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ISOLATION OF MYCOPLASMA GALLISEPTICUM AND MYCOPLASMA SYNOVIAE FROM NATIVE, ROSS AND LOHMANN CHICKENS IN HAIL REGION OF SAUDI ARABIA

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SUMMARY

In this study, *M. gallisepticum* and *M. synoviae* were isolated from different breeds of chickens in Hail region of Saudi Arabia. Out of 200 samples (trachea, lungs, air sacs and synovial swabs) taken from native chickens of different ages, 100 pure mycoplasma isolates were recovered. When typed by morphological, biochemical and serological methods, 39 isolates were identified as *M. synoviae*, 31 isolates were identified as M. gallisepticum..Of 60 samples from Lohmman layers, 11 pure isolates were obtained and 6 of them were identified as *M. synoviae*, 1 was *M. gallisepticum*. Of 140 samples collected from Ross chickens and breeders, only samples collected from breeders aging 8-14 weeks (40 samples) resulted in positive mycoplasma isolation. Fifteen pure mycoplasma isolates were recovered of which 3 were identified as *M. gallisepticum*, 4 were identified as *M. synoviae*. Tracheal samples and synovial fluids resulted in the highest isolation rates from native and Lohmman chickens, respectively while synovial fluid and tracheal samples resulted in equal rates of Mycoplasma isolation from Ross chickens. Agar media containing tryptone soy agar base resulted in bigger Mycoplasma colonies and nipples.

Keywords: Mycoplasma *gallisepticum*, *Mycoplasma synoviae*, Saudi Arabia, chickens

INTRODUCTION

Mycoplasmas belong to a genus within the class Mollicutes, which are the smallest known prokaryotes capable of self-replication (Ryan and Ray, 2004). They have a very small genome, and have evolved to this 'minimalist' status by losing non-essential genes, including those involved in cell wall synthesis (Hutchison and Montague, 2002). However, the Mollicutes exploit their limited genetic material to the maximum and many are successful pathogens in man, animals, birds and plants. The pathogenesis of mycoplasmas are not fully understood, but they are successful pathogens because they can enter the host and multiply, evade the defense mechanisms, cause

damage and escape to infect new hosts. In poultry flocks there is both horizontal and vertical transmission, the former being encouraged by intensive husbandry and stress factors (Bradbury, 2005). Mycoplasma infections are of great concern in avian medicine as . *gallisepticum* (MG) and *M. synoviae* (MS) are the cause of considerable economic losses in the poultry industry (Wang et al., 1997; Feberwee et al., 2005). It should be noted that *M. meleagridis* and *M. iowae* can cause disease in poultry, but MG and MS are considered to be the most important (OIE, 2008). MG causes chronic respiratory disease of chickens and infectious sinusitis in turkeys. MS causes infectious synovitis and/or mild upper respiratory disease. Transmission is either direct, from bird to bird or through the egg, or indirect. Diagnosis is based on isolation and identification of mycoplasmas, according to biochemical, serolotyping or molecular biology tests, or serological examination of host sera by slide agglutination, haemagglutination inhibition or enzyme-linked immunosorbent assay (ELISA) tests (Stipkovits and Kempf, 1996). Mycoplasmas are well known for their interactions with other infectious agents and environmental factors in producing clinical disease. Mixed infections of bacterial or viral origin, play an important role in the spread of MG and MS within chicken flocks or in the induction of clinical respiratory mycoplasmosis. In Saudi Arabia, poultry industry represents a huge business in the private sector to cover a considerable side of the human needs for chicken meat and eggs. Concerning avian mycoplasmosis, there is no data available about its incidence among chicken flocks in Saudi Arabia and its economic impacts on the productivity. Therefore, the present study was carried out to isolate MG and MS from native and foreign breeds of chicken flocks in Hail, a region that contains huge poultry farms (broilers, layers and parents) in Saudi Arabia.

MATERIALS AND METHODS

CHICKENS

Three chicken breeds investigated in this study included native balady (age 16 - 28 weeks) , Ross (broiler aging 3-7 weeks) and Lohmman (layers aging more than 50 weeks). Chickens belonged to commercial flocks in different localities in Hail region from which, clinical suspected birds were collected. Birds were taken to the laboratory for postmortem examination and sample collection for mycoplasma isolation after slaughtering.

MEDIA

Solid and liquid media were prepared for isolation of MG and MS from chicken samples. Different media were prepared for comparative purpose. Media prepared and used in this study were: modified Frey's broth and agar (Frey et al., 1968), modified PPLO agar and broth (Hayflick, 1965), and soy agar broth medium (Attia, 1988). For preparation of mycoplasma media, the base was autoclaved at 121°C for 15 minutes and kept at 56°C in a water bath for 30 minutes before addition of supplements sterilized by filtration. The pH was adjusted to 7.8 and the medium was distributed into suitable containers.

MEDIA SUPPLEMENTS

Supplements added to the bases of mycoplamma media were fresh yeast extract prepared from brewers yeast (Si lesaffre, France), dextrose (BDH, England), L-cysteine Hydrochloride (Fisher Biotech., USA), NAD (Nicotinamide adenine dinucleotide, Sigma, USA), horse serum (VACSERA, Agouza, Giza, Egypt), thallium acetate (BDH, England), phenol red (Winlab, UK) and Penicillin-G sodium (Sigma , USA).

MYCOPLASMA ISOLATION AND PURIFICATION

Standard methods were employed to isolate and purify mycoplasmas from chicken samples (Stipkovits et al., 1975; Attia, 1988; Helail, 2002). Tracheas, lungs, air sacs, and synovial fluid were aseptically taken for isolation of MG and MS. Samples were spread onto agar plates and inoculated into broth. Serial subcultures, purification and cloning were carried out to ensure successful isolation. Inoculated plates and bottles were incubated at 37°C, where the plates were held in moistened candle jar for 2 weeks and examined microscopically by the inverted microscope every 2 or 3 days. Swabs from synovial fluid were cultivated in Frey's medium with NAD and L- cysteine-HCl for isolation of MS. All cultures were spread onto solid medium without penicillin and thallium acetate to exclude the L-forms of bacteria.

IDENTIFICATION OF MYCOPLASMA ISOLATES

Mycoplasma isolates were identified on the bases of colonial morphology (Quinn et al., 2002), digitonin sensitivity (Erno and stipkovits, 1973), glucose fermentation (Sabry, 1968; Helail, 2002) and tetrazolium reduction (Erno and Stipkovits, 1973; Helail, 2002). Film and spot formation (Edward, 1950) and NAD requirement (Frey et al., 1968) were carried out for confirmation of MG isolates. Growth inhibition test (Clyde, 1964) using MG- and MS-specific antisera (Animal Health Service Ltd., Netherlands) was done to ensure the biochemical identification results.

RESULTS

Of 200 different samples taken from native chickens of different ages, 35 samples resulted in positive mycoplasmal growth (Table 1). Positive samples represented 13 tracheas, 11 lungs, 6 air sacs and 5 synovial swabs. From the same table, 6 out of 140 samples from Ross chickens resulted in isolation of Mycoplasmas (2 tracheas, 1 lung, 1 air sac and 2 synovial swabs). Out of 60 samples from Lohmman chickens, 4 produced Mycoplasma isolates (1 trachea, 1 air sac and 2 synovial fluid samples). On microscopic examination of the positive agar cultures, there were mixed types of colonies in most of them. After purification, 100, 15 and 11 pure mycoplasmal isolates were recovered from native, Ross and Lohmman chicken samples, respectively. When typed

by morphological, biochemical and serological methods, native chicken isolates were found to be 39 isolates of *M. synoviae*, 31 isolates of *M. gallisepticum*, while 30 isolates were neither *M. synoviae* nor *M. gallisepticum* (Table 2). Of samples taken from Lohmann layers, 11 pure isolates were obtained and 6 of them were identified as M. synoviae, 1 was *M. gallisepticum*, while 4 were neither *M. synoviae* nor *M. gallisepticum* (Table 2). Samples collected from Ross chickens and breeders resulted in 15 pure mycoplasma isolates of which 3 were identified as *M. gallisepticum*, 4 were identified as *M. synoviae* while 8 were neither M. synoviae nor *M. gallisepticum*. In native chickens, tracheal samples resulted in the highest isolation percentage (26%) followed by lung samples (22%), air sacs (12%) and synovial fluid (10%). Of the Ross breed samples, trachea and synovial fluid samples were equal in the rate of Mycoplasma isolation followed by lung and air sac samples. Concerning Lohmman breed samples, synovial fluid samples resulted in the highest rate of Mycoplasma isolation and no Mycoplasma isolates were recovered from lung samples (Figure 1). On solid media, bigger colonies and nipples were obtained wit MG and MS colonies on agar containing tryptone soy base (Figure 2). Of liquid media, PPLO broth base resulted in the Mycoplasma richest culture.

DISCUSSION

The eradication of avian mycoplasma infection can be achieved through improvements in hygiene and management practices, therapeutic treatment of breeder layers and/or of hatching eggs and better monitoring procedures (Stipkovits and Kempf, 1996). The target of this study was to investigate the incidence of mycoplasmosis among broiler, layer and breeding chickens of different breeds in Hail, a region that has a large contribution to the poultry industry in Saudi Arabia. As MG and MS are the commonest mycoplasmas affecting chickens, they were the targets in the identification steps (Sato, 1996; Stipkovits and Kempf, 1996; Branton et al., 1997). Out of 200 native chicken, 35 samples resulted in growth of mixed mycoplasmas. In contrast, only 6 out of 140 Ross chicken samples and 4 out of 60 Lohmman chicken samples resulted in growth of mycoplasma colonies (Table 1). Tracheal samples produced the highest isolation rates of mycoplasmas from native chickens (26%) followed by lung samples (22%). Samples of Ross and Lohmman breeds differed from those of the native breed in mycoplasma isolation (Figure 1). From the mixed cultures, 126 pure mycoplasma isolates were recovered of which 100, 15 and 11 isolates were obtained from samples of native, Ross and Lohmman chickens, respectively (Table 2). Of the mycoplasma isolates, 35 (27.77%) were identified as *M. gallisepticum* and 49 (38.88%) were identified as *M. synoviae* while 42 (33.33%) were other mycoplasmas. MG isolation rates were 31%, 20% and 9.1% from native, Ross and Lohmman chickens, respectively. In contrast, MS isolation rates were 39%, 26.66% and 54.54%, from native, Ross and Lohmman chickens, respectively. Concerning isolate characters, MG and MS showed their well known characteristics with morphological, biochemical, nutritional and serological identification methods. Incidence of MG infection with the highest rate of isolation among native chickens can

be attributed to many factors of which the hygienic measures and rearing conditions are the most important. In one study, a significant decrease in MG isolations from Leghorn hens was observed in trials from swabs obtained when hens were housed on dry litter floors as compared with swabs taken from the same hens after 18 days or 21 days of confinement in isolation units (Branton et al., 1989). Isolation of MS with the highest rates from Lohmman chickens can be explained by suggestions of previous reports. In one study, MS were experimentally investigated for their virulence in Mycoplasma-free broiler chickens. Based on differences of the virulence, the isolates were classified to four categories: 1. highly virulent, 2. virulent, 3. moderately virulent and 4. slightly virulent. It was suggested that the MS strains tested differ in their potential capacity to invade systemically and produce acute septicaemia (Hinz et al., 2003). This means that less virulent MS can be isolated from clinically normal chickens. This was reported by Bradbury et al. (2001) who isolated MS from the tracheas of seven clinically normal pheasants found in the vicinity of a chicken farm infected with *M. synoviae*, but not from 120 pheasants and partridges with respiratory disease. Concerning isolation media, tryptone soy agar base is preferable for clearer Mycoplasma colonies and PPLO broth base is good for more Mycoplasma colony forming units per ml which is an important issue for antigen preparation purposes. Conclusively, the results obtained in this study threw a spot of light on chicken mycoplasmosis in Hail region, which constitutes a very important region for poultry industry in Saudi Arabia. Both MG and MS were successfully isolated and identified in pure cultures which is important for further epidemiological investigation.

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Table (1): Mycoplasma positive cultures obtained from samples of different chicken breeds in Hail region

Sample	Native		Ross		Lohmman	
	No.	Mycoplasma isolation	No.	Mycoplasma isolation	No.	Mycoplasma isolation
Trachea	50	13	35	2	15	1
Lungs	50	11	35	1	15	-
Air sacs	50	6	35	1	15	1
Synovial swabs	50	5	35	2	15	2
Total	200	35	140	6	60	4

*All cultures were obtained from samples of birds aging 8-14 weeks

Figure (1): Mycoplasma isolates recovered from different samples of native, Ross and Lohmman chickens

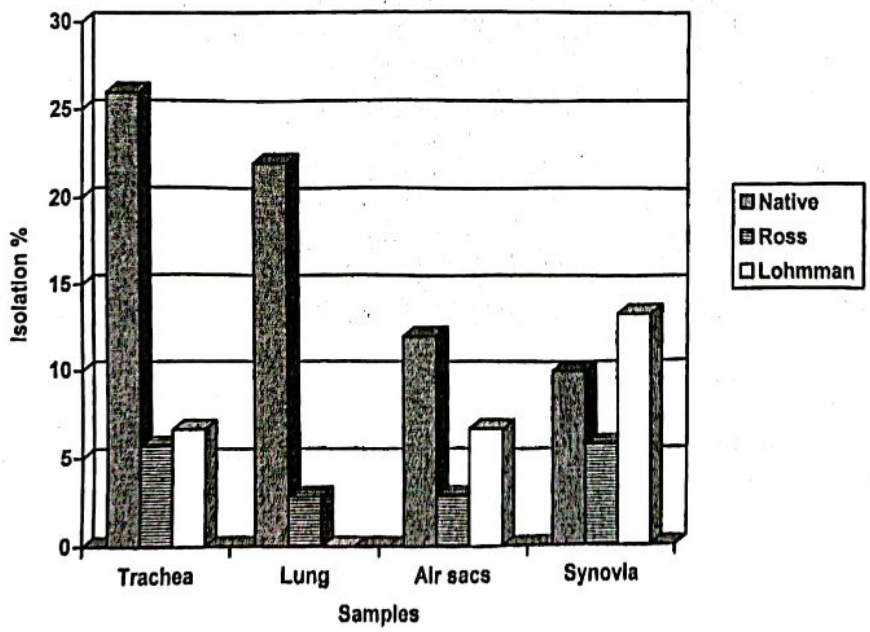
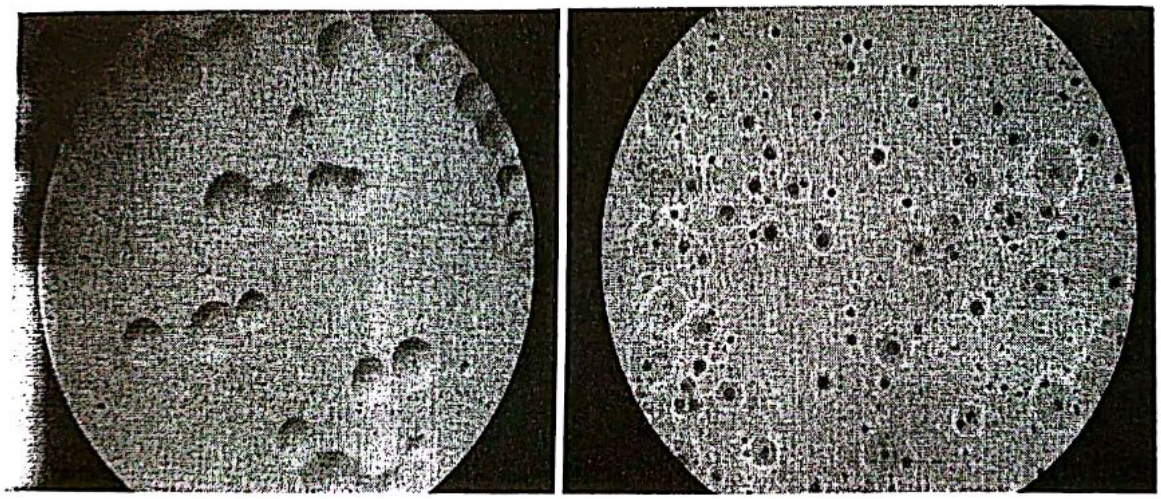


Table (2): *M. gallisepticum and M. synoviae* recovered from different chicken breeds in Hail region

Chicken breed	Mycoplama isolates	*M. gallisepticum*		*M. synoviae*		Other mycoplasmas	
		No.	%	No.	%	No.	%
Native	100	31	31.00	39	39.00	30	30.00
Ross*	15	3	20.00	4	26.67	8	53.33
Lohmman	11	1	9.10	6	54.55	4	36.36
Total	126	35	27.78	49	38.89	42	33.33

*All cultures were obtained from samples of 8-14 weeks old breeders

Figure (2): Microspic mycoplasma colonies



Large *Mycoplasma gallisepticum* colonies on tryptone soya agar (X40)

Mycoplasma synoviae colonies on tryptone soy agar showing the large **nipple (X40)**

عزل ميكوبلازما جاليسبتيكام و ميكوبلازما ساينوفي من دجاج محلي و روس و لوهمان بمنطقة حائل بالمملكة العربية السعودية

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الملخص العربي

استهدفت هذه الدراسة الاستبيان عن مدى انتشار مرض مايكوبلازما الطيور بين قطعان الدجاج في منطقة حائل. وتم ذلك عن طريق عزل كل من مايكوبلازما جاللليسيبتيكم و مايكوبلازما ساينوفي كمسببين رئيسيين للمرض في الدجاج. تم تجميع عينات من قطعان دجاج محلي، روس، ولوهمان ذات أعمار مختلفة وشملت العينات قصبات هوائية، رئة، أكياس هوائية، و مسحات من السائل المفصلي. من ٢٠٠ عينة دجاج محلي تم الحصول على ١٠٠ معزولة مايكوبلازما صنفت ٣١ منها كمايكوبلازما جاللليسيبتيكم و ٣٩ مايكوبلازما ساينوفي. ومن ٦٠ عينة لوهمان تم الحصول على ١١ معزولة مايكوبلازما صنفت معزولة واحدة منها كمايكوبلازما جاللليسيبتيكم و ٦ مايكوبلازما ساينوفي. أما عينات الروس و عددها ١٤٠ فأسفرت عن عزل ١٥ معزولة مايكوبلازما صنفت ٣ منها كمايكوبلازما جاللليسيبتيكم و ٤ مايكوبلازما ساينوفي. و قد أنتجت عينات القصبة الهوائية أكبر نسبة عزل للميكوبلازما في الدجاج المحلي بينما احتلت عينات السائل المفصلي أعلى نسبة عزل في اللوهمان و تساوت عينات القصبة الهوائية والسائل المفصلي في عينات دجاج الروس. و أظهرت البينات المحتوية على أساس أجار تريبتون الصويا مستعمرات ميكوبلازما و حلمات أكبر.