals in order to survive. While, the mechanisms under which these pathogens can survive and proliferate under such extreme environmental conditions are largely unknown, a little research has been carried out on disease and disease related problems in ostrich. Present investigation/study is first ever attempting of its nature in ostrich birds in Quetta.*

**Key Words:** Ostrich, Egg, Gastrointestinal, Reproductive, Bacteria.

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## INTRODUCTION:

Ostrich is the largest living bird in the world [1]. The adult ostrich is approximately 2.75 meter in height and 150 kg body weight. The young ostriches are mottled brown, yellow, orange and cream with black quills on the back [2]. They prefer to eat natural dietary habits and mainly green grasses, berries, seeds, succulent plants and invertebrates [3]. Ostriches are monogastric herbivores have single chambered stomach which has the ability to utilize forage [4]. The ostrich farming industry has expanded considerably and even is facing a competitive market for its products now. However, it seems that the ostrich industry needs much improvement in different aspects of production [5]. Several factors such as reasonable fertility, hatchability, and chicks' livability rates need to be improved because these factors have been shown to have considerable influence on the profitability of ostrich farming. Bacterial contaminations of eggs, dead-in egg shell embryos, and yolk sac infection have been associated with lower hatchability rate [6].

There are no severe infectious or contagious diseases are reported in ostrich birds but ostriches can acquire infections from other species of birds and mammals [7]. Usually they are less sensitive than the original hosts to infectious agents and need additional factors and circumstances to manifested clinical diseases. Most Ostrich diseases are multi-factorial such as yolk-sac retention and also factors which are involved in this condition such as poor hygiene during egg handling and incubation which allows the bacteria to penetrate in the egg shell [7].

Prevention of infection is a better course of action. Nest should be kept dry and covered with clean sand from time to time. Eggs should be collected before they have time to cool down, however, during cooling the egg contents will be shrinked and bacteria from a wet nesting site can be pulled into the egg shell. Therefore, incorrect egg washing could facilitate the penetration of bacteria and microbial spores in the egg shell [7][8]. Complete healed navels of hatchings should be routinely disinfected by using a topical disinfectant or antiseptic solutions. Currently, little information is available about the bacterial contaminations of ostrich eggs, in Balochistan ostrich farming industry. This is the first comprehensive study in Balochistan on the status of bacterial contaminations of ostrich eggs, gastrointestinal and reproductive tract secretion.

## MATERIALS AND METHODS:

A total 150 samples were collected from (50) ostrich eggs, (50) gastrointestinal tract and (50) reproductive

tract secretions for the isolation of different types of incriminating bacteria.

## PREPARATION OF MEDIA AND REAGENTS

The commercially available media such as brain heart infusion medium (BHI broth), nutrient medium, MacConkey medium, eosin methyl blue (EMB) medium were prepared according to the instruction of the manufacture, the pH of the media was adjusted at 7.4 - 7.6 with one molar NaOH and one molar HCL.

## PURIFICATION OF CULTURE

Mixed culture which was grown on respective media, further purified on the same media (separate plate of fresh agar) then again kept in incubator at 37 °C for 24 hours. This purification was repeated until to get pure colonies. After obtaining pure culture, the Gram's staining technique was performed to differentiate gram positive or gram negative bacteria.

## BIOCHEMICAL TESTS

Different selective media were used and biochemical tests were performed for the identification of bacteria, which were isolated from eggs, gastrointestinal tract and reproductive tract of ostrich. The Motility Test, Methyl Red & Vogues Proskauer Test (MR & VP), Indole Test, Catalase, Mannitol fermentation, Citrate Utilization Test, ONPG Test, Urease Test, Coagulase Test and Oxidase Test were performed for the primary isolation, identification and biochemical characterization of bacteria.

## RESULTS:

50 samples from eggs, 50 samples from reproductive tract and 50 samples from gastrointestinal tract secretions of ostrich birds were used for bacterial culture to isolate, identify and biochemical characterization for the different microorganisms.

## SAMPLES PROCESSING

The samples of external and internal part of the eggs, reproductive tract and gastrointestinal tract secretion were collected from animal house and transferred to Center for Advanced Studies Vaccinology and Biotechnology (CASVAB) for isolation and identification of pathogenic bacteria.

## BIOCHEMICAL TESTS

Different selective media were used and biochemical tests were performed for the identification of bacteria, which were isolated from eggs shells, eggs yolk, eggs albumen, gastrointestinal tract and reproductive tract of ostrich (Table 1).

**Table- 1. Biochemical characterization of microorganisms.**

Characteristics	<i>Staph. aureus</i>	<i>E. coli</i>	<i>Citrobacter</i>	<i>Klebsiella</i>	<i>Serratia</i>
Sucrose	+	+	+	+	+
Oxidase	-	-	+	-	-
Mannitol	+	+	+	+	+
Methyl Red (MR)	+	+	+	-	-
Voges Proskauer (VP)	+	-	-	+	+
Lactose Fermentation	+	+	+	+	-
Catalase	+	+	-	-	+
Citrate (Simmons)	+	-	+	+	+
Indole	-	+	-	+	-
Motility	-	+	+	-	+
Phenylalanine (PA)	-	-	-	-	-
Urease	+	-	+	+	-
Coagulase	+	NA	NA	NA	NA
Hemolysis	+	NA	NA	NA	NA

**Isolated organisms from egg shell, egg yolk and egg albumin**

The external part of the egg (egg shell) was contaminated 24% and internal part of the eggs was contaminated 14% with different bacteria (Table 2). It is observed that the isolated microorganisms among the egg shell were *E. coli* 12%, *Citrobacter* 6%, *Staph. aureus* 2% and *Klebsiella* 4% were respectively. In egg yolk isolated bacteria was observed *E. coli* 6% and *Citrobacter* 2% while in egg albumin isolated bacteria were *E. coli* 4% and *Klebsiella* 2% respectively (Table 2).

**Table- 2: Isolated organisms from egg shell, egg yolk and egg albumin**

Characteristics of organisms	Egg shell Isolates (% age)		Egg yolk Isolates (% age)		Egg albumin Isolates (% age)	
	<i>E. coli</i>	6	12%	3	6%	2
<i>Citrobacter</i>	3	6%	1	2%	-	-
<i>Staph. aureus</i>	1	2%	-	-	-	-
<i>Klebsiella</i>	2	4%	-	-	1	2%
Total	12	24%	4	8%	3	6%

**Isolated organisms from reproductive tract and gastrointestinal tract secretions**

From the reproductive tract isolated bacteria were *E. coli* 2%, *Klebsiella* 2% and *Serratia* 2% while from the gastrointestinal tract secretion samples isolated bacteria were *E. coli* 4%, *Citrobacter* 2% and *Klebsiella* 2% respectively (Table 3).

**Table-3. Isolated organisms from reproductive tract and gastrointestinal tract secretions**

Characteristics of organisms	Reproductive tract Isolates (% age)		Gastrointestinal tract Isolates (% age)	
<i>E. coli</i>	1	2%	2	4%
<i>Citrobacter</i>	-	-	1	2%
<i>Klebsiella</i>	1	2%	1	2%
<i>Serratia</i>	1	2%	-	-
Total	3	6%	4	8%

**DISCUSSION:**

Ostrich birds and eggs were used in this study for sample collection and the samples were collected from external and internal part of the eggs, reproductive tract secretions and gastrointestinal tract secretions for the bacteriological isolation and identification. In present study five different types of bacteria were isolated from both part of the eggs, reproductive tract secretions and gastrointestinal tract secretions, which include *Escherichia coli*, *Citrobacter*, *Staphylococcus aureus*, *Serratia* and *Klebsiella*. Similar findings were reported by Cabassi *et al.*, [9] the high prevalence of *Enterobacter* species and *Escherichia coli* in ostrich eggs with reproductive disorders. In the present study *Escherichia coli* (12%), *Citrobacter* (6%), *Staphylococcus aureus* (2%) and *Klebsiella* (4%) found in egg shell while in egg yolk *Escherichia coli* (6%), *Citrobacter* (2%) were observed but in egg albumin *Escherichia coli* (4%) and *Klebsiella* (2%) were found. Our results are corroborating with the findings of Cabassi *et al.*, [9] who has reported that several bacterial diseases are causing infection in ostrich eggs and their life.

In some cases it has been reported that bacterial origin is secondary to septicemia from enteritis via portal circulation [9]. Hence, contamination of *Serratia* and *Citrobacter* species are most commonly concerned in public health and these agents are resistant to some antimicrobial drugs and disinfectants [10].

Moreover, several systemic diseases are involved caused by bacteria in human with immunodeficiency syndrome [11]. However, Citrobacteriosis is rare in ratites but highly fatal disease, primarily caused by *Citrobacter* infection that affects ostrich chicks [12].

The present study results suggest that *Escherichia coli*, *Citrobacter*, *Serratia* and *Staphylococci* infect the eggs by fecal contamination due to egg shell deformity. Similarly it is reported that reproductive failure usually occurs due to poor quality eggs and allowing the egg contamination by bacteria which is present in gut flora [10]. There are few studies reported on reproductive diseases of ostrich, therefore further studies are required to confirm the vertical transmission of bacteria and possible impacts of the contamination by the microbial organisms on ostriches reproduction.

**CONCLUSION:**

The bacterial infections are one of the leading cause of reproductive problems in ostriches throughout

world. A number of bacterial pathogens are involved in gastrointestinal, reproductive tract infection in animals and human including *Escherichia coli*, *Klebsiella*, *Serratia*, *Citrobacter* and *Staphylococcus aureus*. These organisms are important and recognized to be a most common cause of eggs, gastrointestinal tract and reproductive tract infection in ostrich birds. In the present study it is concluded that the isolation of pathogenic organisms are very important in ostrich birds to avoid contaminations in eggs and develop awareness in ostrich farmers to increase the production of ostriches. Therefore, it is important to recommend farmers and other worker in the field on the need of re-evaluation of diseases in ostriches. Among pathogens is an evolving process, routine surveillance and monitoring studies should be conducted to provide farmers with knowledge about the most important bacterial and viral identification in ostriches. The ostrich farmers and producer should be educated about the impact of feed ingredients on growth and development and also to make sure the capacity of the birds to utilize each nutrient and expected performance outcomes.

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