Seroprevalence of Newcastle disease and other infectious diseases in backyard chickens at markets in Eastern Shewa zone, Ethiopia

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ABSTRACT This study was conducted to estimate the seroprevalence of Newcastle disease (ND), *Pasteurella multocida* (PM) infection, *Mycoplasma gallisepticum* (MG) infection, and infectious bursal disease (IBD) and to assess the level of concurrent seropositivity during the dry and wet seasons of the year 2010. In total, 234 and 216 sera were collected during the dry and wet seasons, respectively, from unvaccinated backyard chickens at 4 live poultry markets in 2 woredas (districts) of Eastern Shewa zone, Ethiopia, and were tested using commercial ELISA kits. The overall seroprevalence of ND, PM, MG, and IBD was 5.9, 66.2, 57.7, and 91.9%, respectively, during the dry season, and 6.0, 63.4, 78.7, and 96.3%, respectively, during the wet season. The seroprevalence of MG was higher ($P < 0.001$) during the wet season than during the dry season and higher ($P = 0.002$) in Adami-Tulu-Jido-Kombolcha woreda (74%) than in Ada'a woreda (60%). Area and season had no significant effect on the seroprevalence of ND, IBD, and PM, indicating the widespread presence of those pathogens throughout the year in the study area. Of all the chickens tested, 85.6% had antibodies concurrently to more than one of the pathogens investigated. Birds were concurrently seropositive to more diseases during the wet season (median = 3) than during the dry season (median = 2; $P = 0.002$). As serology is not able to distinguish between strains, further studies are warranted to better understand the circulating strains, their interactions, and their economic effect on backyard poultry production in Ethiopia.

Key words: Newcastle disease, infectious disease, backyard chicken, Ethiopia

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INTRODUCTION

Like elsewhere in the developing world, backyard poultry rearing is a common practice in rural Ethiopia. Village backyard poultry, characterized by traditional production methods and local breeds, represents 98% of the total Ethiopian poultry population of 38 million (CSA, 2008). The sector provides eggs and poultry meat to most rural and many urban consumers (Tadelle, 1996). However, the productivity of backyard chickens is hampered by several factors, including a variety of infectious diseases. In Ethiopia, an estimated 40 to 60% of newly hatched chicks die before reaching maturity, mainly due to disease and predation (Tadelle et al., 2003). An ongoing questionnaire-based survey (our unpublished data) has revealed that farmers in Eastern Shewa Zone consider any disease that causes moderate to high mortality to be Newcastle disease (**ND**), known locally as Fengil or Fenqil, as also reported elsewhere (Sonaiya and Swan, 2004). It is therefore likely that many other infectious diseases have, for a long time, been described incorrectly as ND. Mortalities of backyard chickens may also result from other viral or bacterial diseases, either individually or concurrently. Several viral and bacterial infectious diseases, including ND, *Mycoplasma gallisepticum* (**MG**) infection, infectious bursal disease (**IBD**), and Marek's disease have been described in commercial poultry farms in Ethiopia (Alamargot, 1987; Nasser et al., 2000; Lobago and Woldemeskel, 2004; Zeleke et al., 2005a,b; Chanie et al., 2009), but their occurrence in backyard poultry production systems has rarely been documented. The key to increasing profitability of backyard poultry production is to know which diseases are prevalent in an area (Bell, 2009).Workers in other African countries have documented the presence of many viral and bacterial diseases in backyard production systems (Bell et al., 1990; Kelly et al., 1994; Chrysostome et al., 1995; Idi et al., 1999; Orjaka et al., 1999; Muhairwa et al., 2001; Ndanyi, 2005; Mushi et al., 2006; Mbuthia et al., 2008). It is therefore also likely that infectious diseases,

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such as ND, IBD, mycoplasmosis, and pasteurellosis, could play a role, individually or concurrently, in backyard poultry health in Ethiopia. In addition, backyard poultry could be a potential reservoir of these pathogens that could jeopardize the development of semi-commercial poultry production in Ethiopia.

Newcastle disease is caused by avian paramyxovirus serotype 1 (**APMV-1**), which, with viruses of the other 8 APMV serotypes (APMV-2 to APMV-9), have been placed in the genus *Avulavirus*, belonging to the subfamily *Paramyxovirinae*, family *Paramyxoviridae* (OIE, 2009). It has a worldwide distribution and is regarded as one of the most important constraints to the development of backyard poultry production (Alexander et al., 2004; OIE, 2009). Serological and virological evidence has shown the presence of the disease in backyard poultry in many African countries (Bell et al., 1990; Echeonwu et al., 1993; Alders et al., 1994; Chrysostome et al., 1995; Orjaka et al., 1999; Servan de Almeida et al., 2009; Snoeck et al., 2009), and the disease has long been known to be endemic in Ethiopia (NVI, 1974). Limited previous serological surveys of ND based on haemagglutination inhibition (**HI**) titer antibody detection in backyard chickens in Ethiopia have found that 11 to 38% of chickens had detectable antibodies (Tadesse et al., 2005; Zeleke et al., 2005b; Regasa et al., 2007).

Infectious bursal disease (IBD) is a highly contagious, immunosuppressive infection of immature chickens with a worldwide distribution (Sharma et al., 2000). Two serotypes of IBD virus strains are described: 1 and 2. Serotype 2 strains are classified as apathogenic, and serotype 1 strain, pathogenic to chickens, is classified into several pathotypes, from mild to hypervirulent, according to their virulence (van den Berg et al., 2004). Infectious bursal disease outbreaks among backyard chickens have been reported in China (Fa, 1993), Indonesia (Parede, 1992), Vietnam (Vui et al., 2002), and Ecuador (Sonia et al., 2006). It was also reported from several countries in Africa, including Zimbabwe (Kelly et al., 1994), Niger (Idi et al., 1999), Kenya (Ndanyi, 2005), Egypt (Azzam et al., 2004), Mauritania (Bell et al., 1990), and Botswana (Mushi et al., 2006). The disease was first diagnosed in Ethiopia in 2002 in commercial poultry (Zeleke et al., 2005b) and thereafter in a government-owned poultry multiplication center (Woldemariam and Wossene, 2007) and a commercial broiler farm (Chanie et al., 2009). In Ethiopia, IBD in backyard chickens has been serologically documented from the northwest and central parts of the country (Mazengia et al., 2009; Degefu et al., 2010).

Mycoplasma gallisepticum (MG), together with *Escherichia coli*, is the cause of chronic respiratory disease in chickens and is the most economically important of the avian *Mycoplasma* spp. (Bradbury, 2001). Mycoplasmas are also well known for their interactions with other infectious agents, such as ND virus, and environmental factors in producing clinical disease (Kleven, 1998). The disease has been reported, by serology or isolation of the agent, in backyard poultry in a few African countries, including Niger (Idi et al., 1999), Zimbabwe (Kelly et al., 1994), Botswana (Mushi et al., 1999), Benin (Chrysostome et al., 1995), and Kenya (Ndanyi, 2005) as well as from Ecuador (Sonia et al.,2006) and Argentina (Xavier et al., 2011) and in fancy-breed poultry flocks in Switzerland (Wunderwald and Hoop, 2002) where the management system is equivalent to the one in backyard poultry flocks. In Ethiopia, the disease was reported recently and MG was isolated from commercial broiler farms (Chanie et al., 2009). To our knowledge, there are no reports of its presence in backyard chickens in Ethiopia.

Fowl cholera, caused by *Pasteurella multocida* (**PM**), is another disease of significant economic importance with a worldwide distribution (Christensen and Bisgaard, 2000).The characteristic signs of the disease are respiratory rales, coughing, and nasal discharge. Fowl cholera is considered a leading killer of domestic and wild birds in Asia (Rimler and Glisson, 1997). However, literature on the epidemiology and significance of infections caused by PM in poultry in developing countries is scanty, with reports in backyard chickens in Thailand (Thitisak et al., 1989), Zimbabwe (Kelly et al., 1994), Tanzania (Muhairwa et al., 2001), and Kenya (Mbuthia et al., 2008). There are no published reports of this disease in either commercial or backyard chickens in Ethiopia.

The paucity of information on the presence and prevalence of the above diseases in backyard chickens may reflect a lack of resources for disease surveillance and control in backyard production systems. In addition, the diagnostic coverage of poultry diseases in Ethiopia is limited to the extent that, even from commercial farms, only a few cases are brought to either the National Animal Health Diagnostic and Investigation Center (**NAHDIC**) or the National Veterinary Institute. Most poultry outbreaks, particularly in more remote parts of the country, remain undiagnosed and dead chickens are simply discarded. Therefore, information on the prevalence and significance of infectious poultry diseases can only readily be obtained through indirect serological studies on apparently healthy chickens. It is difficult to design and implement chicken health development programs without an understanding of the diseases present in the backyard poultry production system. Hence, this study was implemented to determine the seroprevalence of ND and other major poultry diseases potentially affecting backyard poultry health in Ethiopia, to determine the level of concurrent seropositivity to multiple pathogens, and to determine any seasonal or geographic patterns of seroprevalence.

MATERIALS AND METHODS

Study Area, Study Design, and Sample Size

Administratively, Ethiopia is subdivided into regions that are again subdivided into zones and then woredas

(districts). The study was conducted in the Eastern Shewa zone of the Oromia region, in the woredas of Adami-Tulu-Jido-Konbolcha (**ATJK**) and Ada'a. To have animals coming from a diversity of sources, 4 rural markets (2 from each woreda) were targeted. Accordingly, Dire and Tuludimitu markets in Ada'a woreda and Adami Tulu and Bulbula markets in ATJK woreda were selected.

A cross-sectional serological survey was carried out twice during 2010: in January, representing the dry season, and in September, representing the wet season.

Because various prevalences had been reported for ND virus antibodies in previous studies, ranging from 11% (Regasa et al., 2007) to 38% (Tadesse et al., 2005), an expected seroprevalence of 20% was assumed for ND. Because little or no information was available for the other diseases, the same expected seroprevalence as for ND was assumed. The sample size was therefore calculated to estimate a prevalence of 20% with 95% confidence and 10% absolute error; this gave a required sample size of 62 birds for each market, during each season. On each sampling occasion, a market was visited twice, on 2 consecutive market days. Apparently healthy chickens, greater than 2 mo of age, were purchased, individually identified using a leg band, and transported to NAHDIC. In addition, individual sellers were interviewed regarding chicken disease occurrence during the previous 6 mo (with symptoms including mass mortality, respiratory distress, diarrhea, ocular and nasal discharges, or nervous signs) and vaccination history in their village of origin.

Sampling Procedure and Sample Analysis

Immediately after arrival at the NAHDIC laboratory, blood samples were collected from the brachial vein in 3-mL disposable syringes, left horizontally for 3 hr, and then vertically for the serum to ooze out. Serum was collected in 2-mL cryovial tubes and kept at −20°C until testing.

Serum samples were analyzed using commercial ELISA kits for the presence of antibodies to ND (Svanovir NDV-Ab Elisa, Svanova Veterinary Diagnostics, Uppsala, Sweden), MG (*Mycoplasma gallisepticum* Ab test kit, Svanova Veterinary Diagnostics), IBD (LSI IBD ELISA, Laboratoire Service International, Lissieu, France), and PM (Synbiotics ProFLOK *Pasteurella multocida*, Synbiotics Corp., San Diego, CA), according to the manufacturers' instructions.

The ND virus antibody and MG ELISA work on the principle of blocking ELISA and are developed to detect specific antibodies against PMV-1 and MG, respectively, in serum. The sample and control optical density (**OD**) values were read using an ELISA reader (BDSL, Immunoscan, Lab System, Switzerland) at 450 nm. From OD values, the percentage of inhibition (**PI**) was calculated for control and test samples using the following formula:

$$\mathrm{PI} = [(\mathrm{OD}_{\text{negative control}} - \mathrm{OD}_{\text{sample}})/\mathrm{OD}_{\text{positive control}}] \times 100/\mathrm{OD}_{\text{negative control}}.$$

The LSIVET IBD and ProFLOK PM antibody ELISA are based on the principle of indirect ELISA. The sample and control OD values were read using an ELISA reader at 405 nm. For each sample, the sample-to-positive (S/P) ratios were calculated from OD values by the formula:

$$\text{S/P ratio} = (\mathrm{OD}_{\text{sample}} - \text{negative control mean OD})/(\text{positive control mean OD} - \text{negative control mean OD}).$$

Data Analysis

Data from the laboratory analyses were stored in a spread sheet, and PI or S/P values were computed as above. The seroprevalence of each disease, for each market, woreda, and season, with binomial exact 95% confidence intervals, were calculated. Seroprevalence was compared between woredas and seasons using Fisher's exact test. The median number of diseases for which birds were seropositive was compared between seasons using the Wilcoxon rank-sum test. A significance level of $\alpha = 0.05$ was used. Analyses were done using STATA 11 (Stata Corp., College Station, TX).

RESULTS

Serum samples from a total of 250 and 229 apparently healthy chickens were collected during the dry and wet seasons, respectively. However, only 234 and 216 sera, respectively, were analyzed because of the limited quantity and quality of serum obtained from some of the birds.

From the interviews we learned that none of the sellers had ever vaccinated their chickens. However, 23.4 and 30% of sellers, during the dry and wet seasons respectively, claimed to have had disease among their poultry flocks during the previous 6 mo, with a combination of signs including depression, inappetance, diarrhea, respiratory distress, and paralysis in some cases.

Table1 shows the prevalence of ND, PM, MG, and IBD antibodies in the different markets and woredas during the dry season. The seroprevalence of ND ranged from 4.3% (Bulbula) to 7.8% (AdamiTulu), but no significant difference was seen between woredas. For PM, the seroprevalence varied from 56.8% (Tuludimitu) to 78.5% (Bulbula) and was higher in ATJK (72.4%) than in Ada'a (58%) woreda ($P = 0.025$). The seroprevalence of MG had a wider variation, from 32.4% (Tuludimitu) to 67.2% (AdamiTulu) and was also significantly higher in ATJK (63.4%) than in Ada'a (50%) woreda ($P = 0.045$). The IBD seroprevalence was high in all of the markets and did not differ significantly between woredas.

Table 1. Seroprevalence of Newcastle disease (ND), *Pasteurella multocida* (PM), *Mycoplasma gallisepticum* (MG), and infectious bursal disease (IBD) in backyard chickens at markets in Eastern Shewa zone, Ethiopia, during January 2010 (dry season)

		Seropositive (%; exact 95% CI)			
Woreda/market	n	ND	PM	MG	IBD
ATJK[1] woreda	134	5.9 (2.6;10.0)^{A}	72.4 (64;79.5)^{B}	63.4 (54.6;72.0)^{B}	94.8 (89.5;98)^{A}
AdamiTulu	64	7.8 (2.6;17.4)^{a}	65.6 (52.6;77.0)^{a}	67.0 (54.3;78.4)^{a}	100 (94.3;100)^{b}
Bulbula	70	4.3 (0.9;12.0)^{a}	78.5 (67.1;87.5)^{a}	60.0 (47.6;71.5)^{a}	90.0 (80.4;95.9)^{a}
Ada'a woreda	100	6.0 (2.2;12.6)^{A}	58.0 (47.7;67.8)^{A}	50.0 (39.8;60.2)^{A}	88.0 (79.9;93.6)^{A}
Dire	63	6.3 (1.8;15.5)^{a}	58.0 (45.6;71.0)^{a}	60.3 (47.2;72.4)^{b}	87.3 (76.5;94.4)^{a}
Tuludimitu	37	5.4 (0.6;18.2)^{a}	56.8 (39.5;73.0)^{a}	32.4 (18.0;49.8)^{a}	89.2 (74.6;97.0)^{a}
Total	234	5.9 (3.3;9.8)	66.2 (59.7;72.0)	57.7 (51.1;64.0)	91.9 (87.6;95.0)

^{A,B}Woredas with different superscripts differ significantly ($P < 0.05$).

^{a,b}Within woreda, markets with different superscripts differ significantly ($P < 0.05$).

[1]ATJK = Adami-Tulu-Jido-Kombolcha.

Table 2 shows the prevalence of ND, PM, MG and IBD antibodies in the different markets and Woreda as during the wet season. The seroprevalence of ND showed wider variation between markets than during the dry season, ranging from 3.2% (Bulbula) to 10.3% (Tuludimitu). No significant difference was seen between woredas but a relatively lower proportion of seropositive chickens were recorded in ATJK (4.2%) than in Ada'a (8.0%) woreda. For PM, the prevalence varied from 60.3% (Dire) to 72.4% (Tuludimitu). Fairly similar seroprevalence (63%) was recorded between woredas. The seroprevalence of MG varied from 66.1% (Dire) to 87.5% (AdamiTulu) and was higher ($P = 0.007$) in ATJK (85.7%) than in Ada'a (70.1%) woreda. During the wet season, IBD seroprevalence was closely similar between woredas: 96.6% in ATJK and 95.9% in Ada'a, and it did not differ significantly between markets.

During both wet and dry seasons, a fairly similar proportion (6%) of chickens had antibodies against NDV. But during both seasons, the proportion of chickens that were seropositive for PM, MG, and IBD could be considered high, reflecting the widespread prevalence of those diseases. Both overall and within woredas, the seroprevalence of MG was higher during the wet season than during the dry season ($P < 0.001$).

The distribution of the number of diseases under investigation to which an individual bird was concurrently seropositive is shown in Figure 1. It was found that 81.2 and 91.2% of the chickens tested had antibodies to at least 2 of the diseases under investigation during the dry and wet seasons, respectively. The median number of diseases to which the birds were seropositive during the dry and wet seasons were 2 and 3, respectively, which differed significantly ($P = 0.002$). Overall, less than 1% of the chickens were seronegative for all 4 diseases, while 2.67% had antibodies to all 4 diseases investigated. Most of the concurrent seropositivity (>75%) was due to IBD with either MG or PM or both. No bird was seropositive for ND alone.

DISCUSSION

In unvaccinated flocks, positive serological results are clear evidence that the birds have been exposed to the infectious agent under investigation, although without identifying the infecting strains. In the present study, we confirmed from the sellers during purchase that none of them had vaccinated their chickens for any poultry diseases. Hence, the presence of antibodies to ND, PM, MG, and IBD was considered evidence of exposure to natural infection.

The study revealed that the prevalence of ND antibodies in backyard chickens was generally low, around 6%. This is considerably lower than previous reports by Zeleke et al.(2005b) and Tadesse et al.(2005), who reported prevalences of 19.8% in the southern and Rift

Table 2. Seroprevalence of Newcastle disease (ND), *Pasteurella multocida* (PM), *Mycoplasma gallisepticum* (MG), and infectious bursal disease (IBD) in backyard chickens at markets in Eastern Shewa zone, Ethiopia, during September 2010 (wet season)

		Seropositive (%; exact 95% CI)			
Woreda/market	n	ND	PM	MG	IBD
ATJK[1] woreda	119	4.2 (1.4;9.5)^{A}	63.0 (53.7;71.7)^{A}	85.7 (78;91.4)^{B}	96.6 (91.6;99)^{A}
AdamiTulu	56	5.4 (1.1;15.8)^{a}	62.5 (48.5;75.0)^{a}	87.5 (76;94.8)^{a}	94.6 (85.1;98.8)^{a}
Bulbula	63	3.2 (0.4;11.0)^{a}	63.4 (50.4;75.3)^{a}	84.1 (72.7;92.1)^{a}	98.4 (91.5;100)^{a}
Ada'a woreda	97	8.0 (3.6;15.6)^{A}	63.9 (53.5;73.4)^{A}	70.0 (59.9;78.9)^{A}	96.0 (89.7;98.8)^{A}
Dire	68	7.4 (2.4;16.3)^{a}	60.3 (47.6;72.0)^{a}	66.1 (53.6;77.2)^{a}	94.1 (85.6;98.4)^{a}
Tuludimitu	29	10.3 (2.2;27.4)^{a}	72.4 (52.7;87.3)^{a}	79.3 (60.3;92)^{b}	100 (88.1;100)^{a}
Total	216	6.0 (3.2;10.0)	63.4 (56;67.0)	78.7 (72.6;84.0)	96.3 (93;98.4)

^{A,B}Woredas with different superscripts differ significantly ($P < 0.05$).

^{a,b}Within woreda, markets with different superscripts differ significantly ($P < 0.05$).

[1]ATJK = Adami-Tulu-Jido-Kombolcha.

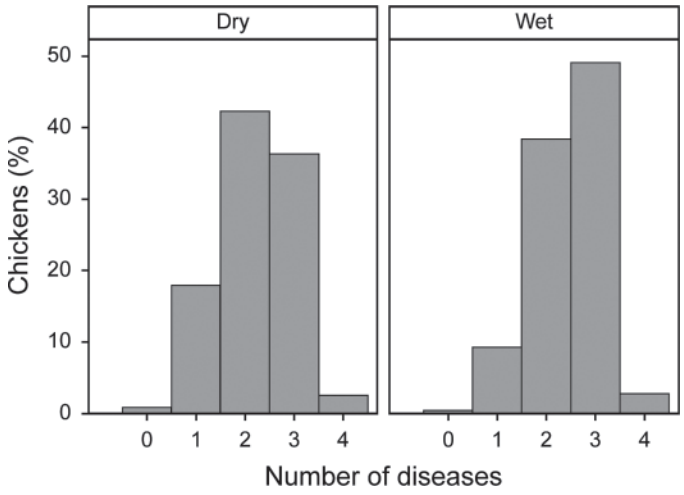

Figure 1. Seasonal distribution of the number of diseases to which an individual bird was seropositive in backyard chickens at markets in Eastern Shewa zone, Ethiopia, tested for antibodies to Newcastle disease, *Pasteurella multocida*, *Mycoplasma gallisepticum*, and infectious bursal disease.

Valley districts and 32.2% in central Ethiopia, respectively, but our results were closer to those reported by Regasa et al. (2007) in southern Ethiopia (11%). Our results are also consistent with seroprevalences in backyard poultry of 4.8% in Mauritania (Bell et al., 1990), 2.2% in Mexico (Gutierrez-Ruiz et al., 2000), 4.8% in California (McBride et al., 1991), and 5% in South Africa (Thekisoe et al., 2003). When chickens are affected by a velogenic ND virus that results in very high mortality, one is likely to find few or no survivors with antibodies. Up to 30% of market sellers claimed to have observed poultry disease signs (sudden death, diarrhea, and nervous signs) resembling Newcastle disease during previous months. Moreover, the fact that high ELISA PI values (as high as 94) were recorded for most of the positive individual animal sera, in the absence of vaccination, suggests that velogenic virus outbreaks might have killed most chickens in the villages and left few survivors with high antibody titer (Chrysostome et al., 1995; Alexander et al., 2004). According to Awan et al. (1994), low ND HI antibody prevalence is suggestive of an interepidemic phase and this could partly explain the high proportion of seronegative chickens in the present study. The seroprevalence was far lower than that reported from Ecuador (97%; Sonia et al., 2006), Tanzania (46.1%; Yongolo et al., 2001), Zambia (36.9%; Alders et al., 1994), Zimbabwe (27%; Kelly et al., 1994), and Bangladesh (88%; Biswas et al., 2009). This could be explained by differences in study settings or by exposure to mild virus strains that induced immunity but did not kill many chickens. The presence of lentogenic, or possibly mesogenic, ND in backyard chickens in an area may result in a constant cycle of infection that periodically boosts the immunity of all exposed chickens, resulting in a higher proportion of chickens with antibodies (Sagild and Haresnape, 1987; Martin, 1992). Another reason for variation between studies could be subjectivity and variation in HI cut-off values used for the interpretation of the result. For instance, some authors considered an HI titer $\geq 1\log_2$ as positive (Alders et al., 1994; Chrysostome et al., 1995; Bouzari and Mousavi Morekani, 2006; Biswas et al., 2009), whereas others used cut-off titers of $3\log_2$ (Tadesse et al., 2005; Zeleke et al., 2005b) or of $4\log_2$ (Gutierrez-Ruiz et al., 2000). Given the periodic outbreaks and probable high mortality among birds affected by ND, our serological findings are likely to be a reasonable indication of the level of ND virus antibody in individual backyard chickens. There was no observed seasonal or geographic variation in seroprevalence, in the present study, suggesting that the disease is widespread and occurs throughout the year in the study area.

Our study revealed a high seroprevalence of fowl cholera (65%), and this constitutes the first report of fowl cholera seroprevalence in Ethiopia. Our finding is in close agreement with that of Kelly et al. (1994), who documented a prevalence of 52% in backyard chickens in Zimbabwe. The high prevalence may be due to infection of backyard chickens by less virulent strains, with or without any clinical signs or significant mortality. This has also been described after challenge with a low virulence strain causing signs of chronic fowl cholera in Kenya (Mbuthia et al., 2008). Biswas et al. (2005) reported proportional mortality from PM of only 6.7% from a longitudinal study in free-range scavenging chickens' in Bangladesh. Mortality is not a typical outcome of fowl cholera in backyard chickens but it may decrease feed efficacy (Mbuthia et al., 2008). Hence, there is a high probability that infected birds will seroconvert and remain convalescent carriers, explaining the observed high prevalence (Muhairwa et al., 2000). Surviving birds from diseased flocks may therefore be a risk to naïve birds. Investigations indicate that carriers of PM may exist within poultry flocks with or without a history of previous outbreaks of fowl cholera (Christensen and Bisgaard, 2000; Mbuthia et al., 2008). Mbuthia et al. (2008) were able to isolate PM from 6.2% of healthy-looking chickens from free-range family poultry farms and at market slaughter slabs in Kenya. Sharing drinking water and feed, which is the common practice in backyard poultry systems in Ethiopia, can facilitate transmission of bacteria between birds.

The MG prevalence observed in this study averaged 67.7%. This high prevalence is in close agreement with reports from Benin (62%; Chrysostome et al., 1995), Botswana (57.8%; Mushi et al., 1999), South Africa (63%; Thekisoe et al., 2003), Ecuador (73%; Sonia et al., 2006), and Bangladesh (58.9%; Sarkar et al., 2005). Lower seroprevalence was reported in village chickens in Malaysia (18.6–25.7%; Shah-Majid, 1996; Faisal et al., 2011) and Zimbabwe (<33; Kelly et al., 1994). Seasonal variation of MG seroprevalence was observed in our study, with higher seroprevalence observed during the wet season (78.7%) than during the dry season (57.7%). This could be due to cold and wet conditions

that stress the birds and make them more susceptible to respiratory infections. Sarkar et al. (2005) also found that MG had a seasonal pattern in Bangladesh, where the seroprevalence was higher during winter than summer. Seroprevalence of MG was also higher in ATJK (74%) than in Ada'a (60%) woreda. This could be associated with the more dusty nature of the areas in ATJK than in Ada'a as well as to differences in hygienic conditions. Lack of cleaning and hygienic practices in chicken houses may result in ammonia build-up in the wet season that predisposes them to respiratory system infection, such as mycoplasmosis (Johnson, 1983). However, this was not assessed in the present study. The occurrence of an MG outbreak in commercial broiler chickens in Ethiopia was associated with overcrowding, poor housing, poor sanitation, and changes in environmental factors (Chanie et al., 2009). Mycoplasmas are also well known for their interactions with other infectious agents and environmental factors in producing clinical disease (Kleven, 1998). Hence, it appears that mycoplasmas, along with other bacterial or viral pathogens, may be responsible for a considerable proportion of the respiratory signs that were reported in poultry.

The survey also indicated that IBD is widespread among village chickens in the study area, with a seroprevalence of 94%. This agrees closely with reports by Degefu et al.(2010) from Ethiopia, Sonia et al. (2006) from Ecuador, and Idi et al. (1999) and Karunakaran et al. (1993) from India, who reported seroprevalences of 76.6, 100, 74, and 73.7% respectively. However, relatively lower IBD seroprevalences were recorded in Mauritania (15.8%; Bell et al., 1990), Zimbabwe (55%; Kelly et al., 1994), Kenya (49.3%; Ndanyi, 2005), and Botswana (66.2%; Mushi et al., 2006). The higher seroprevalence of the disease in the study area, in the apparent absence of mortality, could be due to an IBD virus of lower pathogenicity, unlike the case reported from the Amhara region of Ethiopia (Mazengia et al., 2009) or the outbreak in a commercial broiler farm with evident mortalities (Zeleke et al., 2005a). It is also possible that the birds were infected with IBD virus as adults, at which stage they simply seroconvert without any apparent clinical disease. With such a high seroprevalence and low mortality of infected birds, there is the possibility of genetic resistance among indigenous breeds of chickens in Ethiopia, as reported from Egypt (Hassan et al., 2004). This is difficult to demonstrate using serological studies, but further studies could be undertaken to investigate this.

In general, there was very little variation between seasons in the seroprevalence of PM, IBD, and ND in this study, suggesting the endemicity of those diseases throughout the year in backyard chickens. But variation in the seroprevalence of PM, MG, and IBD was observed between areas during the dry season of the year, suggesting that there could be variation in disease incidence that could be explained partly by variation in local conditions and variation in age structure of flocks in different areas. This could be further substantiated with longitudinal studies that should also take into account the various potential confounders.

In this study, we observed a high percentage of concurrent seropositivity to multiple infectious agents. Although we could not determine whether there had been concurrent infection, most of these involved the immunosuppressive IBD. Although the exact role of each disease is not very clear, the fact that the vast majority of chickens tested had antibodies to 2 or more of the diseases under investigation suggests that their health was effected by multiple infectious pathogens. A similar observation was made in a commercial production system in Ethiopia (Chanie et al., 2009). Biswas et al. (2009) in a serological investigation had reported the existence of various viral diseases in small-holdings in Bangladesh. Bell et al. (1990), in an investigation of the disease status of village chickens in Mauritania, confirmed serologically that village chickens were exposed to NDV, IBD, and *Salmonella pullorum* to varying degrees. Similarly, a study from Zimbabwe demonstrated that backyard chicken flocks had been exposed to several viral and bacterial diseases (Kelly et al., 1994). Biswas et al. (2005) reported the existence of a combination of different viral and bacterial diseases in small-holdings in Bangladesh and concluded that death was the synergic cooperation between different pathogens. Hence, it is possible that the mortalities among backyard chickens in Ethiopia could be explained not only by periodic occurrences of velogenic NDV but also by the synergic effect of other disease-causing agents. Synergism has been demonstrated between MG, ND, and infectious bronchitis (Bradbury, 1984).Carpenter et al. (1991), in a study of turkey flocks, indicated that flocks that had antibodies to ND virus or *Mycoplasma meleagridis* had an increased risk of having an outbreak of fowl cholera. It is generally believed that concurrent infection renders backyard chickens more susceptible to ND infection (Martin, 1992). The fact that mortality was high even in ND-vaccinated flocks indicates that mortalities are the synergic effect of concurrent infections (Kyvsgaard et al., 1999). Kleven (1998) concluded that control of the clinical manifestations of mycoplasma infections is simplified when concurrent infections are minimized and optimum environmental conditions are provided. However, a serological study such as ours cannot provide an accurate indication of concurrent infection of ND with other diseases, first because most chickens might have died due to virulent strains of ND, and second because serology cannot indicate precisely when the infectious agent was present. Nevertheless, the high prevalence of concurrent seropositivity and the fact that about 2.5% of the chickens had antibodies to all of the 4 diseases suggests that backyard chickens are under constant pressure due to several infectious diseases.

In conclusion, our study revealed that several infectious poultry diseases are widespread in backyard chickens in Ethiopia. These diseases are considered to have a significant economic implication individually or con-

currently. It is also likely that other infections occur in addition to the ones investigated (Tadesse et al., 2004). As serology is not able to demonstrate which strains are circulating, further work is recommended to better understand the circulating strains or pathotypes and the epidemiology of these diseases. Second, improvement of village chicken production is at least partly dependent on successful control of some or all of those diseases. Further study is necessary to understand the interactions of these infectious poultry diseases and to estimate their impact on the backyard poultry production system.

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