Serological survey and reproductive performances in buffaloes under fixed time artificial insemination

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ABSTRACT - During an oestrus synchronization trial on heifer and mixed-parity Mediterranean Italian buffaloes, some analyses on health parameters were performed to correlate them to reproductive performances (pregnancy rate and birth rate). Serum sampling were performed every year for two years to be used in serological techniques for the diagnosis of Chlamydia spp. (ELISA), Neospora (ELISA), BHV1 (ELISA gE glycoprotein) and BVDV (ELISA). On the total number of 465 observed buffaloes, the seroprevalence obtained for each disease has respectively been: 24 %, 25 %, 25 % and 22 %. Specific antibodies were not spread over the 13 farms in an homogeneous manner, farm prevalence differences are evidenced by high standard deviations in the mean farm seroprevalence: 42 ± 27.8, 26 ± 26.9, 20 ± 13.8, 9.8 ± 21.1. Among the buffaloes which resulted seropositive to Chlamydia, 33 % were pregnant, 32 % were those with Neospora, 32 % those with BHV1 and finally 29 % of the animals positive to BVDV resulted pregnant. Taking into account the animals that delivered normally, 41 % were positive to Chlamydia, 18 % to Neospora, 18 % to BHV1 and 19 % to BVDV.

Key words: Bubalus bubalis, Artificial insemination, Reproductive disease.

INTRODUCTION - The water buffalo is well known to be susceptible to the most viral, bacterial and parasitic diseases that afflict cattle, while the pathogenesis and the disease effects on buffalo and its productivity are sometimes less evident. The animal reproductive efficiency, in particular, is the most important factor in a farm profitability, however few data referred only to the buffalo reproductive organ infections are available. Among alphaherpesviruses infecting ruminants, the prototype is bovine herpesvirus 1 (BoHV-1), a pathogen of cattle associated with two major syndromes, called infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV), as well as a variety of clinical signs, such as conjunctivitis, encephalitis and abortions. Seroepidemiological surveys have been performed in other species of domestic or wild ruminants in order to investigate whether these animals could be potential BoHV-1 reservoirs. Antibodies against BoHV-1 have been detected in many ruminant species (Thiry et al., 2006) including buffalo (Cavirani et al., 1997).
The bovine viral diarrhoea (BVDV) infection is a major worldwide problem affecting different species of ruminants (Pringle, 1999). The disease is associated both with acute and persistent infections and, depending on epidemiological circumstances, may manifest as outbreaks affecting large numbers of animals or a continual low incidence of cases within endemically infected herds. The significant economic impact inflicted is due to productive and reproductive losses: by a reduced milk yield, reduced conception rate, abortion, foetus mummification, congenital malformations, weak calves and increased animal mortality. Except for cattle, other species that have been infected with BVDV include sheep, goats, lamas, pigs, giraffe, captive and free-ranging deer, antelope, elk, buffalo, water buffalo, reindeer and wildebeest (Brownlie et al., 1989).

Neosporosis is a widespread protozoan infection which causes foetal and neonatal mortality in livestock and companion animals, very little is known about the presence and consequences of this protozoan infection in water buffalo. High rate of neonatal transmission of *N. caninum* in buffaloes was proved (Rodrigues et al., 2005), it is the only known mode of natural infection in cattle neosporosis but it is currently uncertain whether seronegative water buffalo can acquire *N. caninum* through horizontal transmission. Adult buffaloes showed a higher positivity; when compared with younger ones significant differences were found between buffaloes >5 and those <5 years of age, differences among buffaloes are highly significant when their age is considered. The high prevalence of seropositive adult buffaloes in comparison with younger ones (38.6% buffaloes aged 5–6 years and 43.1% animals over 6 years) suggested that transplacental route might not be the only mode of transmission of *N. caninum*. Since similarly to *T. gondii*, *N. caninum* has a two-host life cycle in which the infection might be acquired through ingestion of coccidial oocysts shed by the definitive host which is dog. It is possible that some buffaloes might get infected in this way. Guarino et al. (2000), think that some cases of abortion might be associated to neosporosis in buffalo, considering neosporosis as one of the possible causes of abortion in buffalo.

Chlamydiosis is due to Chlamydia psittaci a small bacterium that causes reproductive disorders, abortion, respiratory diseases, kerato conjunctivitis (Baldi et al., 1997). Its isolation in buffalo calves has been associated to encephalomyelitis and death after 10 to 15 days, sometimes occurring in two to three days (Galiero and Sica, 1997). The aim of this work was to verify the presence of correlations between reproductive performances and serological results of the principal buffalo female reproductive pathogens.

**MATERIAL AND METHODS** - The trial took place in the period February-April of the biennium 2004-2005, during the development of a project aimed at comparing two oestrus synchronization protocols (PRID and Ovsynch), in buffalo farms located in the Latium Region (Centre of Italy). In 13 buffalo herds certified as brucellosis, tuberculosis and leucosis-free and performing no vaccination program, on a total number of 465 female buffaloes, 145 heifers and 320 adult cows, the following serological tests were carried out: BHV1 (ELISA gE glycoprotein), BVDV (ELISA), *Chlamydia* spp. (ELISA), *Neospora* spp. (ELISA). Within each farm, after collecting serum samples, buffaloes were randomly divided into two groups: group P treated with a progesterone releasing intravaginal device (PRID) associated to PMSG and cloprostenol (PGF2α analogue); group G receiving GnRH and cloprostenol (Ovsynch protocol). All the buffaloes were artificially inseminated on the 72nd and the 96th hours from PRID removal (group P) and on the 60th and 84th hours from...
PGF2α injection (group G). Pregnancy was diagnosed by rectal palpation 40 and 60 days after artificial insemination (AI). Each animal was followed up from a reproductive point of view to quantify: A.I. pregnancies and deliveries in order to quantify: A.I. pregnancies (AIP), natural breeding pregnancies (NBP), repeat breeders (RB), early losses (EL), miscarriages (M), perinatal deaths (PD). Data were statistically analysed using SPSS/PC statistics package to quantify serological results for each disease, to compare A.I. results (according to synchronization protocols and bull semen employed), and to test the relationship among reproductive performances and serological results concerning the above mentioned diseases.

**RESULTS AND CONCLUSIONS** - Neither the oestrus synchronization protocol nor the bull semen employed affected A.I. pregnancies. In the same way there was no significant difference in conception rates among the different farms. Serological results expressed as % of positive buffaloes related the total tested animals (465) and to the buffaloes considered in each farm, are shown in Table 1. Farm distribution was not homogeneous but due to the fact that sampling has been suggested only by the entrance of the animals in the reproductive trial, the seroprevalence cannot be considered indicative of health situation neither in farms nor in the Region.

A.I. pregnancies were not influenced by serological positivities as results in Table 2.

Table 1.  Positives (%) at serological exams

| Serol. test | Total + ( %) | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|------------|-------------|---|---|---|---|---|---|---|---|----|----|----|----|
| Neospora   | 24.5        | 100 | 64.3 | 42.9 | 83.3 | 11.8 | 9.3 | 33.3 | 8.3 | 23.1 | 40 | 50 | 38.9 | 34.5 |
| Chlamydia  | 23.7        | 0  | 0  | 12.2 | 8.3 | 30.9 | 8.3 | 96.3 | 17.9 | 25  | 50 | 7.1 | 44.4 | 39.7 |
| BHV1       | 24.9        | 16.7 | 25 | 49.0 | 25 | 41.2 | 21.7 | 29.6 | 17.2 | 13.5 | 10 | 7.1 | 0  | 8.6 |
| BVDV       | 22.0        | 0  | 3.6 | 0  | 0  | 0  | 0  | 76.6 | 1.9 | 10  | 0  | 16.7 | 19.0 |

Table 2.  A.I. results related to serological tests.

| %            | Neospora +/- | Chlamydia +/- | BHV1 +/- | BVDV +/- |
|--------------|--------------|---------------|----------|----------|
| Not Pregnant | 68.3/68.4    | 67.3/68.7     | 69.3/67.9 | 70.3/67.9 |
| Pregnant     | 31.7/31.6    | 32.7/31.3     | 30.7/32.1 | 29.7/32.1 |
|              | 100/100      | 100/100       | 100/100  | 100/100  |

Reproductive performances were gathered in two main groups: RP+EL+M = N (Negative) and AIP+NBP+PD = P (Positive) (Table3). Comparing these groups for test results, no relationship was found for BHV1 and BVDV, but positives to Neospora had lower probability to succeed in a positive reproductive performance (P<0.05) doubling the possibility to have a negative one; Chlamydia serological results, on the contrary, showed how positive animals have greater probability to have good performances (P<0.05) but it can be explained by the
fact that positive animals are mainly represented by adult cows that usually have better performances in this kind of trial.

**Table 3. Reproductive performances related to serological results.**

| % | Neospora +/- | Chlamydia +/- | BHV1 +/- | BVDV +/- |
|---|-------------|--------------|---------|---------|
| N | 69.2/50.9   | 30.6/64.0    | 62.0/52.6 | 66.7/53.3 |
| P | 30.8/49.1   | 69.4/36.0    | 38.0/47.4 | 33.3/46.7 |
| 100/100 | 100/100 | 100/100 | 100/100 |

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